Breath Tests

&
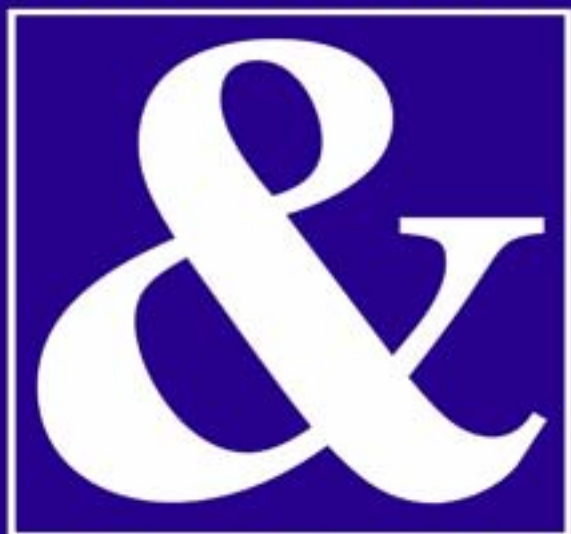
Gastroenterology

Second Edition
1998

Lyle H. Hamilton, Ph.D.
QuinTron Instrument Company

Milwaukee, WI 53215 USA

Preface to Second Edition

Breath-testing has developed into an important diagnostic tool in the practice of gastroenterology over the past 20 years. For more than a dozen years before that, QuinTron Instrument Co. worked in the field of pulmonary instrumentation. In 1976 Dr. Noel Solomons (doing work in the field of nutrition in Guatemala) saw one of our gas chromatographs which was developed for respiratory gas analysis, and asked whether it would measure trace concentrations of breath hydrogen. At the suggestion of Dr. G.B. Spurr, to whom we are indebted for the contact, Dr. Solomons came to Milwaukee for a weekend and we demonstrated that it could (barely) do the job he wanted done. We started to manufacture GCs for breath tests in 1978. Our Engineer, Mr. Thomas Christman, adapted the instrument for a solid-state sensor specific for H_2 and in 1981 we introduced the MicroLyzers for this special application. From that time forward, QuinTron became dedicated to this special field, and we developed the line of instruments and accessories which are now marketed around the world.

From the beginning we were involved in answering the questions of physicians and technical staff about techniques of breath-gas analyses for the special field of disaccharide malabsorption. I felt at home addressing their questions about disaccharide malabsorption, bacterial overgrowth and intestinal transit time because I had already spent 20 years teaching physiology to medical students, residents and Fellows at the Medical College of Wisconsin and the Zablocki VA Medical Center in Milwaukee.

As our knowledge grew from information in the literature provided by academic gastroenterologists, QuinTron's business grew. We invented new devices for improved sample collection and developed new instruments to meet the needs of workers in the field. Consistent with my academic background, I prepared "tutorials" for workers who needed to understand more about the methods they were being asked to use. In 1992, we formulated a monograph based on the tutorials, with some information added about protocols and descriptions of the basic instrumentation. We then included some references and called it "Breath Trace-Gas Tests and Gastroenterology." It was distributed to customers and others who were interested in the topic.

At about that time, QuinTron was purchased by the E.F. Brewer Co. Within two years they changed leadership and their interests shifted, so a year later QuinTron was returned to the original owners (in the fall of 1996). As part of a fresh start, we reviewed the monograph and corrected errors we found, expanded material, added more references and changed the format slightly. It is once again ready for distribution.

It is unlikely that anyone will read this monograph from cover to cover in one sitting. If they do, they will probably criticize it for replicating material in some chapters. It is designed as a reference document, with only general information from other chapters required for an understanding of each topic, so some replication was necessary. References to the literature are presented at the points where they are relevant. We hope they will be useful.

We are sure that errors and misquoted references have gotten through the proofreading process, and would like to have them drawn to our attention, so proper credit can be given in the 3rd Edition – whenever it is written.

We would like to thank the doctors, nurses, and technicians, around the world, who have supported us, and who made it worth while to get back into the business. We will continue to work hard to retain their confidence and support, and to help expand the field of breath testing in Gastroenterology.

Lyle H. Hamilton, Ph.D.
Emeritus Professor of Physiology,
Medical College of Wisconsin
and Director of Research & Development
QuinTron Instrument Company
Summer 1998

Table of Contents

1. Introduction to Breath-Testing

How Do Breath-Tests Help Diagnose Digestive Problems?

When bacteria metabolize (or ferment) carbohydrates, they produce acids, water and gases. The major gases which are produced from the bacterial metabolism of disaccharides (including lactose, commonly known as milk sugar) include carbon dioxide (CO_2) and hydrogen (H_2).[1] Methane (CH_4) production has been identified in those who fail to produce H_2 following ingestion of non-digestible sugars.[2] Nearly half of H_2 producers also produce CH_4. The feasibility of using the appearance of such gases to study intestinal absorption and intermediary metabolism has been recognized for many years[3] and its use has grown markedly.

In addition to these gases, small concentrations of volatile fatty acids and aromatic gases such as skatole are produced by bacterial fermentation, but they will not be considered further here because they comprise a special field of study and are not relevant to this field of breath-testing.

CO_2 is produced by all cells during metabolism, but *only bacteria* can produce H_2 and CH_4 as metabolic by-products. Thus, if either H_2 or CH_4 are produced in the body, it means that the substrate (the food substance) has been exposed to bacterial fermentation. The breath levels of both gases are correlated with the degree of colonic fermentation, and are useful markers for this process.[4]

In the digestive tract, bacteria are normally found only in the colon. Most of the bacteria ingested with food are killed by the acidity of the stomach, so the small intestine usually has few or no bacteria. However, bacteria can gain access to the small intestine through the stomach or by invading retrograde from the colon.

Achlorhydria, which is a lack of gastric acid production, can permit the passage of food through the stomach and into the small intestine where they can thrive, producing the condition called *bacterial overgrowth*. The invasion of bacteria into the jejunum has been demonstrated after surgery performed to decrease or eliminate gastric acid production.[5] *Intestinal stasis*, or reduced motor activity of the intes-

tine, can permit the retrograde movement of bacteria as a result of flaccidity, from the colon (where they normally exist) back to the ileum where they produce symptoms of bloating and cramps.[6] Chronic diarrhea has been related to contamination of the small intestine from the colon.[7] It can result in serious consequences when bacteria further invade the jejunum, because they metabolize nutritional substrates on which the body depends,[8] in addition to causing discomfort.

For a time, the literature addressed the possibility that breath-tests might serve as a marker for colon polyps or colon cancer. A correlation between CH_4 production and the incidence of colon cancer was reported,[9-11] but other reports indicated that testing for breath CH_4 did not contribute towards identifying patients with colon cancer or premalignant lesions.[12-14] Most evidence now supports the idea that the presence of CH_4 in expired air is too non-specific, and breath-tests are of limited use as a diagnostic aid in this area.

Recent studies have recommended that all patients suspected of having Irritable Bowel Syndrome (IBS) should be tested for disaccharide malabsorption to detect those cases which involve simple malabsorption. Tolliver, et al.,[15, 16] Cierna, et al.[17] and Webster, DiPalma and Gremse[18] showed that over 25% of IBS groups had lactose malabsorption, but only about half of Tolliver's group and none of Cierna's patients associated lactose-containing food with their symptoms. Vernia and co-workers in Italy[19] found 68% of 230 patients with a suggested diagnosis of IBS had lactose malabsorption which was relieved in most cases by dietary control; but the prevalence of lactose malabsorption in Italy depends on the geographic areas studied.[20, 21] Fernandez-Banares[22] reported that over 90% of patients with symptoms of functional bowel disease had malabsorption from either lactose, fructose, sorbitol, fructose + sorbitol or sucrose. Ogata[23] found higher rates (over 90%) of lactose malabsorption in Japanese patients with small intestine-type Crohn's disease than in normal controls (who had an incidence of 50% lactose malabsorption). These papers supported the earlier report by DiPalma and Narvaez,[24] who recognized that a large percentage of IBS patients had unsuspected lactose malabsorption and recommended routine testing of patients with suspected IBS.

All medical students recognize that the colon is involved in salvaging salt and water from the luminal contents. However, the colon is in-

volved in much more *physiology,* and the physiological function of the colon depends on having a high bacterial-count. Bacterial fermentation of fiber which was not digested in the small intestine produces short-chain fatty acids (SCFA) which are absorbed and which are beneficial to health. As much as 10-20% of fiber in foods like cereals and legumes escape digestion in the small intestine and are broken down in the colon.[25] In addition, 5-10% of starch escapes digestion in the small intestine and is metabolized in the colon,[26, 27] thus adding to the efficiency of energy production by such food-stuffs. Some mucin and mucopolysaccharide molecules are sloughed from the wall of the intestines and also contribute slightly to the production of H_2 and CH_4 in the colon.[28]

Colonic bacteria contribute to fecal bulk, and the SCFA which result from fermentation of carbohydrates reduce colonic pH. These factors may reduce the incidence of diarrhea, and may enhance the colonic absorption of metal ions like calcium, magnesium and zinc.

Given that surprisingly large volumes of gas can be generated in the colon, it is important to recognize that the body must continually eliminate significant volumes of gas that are produced in the colon. Such gas has four main fates: (1) a portion of it is lost as flatus, (2) some of it is metabolized; for instance, H_2 can be consumed by intestinal flora in the process of generating CH_4, (3) some of the CO_2 is dissolved in water as carbonic acid (H_2CO_3) and is neutralized or converted to salts, and (4) some of the gas is absorbed into the blood circulating through the colonic tissue and is carried to the lungs, where it is equilibrated with air in the alveoli. In particular, large quantities of CO_2 are eliminated by that route, but both H_2 and CH_4 are also lost through the lungs.

Gases in the blood returning to the lungs are equilibrated with air in the *alveoli*. The air contained in the trachea, the bronchi and bronchioles do not participate in such gas exchange. At the end of an inspiration, the airways reflect room air composition because the air has not been in contact with blood from the tissues. This portion of air, which is the first to be expelled on the following exhalation, is called *dead space* air and should not be included in the sample analyzed for breath-testing. Much of the success of breath-testing depends on having a sample of pure alveolar air for analysis, and attention must be paid to the technique of sampling air for analysis during the test. Most investigators

recognize this fact, and correct for any possible contamination on the basis of the CO_2 concentration in the sample. This point is covered in detail elsewhere.

Breath-hydrogen testing was developed over 25 years ago (see "History of Breath Testing"), and as a result of its application to digestive problems we have become aware of the fact that disaccharide malabsorption and bacterial overgrowth are more frequent contributors to diarrhea and other intestinal symptoms than was formerly suspected. They are the most common cause of "osmotic" diarrhea in adults because disaccharides are so common in the diet. The clinical problem with lactose malabsorption is widespread in some populations. In some geographical areas, such as in North America, milk is used widely by a portion of adults who are expected to drink it but do not know that they will have problems because they are unable to digest it.

It is commonly believed that some people develop a problem with lactose malabsorption as they get older. A few reports of increased malabsorption in older people have been reported,[29, 30] but other studies showed that lactose intolerance in adults was independent of age.[31] Though older subjects (>65 years) had a delayed transit time, it was insufficient to alter lactose tolerance.[32] However, the inability to drink milk is frequently developed by older people who were able to use milk products earlier. It has been proposed that they have "intestinal senescence" which slows down their intestinal motility and makes them more sensitive to any malabsorption present.[33]

Lactose is normally hydrolyzed into glucose and galactose, which are readily absorbed in the jejunum. If the enzyme *lactase* is lacking (or inadequate amounts are produced), the lactose will not be completely hydrolyzed, and the resultant condition is *lactose malabsorption.* Such malabsorption can exist at any level of deficiency. If it is mild, it may not cause a problem with the usual diet. However, when it is severe, small amounts of lactose will remain in the lumen of the intestine and cause distention, discomfort and diarrhea by retaining water. When the sugar gets to the colon, gases produced there cause further distention, cramps, flatulence and general discomfort, along with diarrhea which can range from a mild to an explosive discharge. Those symptoms produce the condition called *lactose intolerance*, which is *lactose malabsorption with discomfort.* Comparing the breath level before and after

ingestion of a specific sugar increases the likelihood that the test is specific for malabsorption of that sugar.

Sensitivity to Symptoms of Lactose Malabsorption

Some investigators have reported that young people and adults have the ability to adapt (at least to some degree) to increased levels of milk in the diet if its exposure is prolonged and gradual. It has been proposed that this is due to increased tolerance to the fermentation products[34] or a reduced production of H_2.[35] Others [36, 37] showed an increase in the activity of fecal beta-galactosidase (the enzyme which hydrolyzes galactose-containing disaccharides). This suggests that there is an adaptation of the colonic flora to the continued exposure to lactose, to explain the reduction in H_2 production with gradually increasing exposure.

Many investigators have reported the appearance of symptoms in patients who do not have sufficient gas production to explain the symptoms on the basis of the generally accepted concept of distension due to H_2 or CH_4 production. It has been proposed that symptoms of cramps and distress following 6g lactose[38] or 240ml milk[39] must be due to some cause other than gas production.

When lactose is hydrolyzed by the lactase enzyme either before milk is consumed or by the simultaneous consumption of the lactase with lactose-containing milk, the symptoms and the production of trace gases are reduced[39, 40] and fecal beta-galactosidase levels are reduced.[41]

The same dose of lactose in yogurt as in milk produced fewer symptoms.[42] This was related to a slower H_2-excretion pattern with a delayed rise-time and a lower rate of rise for the H_2 curve. It should be pointed out that there is, in turn, a slower response to equal volumes of lactose in milk as compared to lactose in water; related in this case to a slower gastric emptying time with milk.

An as yet unexplained observation was reported by Medow and coworkers,[43] in which milk-induced CH_4 responses were not aborted by administering lactase enzyme which blocked the H_2 response. If this observation is supported by other data, it will require a different explanation than the hypothesis of methanogenesis due to colonic flora.

What is Normal for Breath Trace-Gases?

Since carbohydrates which get to the colon can be metabolized by bacteria and produce H_2 and CH_4, it is common for some of these gases to be detected in the alveolar air even when milk has not been consumed. There is some variability in alveolar levels of H_2 and CH_4 throughout the day, and the pattern of trace-gas levels in alveolar air is different for the two gases. The fasting breath-H_2 level is ordinarily below 10 ppm, but larger variability (up to 40 ppm) has been reported.[44] The fasting breath H_2 level may be elevated if the person has ingested slowly digesting foods the day before.[45]

Intestinal motility is reduced during sleep, and H_2 can accumulate as a result of the residual colonic material being exposed to bacteria for a longer period of time. In addition, there is a reduced level of breathing (called *hypoventilation*) during sleep. That will allow H_2 (and presumably CH_4) to accumulate in the tissues and blood because it is not as efficiently eliminated by the lungs. Shortly after awakening and becoming active, the normal fasting breath level falls to lower levels, and gradually rises during the day.[4] The pattern of an increasing level probably reflects the colonic metabolism of carbohydrates which were eaten at breakfast and lunch. The H_2 level falls slightly late in the afternoon, though one would predict a second rise in response to the evening meal.

The pattern of H_2-concentration in the expired air is different if the subject does not eat food. If the patient 'fasts' for the day, the H_2 level falls gradually during the entire period of measurement because no additional carbohydrate has reached the colon. The prolonged gradual fall in H_2 reflects the decreasing availability of carbohydrate for the generation of H_2. No similar temporal pattern has been described for the production of CH_4. In addition, it appears to be less affected by dietary intake,[4] though it is clear that CH_4 responds to disaccharides which escape digestion in the small intestine, and it is part of the normal breath gas response to lactose malabsorption.[4, 43]

The protocols for specific breath-tests for some sugars are presented later in the monograph. The details of those protocols are presented in the appropriate sections, but in general, if there is malabsorption of a specific sugar, when a standardized dose of that sugar is in-

gested there will be a measurable increase in the alveolar concentration of H_2 and/or CH_4. Doses are in the range of 10-50 grams, depending on the sugar used and the results desired. The tests are designed to produce a measurable increase in parts per million (ppm) within a specified time period (usually under three hours, though longer time periods are frequently used for research studies).

The tests for malabsorption are most frequently applied to lactose because it is the most common cause of sugar malabsorption. However, it is also applied to fructose (fruit juices)[46] and sorbitol (an alcohol sugar used as a non-glucose sweetener).[47] Different sugars are used in other special breath-tests, such as lactulose for measurements of intestinal transit time[48] or bacterial overgrowth,[49] or high doses of glucose for the detection of bacterial overgrowth.[50] Many studies have been made with D-xylose (a 5-carbon sugar), proposed to detect bacterial overgrowth or used to measure intestinal integrity. However, there is some reservation about its advantages over other methods.[51, 52] The breath-test can even be applied to sucrose[53] (common table sugar) which has been demonstrated to be a non-absorbed sugar in a few cases. These special applications of the breath-test procedure are presented in separate chapters of the monograph.

References

1.   Bond, J.H., Levitt, M.D. Quantitative measurement of lactose absorption. Gastroenterol. 1976; 70(6):1058-62

2.   Bjrneklett, A.; Jenssen, E. Relationship between hydrogen (H_2) and methane (CH_4) production in man. Scand J Gastroenterol. 1982; 17:985-92

3.   Solomons, N.W.; Schnieder, R.E.; Garcia-Ibanez, R.; Pineda, D.; Viteri, F.E.; Lizarralde, E.; Schoeller, D.; Klein, P.; Rosenberg, I.H.; Calloway, D. Use of tests based on the analysis of expired air in nutritional studies. Arch Latinoam. Nutr. 1978; 28:301-17

4.   Le Marchand, L.; Wilkens, L.R.; Harwood, P.; Cooney, R.V. Use of breath hydrogen and methane as markers of colonic fermentation in epidemiologic studies: arcadian patterns of excretion. Environ Health Perspect. 1992; 98:199-202

5.   Bjrneklett, A.; Fausa, O.; Midvedt, T. Small-bowel bacterial overgrowth in the postgastrectomy syndrome. Scand J Gastroenterol. 1983; 18:277-87

6.   Dooley, C.P.; el Newihi, H.M.; Zeidler, A.; Valenzuela. J.E. Abnormalities of the migrating motor complex in diabetics with autonomic neuropathy and diarrhea. Scand J Gastroenterol. 1988 23:217-23

7. Davidson, G.P.; Robb, T.A.; Kirubakaran, C.P. Bacterial contamination of the small intestine as an important cause of chronic diarrhea and abdominal pain: diagnosis by breath hydrogen test. Pediatrics. 1984; 74:229-35

8. Isaacs, P.E.; Kim, Y.S. The contaminated small bowel syndrome. Am J Med. 1979; 67:1049-57

9. Karlin, D.A.; Jones, R.D.; Stroehlein, J..R.; Mastromarino, A.J.; Potter, G.D. Breath methane excretion in patients with unresected colorectal cancer. J Natl Cancer Inst. 1982; 69:573-6

10. Pique, J.M.; Pallares, M.; Cuso, E.; Viular-Bonet, J.; Gassul, M. Methane production and colon cancer. Gastroenterology. 1984; 87:601-5

11. Scheppach, W.; Webner, RF.; Bartram, P.; Schramel, P.; Kasper, H. Metabolic and nutritional parameters in patients after colonic polypectomy. Digestion. 1988; 41:94-100

12. Segal, I.; Walker, A.R.; Lord, S.; Cummings, J.H. Breath methane and large bowel cancer risk in contrasting African populations. Gut. 1988; 29:608-13

13. Nordgaard, I.; Rumessen, J.J.; Nielsen, S.A.; Gudmand-Hoyer, E. Absorption of wheat starch in patients resected for left-sided colonic cancer. Scand J Gastroenterol. 1992; 27:632-34

14. Le Marchand, L.; Wilkens, L.R. Harwood, P.; Cooney, R.V. Breath hydrogen and methane in populations at different risk for colon cancer. Int J Cancer. 1993; 55:887-90

15. Tolliver, B.A.; Herrera, J.L.; DiPalma, J.A. Evaluation of patients who meet clinical criteria for irritable bowel syndrome. Am J Gastrolenterol. 1994; 89(2):176-8

16. Tolliver, B.A.; Jackson, M.S.; Jackson, K.L.; Barnett, E.D.; Chastang, J.F.; DiPalma, J.A. Does lactose maldigestion really play a role in the irritable bowel? J Clin Gastroenterol 1996; 23(1):15-7

17. Cierna, I.; Cernak, A.; Krajcirova, M.; Leskova, L. Lactose intolerance in the differential diagnosis of abdominal pain in children. Cesk Pediatr 1993; 48(11):651-3

18. Webster, R.B.; DiPalma, J.A.; Gremse, D.A. Lactose maldigestion and recurrent abdominal pain in children. Dig Dis Sci. 1995;40(7):1506-10

19. Vernia, P.; Ricciardi, M.R.; Frandina, C.; Bilotta, T.; Frieri, G. Lactose malabsorption and irritable bowel syndrome. Effect of a long-term lactose-free diet. Ital J Gastroenterol. 1995 (Apr);27(3):117-21.

20. Rogerro, P.; Offredi, M.L.; Mosca, F.; Perazzini, M.; Mangiaterra, J.; Marenghia, L.; Careddu, P. Lactose absorption and malabsorption in healthy Italian children: do the quantity of malabsorbed sugar and the small bowel transit time play roles in symptom production? J Pediatr Gastroenterol Nutr. 1985; 4(1):82-6

21. Burgio, G. R.; Flatz, G.; Barbera, C.; Patan:e, R.; Boner, A.; Cajozzo, C.; Flatz, S. D. Prevalence of primary adult lactose malabsorption and awareness of milk intolerance in Italy. Am J Clin Nutr. 1984; 39(1): 100-4

22. Fernandez-Banares, F.; Esteve-Pardo, M.; de Leon, R.; Humbert, P.; Cabre, E.; Llovet, J.M.; Gassull, M.A. Sugar malabsorption in functional bowel disease: clinical implications. Am J Gastroenterol. 1993 Dec; 88(12):2044-50

23. Ogata, M.; Yao, T. Hypolactasia in Japanese Crohn's patients. Nippon Shokakibyo Gakkai Zasshi. 1992 Nov; 89(11):2655-63

24. DiPalma, J..A.; Narvaez, R.M. Prediction of lactose malabsorption in referral patients. Dig Dis Sci. 1988; 33:303

25. Levitt, M.D.; Hirsh, P.; Fetzer, C.A.; Sheahan, M.; Levine, A.S. H_2 excretion after ingestion of complex carbohydrates. Gastroenterology. 1987; 92:383-9

26. Stephen, A.M. Starch and dietary fibre: their physiological and epidemiological interrelationships. Can J Physiol Pharmacol. 1991; 69:116-20

27. Strocchi, A.; Levitt, M.D. Measurement of starch absorption in humans. Can J Physiol Pharmacol. 1991; 69:108-10

28. Peerman, J.A.; Modler, S.; Glycoproteins as substrates for production of hydrogen and methane by colonic bacterial flora. Gastroenterology. 1982; 83:388-93

29. Saltzberg, D.M; Levine, G.M.; Lubar, C. Impact of age, sex, race and functional complaints on hydrogen (H_2) production. Dig Dis Sci. 1988; 33:308-13

30. Rao, D.R.; Bello, H; Warren, A.P.; Browdn, G.E. Prevalence of lactose maldigestion. Influence and interaction of age, race, and sex. Dig Dis Sci 1994; 39(7);1519-24

31. Rosenkranz, W.; Hadorn, B.; Muller, W.P.; Heinz-Erian, P.; Hensen, C.; Flatz, G. Distribution of human lactase phenotypes in the population of Austria. Hum Genet. 1982; 62:158-61

32. Suarez, F.L.; Savaiano, D.A. Lactose digestion and tolerance in adult and elderly Asian-Americans. Am J Clin Nutr. 1994; 59:1021-4

33. Feibusch, J.M.; Holt, D.R. Impaired absorptive capacity for carbohydrate in the aging human. Dig Dis Sci. 27:1095, 1982

34. Johnson, A.O.; Semenya, J.G.; Buchowski, M.S.; Enwonwu, C.O.; Scrimshaw, N.S. Adaptation of lactulose maldigesters to continued milk intakes. Am J Clin Nutr. 1993 (Dec);58(6):879-81

35. Hertzler, S.R.; Savaiano, D.A.; Levitt, M.D.. Fecal hydrogen production and consumption measurements. Response to daily lactose ingestion by lactose maldigesters. Dig Dis Sci. 1997(Feb); 42(2):348-53

36. Hertzler, S.R.; Savaiano, D.A. Colonic adaptation to daily lactose feeding in lactose maldigesters reduces lactose intolerance. Am J Clin Nutr. 1996 (Aug); 64(2):232-6

37. Hertzler, S.R.; Huynh, B.C.; Savaiano, D.A. How much lactose is low lactose? J Am Diet Assoc. 1996 (Mar); 96(3):243-6

38. Shermak, M.A.; Saavedra, J.M.; Jackson, T., L.; Huang, S.S.; Bayless, T.M.; Perman, J.A. Effect of yogurt on symptoms and kinetics of hydrogen production in lactose-malabsorbing children. Am J Clin Nutr. 1995(Nov); 62(5):1003-6

39. Kotz, C.M.; Fujrne, J.K.; Savaiano, D.A.; Levitt, M.D. Factors affecting the ability of a high beta-galactosidase yogurt to enhance lactose absorption. J Dairy Sci 1994 (Dec); 77(12):3538-44

40.   Montes, R.G.; Bayless, T.M.; Sasvedra, J.M.; Perman, J.A. Effect of milks inoculated with Lactobacillus acidophilus or a yogurt starter culture in lactose-maldigesting children. J Dairy Sc. 1995 (Aug); 78(8):1657-64

41.   Suarez, F.L.; Savaiano, D.A.; Levitt, D.M. A comparison of symptoms after the consumption of milk or lactose-hydrolyzed milk by people with self-reported severe lactose intolerance. N Eng J Med. 1995 (Jul); 333(1):1-4

42.   Jian, T.; Mustapha, A.; Savaiano, D.A. Improvement of lactose digestion in humans by ingestion of unfermented milk containing Bifidobacterium longum. Singapore Med J. 1996 (Feb); 37(1):72-81

43.   Medow, M.S.; Glassman, M.S.; Schwarz, S.M.; Newman, L.J. Respiratory methane excretion in children wit lactase intolerance. Dig Dis Sci. 1993; 38:328-32

44.   Pitzalis, G.; Fancellu, M.G..; Degnelloll, F.; Galastri, E.; Bonamico, M.; Imperatol, C. Prevalence of lactose malabsorption in Roman school children. A H_2 breath test study using a cow's milk. Minerva Pediatr. 1993;

45.   Brummer, R..J.; Armbrecht, U.; Bosaeus, I.; Dotevail, G.; Stockbruegger, R.W. The hydrogen (H_2) breath test. Sampling methods and the influence of dietary fibre on fasting level. Scand J Gastroenterol. 1985; 20:1007-13

46.   Kneepkens, C.M.; Vonk, R.J.; Fernandez, J. Incomplete intestinal absorption of fructose. Arch Dig Child. 1984 (Aug); 59(8):735-8

47.   Corazza, G.R.; Strocchi, A.; Rossi, R.; Sirola, D.; Gasbarrini, G. Sorbitol malabsorption in normal volunteers and in patients with coeliac disease. Gut. 1988 (Jan); 29(1):44-8

48.   Bond, J.H.; Levitt, M.D. Effect of dietary fiber on intestinal gas production and small bowel transit time in man. Am J Clin Nutr. 1978; 31(10 Suppl.):S169-74

49.   Rhodes, J.;M.; Middleton, P.; Jewell, D.P. The lactulose hydrogen breath test as a diagnostic test for small-bowel bacterial overgrowth. Scand J Gastroenterol. 1979; 14:333-6

50.   Kerlin, D.A.; Wong, L. Breath hydrogen testing in bacterial overgrowth of the small intestine. Gastroenterology. 1988; 95:982-8

51.   King, C.E.; Toskes, P.PP. Comparison of the 1-gram [14C]xylose, 10-gram lactulose-H_2, and 80-gram glucose-H_2 breath tests in patients with small intestine bacterial overgrowth. Gastroenterology. 1986; 1447-51

52.   Lembke, B.; Bornholdt, C.; Kirchhoff, S.; Lankisch, P.G. Clinical evaluation of a 25 g D-xylose hydrogen (H_2) breath test. Z Gastroenterol. 1990; 28(10):555-60

53.   Davidson, G.P.; Robb, T.A. Detection of primary and secondary sucrose malabsorption in children by means of the breath hydrogen technique. Med J Austral. 1983 (Jul); 2(1):l29-32

2. The History of Breath-Testing

Being aware of the development of breath-testing will help you understand more about the test and appreciate the way in which the test was developed. It will help explain why gastroenterologists first looked at H_2 as an indicator of bacterial action on food-stuffs and why CH_4 has more recently been added as a tool. Perhaps it will help those who think that the addition of CH_4 as part of the test is only a frill that the academic investigators have added to a formerly very simple (and, therefore, attractive) test.

The introduction of breath-H_2 as an index of lactose malabsorption was made in the decade of the 1970s. In 1975, Newcomer and associates[1] studied breath H_2,[14]CO_2-labeled lactose and blood sugar changes for the measurement of lactose malabsorption. They demonstrated the superiority of H_2 measurements in detecting lactose malabsorption. Bond and Levitt,[2] in 1978, used breath-H_2 to indicate that some disaccharides (complex sugars) were not broken down (hydrolyzed) and absorbed in the small intestine during the digestion of foods. It was based on evidence that the disaccharide reached the colon intact, resulting in a change in the concentration of H_2 in expired air after the sugar was ingested. The most prominent clinical application of the test was for the diagnosis of lactose malabsorption or lactose intolerance. The H_2 breath-test (often referred to as the *HBT* or the *BHT*) has essentially replaced the blood-test, in which the *lack* of a blood glucose response to lactose ingestion was an indication that the lactose was not digested and absorbed. Repeated studies since its first description have shown that the breath-test is superior, and it has been accepted by most gastroenterologists as the method of choice for diagnosing lactose malabsorption.[1, 3, 4, 5]

When the reliability and simplicity of the breath-H_2 test was demonstrated with lactose, it was soon applied to other complex sugars like fructose[6, 7] (from fruits), maltose[8] (from some starches) and sucrose[9] (common table sugar, which is only rarely malabsorbed). The H_2 breath-test has also been used to indicate that some people are unusually sensitive to sorbitol[10] (an artificial sweetener used in sugar-free candy, chewing gum and other dietetic foods), and for the lactulose breath-test (used to measure intestinal transit time[11] or to detect bacterial overgrowth retrograde into the small intestine).[12]

In the last few years, since technology has made it practical to do so, methane has been added as a useful trace-gas for studies of digestive problems. Almost from the beginning, it was recognized that CH_4 was produced during the bacterial metabolism of carbohydrates in the colon, but the lack of a practical analytical technique for the measurement of CH_4 in alveolar air delayed its clinical acceptance.

Methane is an important intestinal gas and should also be measured in studies of carbohydrate malabsorption in order to provide the most information to the physician.

There is a complex interaction between H_2 production and CH_4 production which is not well understood. In particular, the sites for H_2 production are not the same as the major sites for CH_4 production, and this leads to some difficulty in interpreting the patterns of gas appearance in the alveolar air. It has been shown that a relationship exists between H_2 and CH_4 production, in which methanogenic bacteria are able to convert H_2 to CH_4,[13-15] and this exchange occurs in the colon. Therefore, when disaccharides are metabolized by bacteria, sometimes only H_2 is produced, sometimes both H_2 and CH_4 will appear in the alveolar air, and sometimes only CH_4 will be increased. Thus, although knowledge is still incomplete about this interaction, it is important to look at both components for a complete understanding of the breath-test gas response.

With respect to the specificity of trace-gas increases for disaccharide malabsorption, since H_2 and CH_4 are produced only by bacteria, and carbohydrates are the primary substrate for their production, the presence of either of these gases in the breath will signal the breakdown of carbohydrates in the intestinal tract. Colonic bacteria can form H_2 and CH_4 from other nutrients, such as amino acids and endogenous mucin and glycoprotein[16] (produced in the lumen of the gut), but the quantity of gas produced from those sources are inconsequential when compared with that produced by carbohydrates when properly tested.

The response to a challenge-dose of sugar is measured from the control (or pre-dose) baseline, or from the lowest level reached prior to an increase. This is used as a reference level for measuring the response to the challenge dose of the disaccharide ingested.

References

1. Newcomer, A.D.; McGill, D.B.; Thomas, P.J.; Hofman, A.F. Prospective comparison of indirect methods for detecting lactase deficiency. N Engl J Med; 1975; 293(24); 1232-6

2. Bond, J.H.; Levitt, M.D. Effect of dietary fiber on intestinal gas production and small bowel transit time in man. Am J Clin Nutr; 1978 Oct; 31(10 Suppl): S169-S174

3. Douwes, A.C.; Fernandes, J.; Degenhart, H.J. Improved accuracy of lactose tolerance test in children, using expired H2 measurement. Arch Dis Child; 1978 Dec; 53(12): 939-42

4. Newcomer, A.D. Screening tests for carbohydrate malabsorption. J Pediatr Gastroenterol Nutr; 1984; 3(1): 6-8

5. Perman, J.A. Clinical Application of breath hydrogen measurements.   Canad J Physiol Pharmacol; 1991 Jan; 69(1): 111-5

6. Ravich, W.J.; Bayless, T.M.; Thomas, M. Fructose: Incomplete intestinal absorption in humans. Gastroenterology. 1983; 84(1) :26-29

7. Kneepkens, C.M.; Vonk, R.J.; Fernandes, J. Incomplete intestinal absorption of fructose. Arch Dis Child, 1984; 59(8);:735-38

8. Gudmand-H:yer, E.; Krasilnikoff, P.A.; Skovbjerg, H. Sucrose-isomaltose malabsorption. Adv Nutr Res; 1984; 6: 233-69

9. Davidson, G.P.; Robb, T.A. Detection of primary and secondary sucrose malabsorption in children by means of the breath hydrogen technique. Med J Aust; 1983 Jul 9; 2(1): 29-32

10. Hyams, J.S. Sorbitol intolerance: an unappreciated cause of functional gastrointestinal complaints. Gastroenterology; 1983 Jan; 84(1): 30-3

11. Diggory, R.T.; Cuschieri, A. The effect of dose and osmolality of lactulose on the oral-cecal transit time determined by the hydrogen breath test and the reproducibility of the test in normal subjects. Ann Clin Res. 1985;17:331-3

12. Rhodes, J.M.; Middleton, P.; Jewell, D.P. The lactulose hydrogen breath test as a diagnos-tic tool for small bowel bacterial overgrowth. Scand J Gastroenterol. 1979; 14(3):333-6

13. Fritz, M.; Siebert, G.; Kasper, H. Dose dependence of breath hydrogen and methane in healthy volunteers after ingestion of a commercial disaccharide mixture, Palatinit. Br J Nutr; 1985 Sep; 54(2): 389-400.

14. Cloarec, D.; Bornet, F.; Gouilloud, S.; Barry, J.L.; Salim, B.; Galmiche, J.P. Breath hydrogen response to lactulose in healthy subjects: relationship to methane producing status. Gut; 1990 Mar; 31(3): 300-4

15. Peled, Y.; Gilat, T.; Liberman, E.; Bujanover, Y. Development of methane production in childhood and adolescence. J Pediatr Gastroenterol Nutr; 1985 Aug; 4(4): 575-9

3. Genetic Basis for Lactose Malabsorption

The number of people who have lactose malabsorption is surprisingly large. Adults who cannot digest milk sugar make up the *majority* of the world's population. Adults who *can* drink milk without getting sick are likely to be Northern Europeans and their descendants. There are also a few groups in other parts of the world, such as those reported in Pakistan and Northern India, and a few tribes found in Africa, who have a reduced incidence of lactose malabsorption.

The question of why most Northern European adults (and their descendants) can drink milk while great variability exists with respect to adults in most other parts of the world has stimulated research in sociology, anthropology, geography, genetics, biochemistry and other branches of science. Some answers have come from studies of marriages between lactose tolerant and intolerant individuals. The ability to digest milk beyond the age of about three to five years, after which it is gradually reduced over the next several years, is genetically determined, and is a dominant trait. There is apparently a difference in the age at which lactose malabsorption develops in children. It varies from age two in Chinese[1] or age three to five years in Gambian[2] children, to "adolescence" in Native North Americans,[3] with the rest of the world represented between these two extremes. Fundamentally, it depends on a genetically-determined persistence into adulthood of the production of lactase in the small intestine. Lactase is the enzyme which breaks down milk sugar so it can be absorbed. The gene responsible for determining lactase "persistence" regulates lactase phlorizin hydrolase messenger RNA levels.[4] This determines the level of lactase production, and therefore, the lactose-digesting capacity.

This heterozygous dominant trait means that if a child inherits a gene for life-long milk tolerance from one parent and a gene for milk intolerance from the other parent, the child will be able to drink milk as an adult. However, if that child grows up and marries a lactose intolerant individual, it is likely that half of their children will be lactose intolerant.

Some scientists believe that the observed pattern of distribution reflects the history of the group in which a selective nutritional advantage was conferred on a few individuals who extended lactase produc-

tion further into adulthood. The advantage of prolonged milk consumption presumably gave them greater vigor and better health, and permitted them to become the dominant members of the group or tribe. This would modify, and gradually represent, the genetic make-up of the group.

It has been suggested by others that this explanation needs some consideration of additional factors, like social patterns. If the society did not accept milk as an adult food, those few selected "non-malabsorbers" would likely not drink milk in order to become aware of their superiority. Furthermore, that advantage might be diluted by social custom which could provide an alternate solution to having milk available for adults. For instance, in India they have adopted the custom of using yogurt in their diet. Yogurt can be more easily digested by lactose malabsorbers because the process of making yogurt hydrolyzes some of the lactose and avoids the problem induced by lactose in the diet. This approach neutralizes some of the advantages provided to those who can drink milk.

In addition, this simplistic "survival of the fittest" explanation does not fit all the observations, and has not been accepted by everyone, since some ethnic groups which are not milk-consumers are reported to have a reduced incidence of lactose malabsorption.[5] Some investigators believe that a genetically dominant "sport" appeared in early inhabitants of a Scandinavian country, and perhaps in some other regions of the world, to establish the trait of persistent lactase production. The social pattern of community milk consumption by adults likely developed *after* the genetic trait for adult lactase production was established.

To summarize, there is no question about the genetic basis for the pattern of adult lactose malabsorption (which should actually be considered to be the "normal condition"), but there is no general agreement about the origin of the trait of adult "lactose digestibility," and no generally accepted explanation for the geographic or ethnic pattern of malabsorption which is shown in the table which follows.

The breath-test has provided a simple non-invasive test which has been widely used to help determine the distribution of people who have inherited the intolerance for milk. The table shows the ethnic variability reported by investigators from around the world. It presents a collection from the reports which are in the literature. Some data have been

repeated from paper to paper in the literature, with inadequate substantiation. They are included in the table without a reference number in order to present a picture of the general concept of our understanding about the distribution of lactose malabsorption. The references present representative reports, and other papers not cited may well differ in their reported prevalence of malabsorption. No attempt has been made to separate lactose "malabsorption" from "intolerance" populations, since such characterization is frequently not made clear. Such differences, however, may account for some of the discrepancies.

Reported Incidence of Adult Lactose Malabsorption

Ethnic Group	% Intolerant	Reference (if found)
No. American. Caucasian	7-15	
Europeans	10-20	
Northern Europe	6	6
France, Western	24	16
Germany	6-23	12
Austria, Eastern	25	13
" Western	15	13
Hungary	37	14
Gypsies	56	14
Italy	56-85	17-20
Greece	56-75	23, 24
Mediterranean	41	23
Egypt	60-90	23
Jews	60-80	30
Tunisia	80	29
Polynesia	40-75	7, 8
So. American Indians	70-90	31
Uruguay	65	28
Mexican	70-80	
Mexican Children <6 yr	14	33
Mexican Adults	33	33
No. American Indians	60	3
No. American Blacks	70-75	
No. American Blacks	50	25
African Blacks	97-100	
African	63	26
Gabon	60-65	27
Gambi	63	27

Reported Incidence of Adult Lactose Malabsorption

Ethnic Group	% Intolerant	Reference (if found)
Sudan	60-87	28 , 30
Bosotho	85	31
San (Bushmen)	20	5
Bantus	10	5
Pakistan	3-15	
Pakistan	60	15
Chinese	65-98	9, 10
Chinese Children	10-68, 78	9, 11, 34
Japan	92	35
Japan	50-75	36
Northwest India	3-15	
Northern India	40	22
Central India	25-65	
Dravidian Indians	5-100	

References

1. Chang, M.H.; Hsu, H.Y.; Chen, C.H.; Lee, C.H.; Hsu, J.Y. Lactose malabsorption and small-intestinal lactase in Chinese children. J Pediatr Gastroenterol Nutr. 1987; 6:368-72

2. Erinosa, H.O.; Hoare, S.; Spencer, S.; Lunn, P.G.; Weaver, L.T. Is cow's milk suitable for the dietary supplementation of Gambian children: 1. Prevalence of lactose malabsorption. Ann Trop Paediatr. 1992; 12:359-65

3. Caskey, D. A.; Payne-Bose, D.; Welsh, J. D.; Gearhart, H. L.; Nance, M. K.; Morrison, R. D. Effects of age on lactose malabsorption in Oklahoma Native Americans as determined by breath H2 analysis. Am J Dig Dis. 1977 (Feb); 22:113-6

4. Fajardo, O.; Naim, H. Y.; Lacey, S. W. The polymorphic expression of lactase in adults is regulated at the messenger RNA level [see comments]. Gastroenterology. 1994; 106:1233-41

5. O'Keefe, S. J.; Young, G. O.; Rund, J. Milk tolerance and the malnourished African. Eur J Clin Nutr. 1990 (Jul); 44(7):499-504

6. Newcomer, A. D.; McGill, D. B. Irritable bowel syndrome. Role of lactase deficiency. Mayo Clin Proc. 1983 (May); 58:339-41

7. Seakins, J. M.; Elliott, R. B.; Quested, C. M.; Matatumua, A. Lactose malabsorption in Polynesian and white children in the south west Pacific studied by breath hydrogen technique. Br Med J [Clin Res]. 1987 (Oct 10); 295:876-8

8. Arnhold, R.G.; Perman, J.A.; Nurse, G.T. Persistent high intestinal lactase activity in Papua New Guinea. Ann Hum Biol. 1981; 8:41-4

9. Ting, C. W.; Hwang, B.; Wu, T. C. Developmental changes of lactose malabsorption in normal Chinese children: a study using breath hydrogen test with a physiological dose of lactose. J Pediatr Gastroenterol Nutr. 1988 (Nov); 7:848-51

10. Yap, I.; Berris, B.; Kang, J. Y.; Math, M.; Chu, M.; Miller, D.; Pollard, A. Lactase deficiency in Singapore-born and Canadian-born Chinese. Dig Dis Sci 1989; 34:1085-8

11. Quak, S. H.; Raman, G. V.; Low, P. S.; Wong, H. B. Lactase insufficiency in Chinese children detected by oral milk and lactose challenge. Ann Trop Paediatr. 1987 (Jun); 7:100-3

12. Flatz, G.; Howell, J. N.; Doench, J.; Flatz, S. D. Distribution of physiological adult lactase phenotypes, lactose absorber and malabsorber in Germany. Hum Genet. 982; 62(2): 152-7.

13. Rosenkranz, W.; Hadorn, B.; M:uller, W.; Heinz-Erian, P.; Hensen, C.; Flatz, G. Distribution of human adult lactase phenotypes in the population of Austria. Hum Genet. 1982; 62(2): 158-160

14. Czeizel, A.; Flatz, G.; Flatz, S. D. Prevalence of primary adult lactose malabsorption in Hungary. Hum Genet. 1983; 64(4): 398-401

15. Ahmad, M.; Flatz, G. Prevalence of primary adult lactose malabsorption in Pakistan. Hum Hered. 1984; 34:69-75

16. Cloarec, D.; Gouilloud, S.; Bornet, F.; Bruley des Varannes, S.; Bizais, Y.; Galmiche, J. P. Lactase deficiency and lactose intolerance-related symptoms in adult healthy subjects from western France. Gastroenterol Clin Biol. 1991; 15:588-93

17. Zuccato, E.; Andreoletti, M.; Bozzani, A.; Marcucci, F.; Velio, P.; Bianchi, P.; Mussini, E. Respiratory excretion of hydrogen and methane in Italian subjects after ingestion of lactose and milk. Eur J Clin Invest. 1983 (Jun); 13:261-6

18. Ceriani, R.; Zuccato, E.; Fontana, M.; Zuin, G.; Ferrari, L.; Principi, N.; Paccagnini, S.; Mussini, E. Lactose malabsorption and recurrent abdominal pain in Italian children. J Pediatr Gastroenterol Nutr. 1988; 7:852-7

19. Camboni, G.; Corbellini, A.; Quatrini, M.; Conte, D.; Bianchi, P. A. Lactose malabsorption and intolerance in Italians. Clinical implications. Dig Dis Sci. 1986; 31:1313-6

20. Rogerro, P.; Offredi, M.L.; Mosca, F.; Perazzini, M.; Mangiaterra, J.; Marenghia, L.; Careddu, P. Lactose absorption and malabsorption in healthy Italian children: do the quantity of malabsorbed sugar and the small bowel transit time play roles in symptom production? J Pediatr Gastroenterol Nutr. 1985; 4(1):82-6

21. Burgio, G. R.; Flatz, G.; Barbera, C.; Patan:e, R.; Boner, A.; Cajozzo, C.; Flatz, S. D. Prevalence of primary adult lactose malabsorption and awareness of milk intolerance in Italy. Am J Clin Nutr. 1984; 39(1): 100-4

22. Kochhar, R.; Mehta, S. K.; Goenka, M. K.; Mukherjee, J. J.; Rana, S. V.; Gupta, D. Lactose intolerance in idiopathic ulcerative colitis in north Indians. Indian J Med Res. 1993; 98:79-82

23. Brand, J. C.; Darnton-Hill, I. Lactase deficiency in Australian school children. Med J Aust. 1986 (Oct 6); 145:318-22

24. Ladas, S. D.; Katsiyiannaki-Latoufi, E.; Raptis, S. A. Lactose maldigestion and milk intolerance in healthy Greek schoolchildren. Am J Clin Nutr. 1991 (Mar); 53(3): 676-80

25. Johnson, A. O.; Semenya, J. G.; Buchowski, M. S.; Enwonwu, C. O.; Scrimshaw, N. S. Correlation of lactose maldigestion, lactose intolerance, and milk intolerance. Am J Clin Nutr. 1993 (Mar); 57:399-401

26. O'Keefe, S. J.; O'Keefe, E. A.; Burke, E.; Roberts, P.; Lavender, R.; Kemp, T. Milk-induced malabsorption in malnourished African patients. Am J Clin Nutr. 1991; 54:130-5

27. Gendrel, D.; Dupont, C.; Richard-Lenoble, D.; Gendrel, C.; Nardou, M.; Chaussain, M. Milk lactose malabsorption in Gabon measured by the breath hydrogen test. J Pediatr Gastroenterol Nutr. 1989; 8:55-7

28. Bayoumi, R. A.; Saha, N.; Salih, A. S.; Bakkar, A. E.; Flatz, G. Distribution of the lactase phenotypes in the population of the Democratic Republic of the Sudan. Hum Genet. 1981; 57:279-81

29. Filali, A.; Ben Hassine, L.; Dhouib, H.; Matri, S.; Ben Ammar, A.; Garoui, H. Study of malabsorption of lactose by the hydrogen breath test in a population of 70 Tunisian adults. Gastroenterol Clin Biol. 1987 (Aug); 11:554-7

30. Bujanover, Y.; Katz, A.; Peled, Y.; Gilat, T. Lactose malabsorption in Israeli children. Isr J Med Sci. 1985; 21:32-5

31. Tolboom, J. J.; Kabir, H.; Molatseli, P.; Anderson, J.; Arens, T.; Fernandes, J. Lactose malabsorption and giardiasis in Basotho school children. Acta Paediatr Scand. 1987;76:60-5

32. Maggi, R.; Sayagues, B.; Fernandez, A.; Romero, B.; Barusso, P.; Hernandez, C.; Magari:nos, M.; Mendez, G.; Dilascio, C.; Martell, M. Lactose malabsorption and intolerance in Uruguayan population by breath hydrogen test (H2). J Pediatr Gastroenterol Nutr. 1987 (May); 6(3): 373-6

33. Lopez, P.; Rosado, J.L.; Palma, M.; Gonzalez, C.; Valencial, M.E. Poor digestion of lactose. Its definition, prevalence in Mexico, and its implications in milk consumption. Rev Invest Clin. 1996 (Nov); 48 Suppl::15-22

34. Wong, F.H.; Yeung, C.Y.; Fung, K.W.; Tam, A.Y. Breath hydrogen (H2) analysis in southern Chinese children and infants by gas chromatography and a novel automatic sampling system. Singapore Med J. 1996 (Feb); 37(1):72-81

35. Kondo, T.; Liu, F.; Toda, Y. Milk is a useful test meal for measurement of small bowel transit time. J Gastroenterol 1994 (Dec); 29(6);715-20

36. Ogata, M.; Yao, T. Hypolactasia in Japanese Crohn's patients. Nippon Shkokakibyo Gakkai Zasshi. 1992 (Nov);89(11):2655-63

4. Structures of Common Sugars

Sugars are individual molecules (or chains of two or more molecules) having a structure represented by the chemical formula of $[C(H_2O)_x$ less one water molecule if the sugar is a disaccharide]. The water molecule is removed when the two molecules are polymerized and is added back when it is split again (hydrolyzed) back into the individual molecules. Most of the molecules which are called "common sugars" contain 6 carbon atoms (x = 6) with their associated H^+ and ^-OH bonds, giving the common formula of $C_6H_{12}O_6$.

Although several 6-carbon sugars (called *hexoses*) have the same chemical formula, they have different orientation of the atoms contributing to the molecule. The different forms are called "stereoisomers" and their specific orientation provides properties to each molecule which affect the way they are absorbed by the intestine and the way they are metabolized after they are absorbed. So, though they have the same chemical formula, they are *physiologically* very different from each other.

Simple Sugars, or Monosaccharides

When the 6-carbon sugar molecule exists as a single unit hexose, it is called a *monosaccharide*. Glucose (also called dextrose) is a monosaccharide and it is a hexose because it has a 6-carbon chain. It is the sugar which results from intestinal digestion of more complex sugars, and is absorbed into the blood stream where it exists as "blood sugar." It is found, along with *fructose* (a different monosaccharide), in fruits and berries. Fructose is more sweet-tasting than glucose, and is called fruit sugar. *Galactose* is the third hexose monosaccharide which is found commonly in nature. It is, along with glucose, a hydrolysis-product of the disaccharide lactose (discussed in the next section). *Mannose* is a hexose which is found in some special plants, such as the ivory nut. While glucose, fructose and galactose are common foodstuffs, mannose plays but a minor role in animal nutrition.

Pentoses are 5-carbon sugars. They are found more in the plant kingdom than in the animal kingdom, but they are of interest and importance because they are used to diagnose gastrointestinal disturbances. D-x*ylose* is the most commonly used pentose, and is used by some

investigators to evaluate the continuity of the small intestine and to measure its transport capability.

Sorbitol is an alcohol-sugar. It is used as a sweetener in diabetic candies and drinks because it is not digested in the small intestine. However, it is carried to the colon where it is metabolized by bacteria. Many people who are not diabetics use sorbitol-sweetened candy to reduce their caloric intake (since it does not contain glucose). However, the end-products of sorbitol metabolism which are absorbed from the colon provide about the same energy as that obtained from an equivalent amount of sugar, so its use will not benefit weight-loss. Sorbitol is poorly absorbed, and can cause problems if used too generously by some individuals.

Most monosaccharides are absorbed into the capillaries of the intestinal villi by an active transport mechanism. They are combined with phosphoric acid to form hexose monophosphate, which is actively transported across the membranes of the cells lining the intestine. Substances which block the formation of the hexose monophosphate will interfere with the absorption of sugars. There is a gradation in the rate at which different hexoses are absorbed, indicating that there is a *selective, active transport of the sugars.* The order is galactose > glucose > fructose > mannose. Further evidence for the active transport of sugars is provided by the fact that the rate of absorption does not depend on the concentration difference across the membrane. Thus, the rate of absorption remains constant over a long time-period; in fact, until most of the sugar is absorbed.

In addition to having different absorption rates, hexoses also have different pathways for the transport of sugars across the intestinal wall, from the lumen to the lymph channels or blood stream serving the small intestine. For instance, it is known that the transport of fructose involves a different pathway than that for glucose and galactose. It will be shown later that children with glucose malabsorption (due to transport abnormalities) are able to absorb fructose without difficulty, and that there is an interaction between fructose uptake and the glucose transport mechanism. These differences depend on enzymes in the small intestine which are specific in aiding the absorption of different sugars.

The absorption rates for *hexoses* are normally greater than those for *pentoses* (e.g., xylose). However, if the membrane is poisoned so that it is unable to <u>actively</u> transport sugars, the smaller pentose molecule will be absorbed more quickly than the larger hexose molecule. Under those circumstances, the movement of the sugar is passive—dependent on the concentration gradient and the *diffusibility* of the molecule.

Disaccharides

Disaccharides, which are usually a combination of two hexose molecules, with the chemical formula of $C_{12}H_{22}O_{11}$, must be hydrolyzed by the addition of a water molecule to form two single monosaccharides, each with the formula of $C_6H_{12}O_6$, before they can be absorbed into the body to serve as a source of energy. *Disaccharidases* belong to a class of enzymes which are able to hydrolyze disaccharides into two monosaccharides. Enzymes have great specificity, whereby a particular enzyme acts on a particular sugar (substrate) but is inactive on other even closely related sugars.

Natural disaccharides most commonly consist of two hexose molecules. *Sucrose* is a disaccharide composed of glucose and fructose. It is found in sugar cane and sugar beets, and the fructose moiety is responsible for its characteristic sweetness. The enzyme *sucrase* (also called sucrase-isomaltase, saccharase or invertase) is present in both animal and plant tissues, and hydrolyzes sucrose into its two component monosaccharides. *Maltose* is called malt sugar. It consists of *two molecules of glucose*. As suggested from the system nomenclature, maltose is split by the enzyme *maltase*, produced at the brush border of the small intestine. The enzyme is also found in sprouting barley. Maltose is a major component of starch, so it is of considerable nutritional importance. Lactose (milk sugar) is also a disaccharide; it releases glucose and galactose when it is hydrolyzed. Ordinarily, it is hydrolyzed in the small intestine by the enzyme *lactase*, which is released from cells in the brush border. When that enzyme is deficient or absent, the lactose sugar is not hydrolyzed and remains in the intestine, causing the condition called *lactose malabsorption,* about which much will be said in subsequent chapters.

Lactulose is a special disaccharide consisting of galactose and fructose. There is no naturally occurring "lactulase" in the body to hydrolyze the sugar, so when it is ingested, lactulose remains in the small intestine. The sugar is transported to the colon and is metabolized by bacteria there. The products of its metabolism include H_2 and CH_4, which are measured in the test for bacterial overgrowth and for the detection of intestinal transit time.

Tests for Lactose Malabsorption

Although the prevalence of lactose malabsorption has become more apparent since the breath-test has become clinically available, it has long been recognized as a health problem. Several approaches have been used, and some of them are still being used, to diagnose the problem. They vary from overly-simple to unnecessarily complex.

Removing milk from the diet

A naive and simplistic (if not unscientific) alternative to actually testing for lactose malabsorption in suspected patients is to remove milk from the diet and see if it has an effect. There are several problems with that approach:

(1) On query, many patients deny any relationship between diet and the symptoms which are developed after eating a meal.[1] This can result from the fact that there is a period of one to three hours between the ingestion of food and the appearance of symptoms. The physician can be misled by the responses and, consequently, may not adequately pursue the question of lactose malabsorption in patients who might have it. Furthermore, if the patient is skeptical, he may only half-heartedly comply with the instructions to avoid milk products and, thus, complicate the diagnosis even more;

(2) Lactose is present in many unsuspected foods - like hot dogs, some non-dairy coffee creamers, breading for chicken, liverwurst and many candies. Even some drugs use lactose as a filler. Therefore, even if the patient attempts to comply, he may unintentionally continue to ingest lactose and report a lack of success from avoiding milk in the diet;

(3) Many marginally intolerant patients are not convinced that they have lactose intolerance until it is demonstrated by a suitable malabsorption test.[1,2] Generating objective data from an accurate, semi-quantitative test will provide convincing evidence that the problem is real and the cause is known. This evidence will give confidence to the physician and reassurance to the patient;

(4) Because of the calcium, vitamins and other nutritional benefits from drinking milk, it should be an important part of the diet, particularly for women and growing children. A relationship has been reported between lactose malabsorption and post-menopausal osteoporosis,[2] probably through diminished calcium intake by women who avoided drinking milk. Corazza, *et al.,*[3] related reduced bone mass density and low calcium intake to lactose intolerance symptoms in Italian women who avoided milk consumption in order to reduce discomfort. Therefore, it is generally not advisable to arbitrarily withdraw milk from the diet without a demonstrated and unavoidable reason;

(5) The all-or-none treatment is unnecessary for many patients who have lactose malabsorption. The breath-test for malabsorption can provide an indication of the severity of the lactase deficiency and may show that the patient is able to include some milk in the diet without generating symptoms of lactose intolerance.

Histology

Endoscopic techniques can be used to retrieve a biopsy specimen of the jejunal wall, which can be tested for its ability to generate glucose and galactose from lactose applied in vitro. If the monosaccharides are detected after an incubation period, the biopsy specimen was able to produce the lactase enzyme, so the patient was not intolerant, though lactase production might be impaired. Thus, no reliable estimate of the degree of impairment is determined with this test. In addition, it is rejected by many patients because of its discomfort. The histological approach is not a common clinical test for disaccharide malabsorption.

Blood Test

The blood test for lactose malabsorption requires the ingestion of a prescribed dose of 50g lactose (equivalent to drinking a quart of milk),

with serial blood samples drawn at 30-minute intervals for up to two hours for the measurement of a blood glucose change. If there is *no change* in blood glucose over the time period necessary for gastric emptying, digestion and absorption of the sugar, the lactose was not hydrolyzed so glucose could be absorbed. Thus, the lack of a blood sugar response suggests the presence of lactose malabsorption.

There are several major disadvantages offered by the blood test for lactose malabsorption:

(1) In patients with malabsorption, the large dose of lactose required for the blood test will cause severe cramps and diarrhea, making the test worse than the disease, and generating a resolve by the patient to not ever go through it again;

(2) Some laboratory personnel are reluctant to *unnecessarily* process blood samples from patients with gastrointestinal symptoms because such symptoms are frequently associated with AIDS patients;

(3) Patients who are diabetics may have problems with ingesting 50 grams of lactose if it is hydrolyzed to glucose and glycogen by lactose absorbers, and their intake may be less than anticipated if they are expected to, but do not, digest the sugar;

(4) Since the breath-test has been demonstrated to have more sensitivity and accuracy than the blood test, even if the blood test is used, it can lead to more frequent misdiagnosis, thereby adding to the frustration of the physician and to the dissatisfaction and prolonged discomfort of the patient when the problem is misdiagnosed; and

(5) Many patients refuse the test because of the serial blood samples, thus effectively making the test not a choice.

Breath-Tests

Hydrogen and methane are produced in the digestive system *primarily* only by the bacterial fermentation of carbohydrates (sugars, starches or vegetable fibers), so if either of these gases appear in the expired air, it is usually a signal that carbohydrates or carbohydrate fragments have been exposed to bacteria, permitting such fermentation to take place.[4] The generation of H_2 or CH_4 will result in the reabsorption of some of these gases into the blood stream from the site of their digestion, and they will appear in the expired air.

Bacteria are ordinarily not present in significant numbers in the small intestine, where digestion and absorption of sugars takes place. Therefore, when a challenge dose of lactose is ingested, the level of hydrogen in alveolar air will rise significantly within one to two hours (depending on the intestinal transit time) <u>only if the sugar is not digested</u> and, therefore, reaches the colon.

The breath-H_2 test is a simple, non-invasive procedure which is readily accepted by patients and staff,[5] and which has greater reliability and acceptability than the blood test, according to most reports in the literature.[1, 6-10] The lower dose of lactose usually does not cause the discomfort and explosive diarrhea frequently seen by malabsorbers who are given the large dose required for the blood test.[11]

A recent study[12] with over 300 patients showed that G-I symptoms after a lactose challenge are strongly associated with the amount of H_2 excreted, and the relationship between blood glucose change and symptom-severity was less evident.

False-positive breath-tests are rare, and when they occur they are usually caused by improperly doing the test - allowing the subject to smoke, to sleep or to eat shortly before or during the test.[13] Bacterial overgrowth (from the colon retrograde into the small intestine) can also produce a false-positive breath-test, but it is usually preceded by an elevated fasting breath-H_2 level and the response is seen soon after the sugar is ingested (within 20-30 minutes).

The incidence of false-negative results with the breath-test is well below that seen with the blood test.[1, 6, 7] False-negative results are reported to be from 5 to 15% of all lactose malabsorbers,[14-16] due to a variety of causes. Many of the false-negative reports can be avoided by measuring methane in addition to hydrogen[17] because some methanogenic flora convert colonic H_2 to CH_4. If a CH_4 analyzer is not available, an alternate approach is to test suspected false-negative patients with another disaccharide which is known to be carried to the colon. A 10g dose of lactulose will produce a positive breath-hydrogen test in patients who are capable of producing hydrogen, and will indicate when a patient suspected of being a lactose malabsorber is probably a hydrogen-nonproducer.[18] Colonic hyperacidity can severely inhibit bacterial

activity, and can produce a false-negative test. Pretreatment with $MgSO_4$[19] may prevent false-negative tests due to colon hyperacidity. Thus, measuring breath CH_4, repeating the test with lactulose on another day, or reducing colonic acidity if it is suspected (or demonstrated) to be elevated before the test, will reduce the percentage of false-negative results to very acceptable levels.

References

1.   DiPalma, J.A.; Narvaez, R.M. Prediction of lactose malabsorption in referral patients. Dig Dis Sci. 1988; 33:303

2.   Newcomer, A.D.; Hodgson, S.F.; McGill, D.B.; Thomas, P.J. Lactase deficiency: prevalence in osteoporosis. Ann Intern Med. 1978; 2:218

3.   Corazza, G.R.; Benati, G.; DiSario, A.; Tarozzi, C.; Strocchi, A.; Passeri, M.; Gasbarrini, G. lactose intolerance and bone mass in postmenopausal Italian women. Br J Nutr. 1995 (Mar); 73(3):479-87

4.   Levitt, M.D. Production and excretion of hydrogen gas in man. New Engl. J. Med. 1968; 281:122

5.   Metz, G.; Jenkins, D.L.; Peters, T.J.; Newman, A.; Blendis, L.M. Breath hydrogen as a diagnostic method for hypolactasia. Lancet. 1975; 1(7917):1155-7

6.   Davidson, G.P.; Robb, T.A.. Value of breath hydrogen analysis in management of diarrheal illness in childhood: Comparison with duodenal biopsy. J Ped Gastroenterol Nutr. 1985; 4:381-7

7.   Fernandes, J.; Vos, C.E.; Douwes, A, C, ; Slotema, E.; Degenhart, H.J. Respiratory hydrogen excretion as a parameter for lactose malabsorption in children. Amer J Clin Nutr. 1978; 31:597-602

8.   Newcomer, A.D.; McGill, D.B.; Thomas, R.J.; Hofmann, A.F. Prospective comparison of indirect methods for detecting lactase deficiency. New Engl J Med. 1975; 293:1232-6

9.   Douwes, A.C.; Fernandes, J.; Degenhart H.J. Improved accuracy of lactose tolerance test in children, using expired H_2 measurement. Arch Dis Child. 1978; 53:939-42

10.   Solomons, N.W.; Garcia-Ibanez, R.; Viteri, F.E. Hydrogen breath test of lactose absorption in adults: The application of physiological doses and whole cow's milk sources. Amer J Clin Nutr. 1980; 33:545-54

11.   Jones, D.V.; Latham, M.C.; Kosikowski, F.V.; Woodward, G. Symptom response to lactose-reduced milk in lactose-intolerant adults. Amer J Clin Nutr. 1976; 29(6):633-8

12.   Hermans, M.M.; Brummer, R.J.; Ruijgers, A.M.; Stockbrugger, R.W. The relationship between lactose tolerance test results and symptoms of lactose intolerance. Am J Gastroenterol. 1997 (Jun); 92(6):981-4

13.   Solomons, N.W. Evaluation of carbohydrate absorption: The hydrogen breath test in clinical practice. Clin Nutr J. 1984; 3:71-78

14. Filali, A.; Ben Hassine, L.; Dhouib, H.; Matri, S.; Ben Ammar, A.; Garoui, H. Study of malabsorption of lactose by the hydrogen breath test in a population of 70 Tunisian adults. Gastroenterol Clin Biol. 1987; 11:554-7

15. Douwes, A.C.; Schaap, C.; van der Kleivan Moorsel, J.M. Hydrogen breath test in school children. Arch Dis Child.1985; 60:333-7

16. Rogerro, P.; Offredi, M.L..; Mosca, F.; Perazzani, M.; Mangiaterra, V.; Ghislanzoni, P.; Marenghi, L.; Careddu, P. Lactose absorption and malabsorption in healthy Italian children: Do the quantity of malabsorbed sugar and the small bowel transit time play roles in symptom production? J Pediatr Gastroenterol Nutr.1985 (Feb); 4(1):82-6

17. Cloarac, D.; Bornet, F.; Gouilloud, S.; Barry, J.Ll.; Salim, B.; Galmiche, J.P. Breath hydrogen response to lactulose in healthy subjects: relationship to methane producing status. Gut. 1990 (Mar); 31:300-4

18. Robb, T.A.; Goodwin, D.A.; Davidson, G.P. Faecal hydrogen production in vitro as an indicator for in vivo hydrogen producing capability in the breath hydrogen test. Acta Paediatr Scand. 1985 (Nov); 74:942-4

19. Vogelsang, H.; Ferenci, P.; Frotz, S.; Meryn, S.; Gangl, A. Acidic colonic microclimate—possible reason for false negative hydrogen breath tests. Gut. 1988 (Jan); 29:21-6

5. Protocol for Lactose Malabsorption

Some patients do not produce H_2 even though they are malabsorbers. This is presumably because they do not produce H_2, or the H_2 is consumed by methanogenic bacteria. Patients who are strongly suspected of being lactose malabsorbers but do not produce H_2 make up some 5-15% of patients tested. Those patients can be examined further in one of two ways:

(1) Repeat the test with lactulose (which is a disaccharide not hydrolyzed in the intestine, and is, therefore, expected to get to the colon) on another day, to verify that the patient is not able to produce measurable amounts of H_2, or,

(2) Measure CH_4 with a Model DP or Model SC MicroLyzer to detect a response in malabsorbers who generate CH_4 instead of, or in addition to, H_2.

Patient Instructions and Preconditions

a. The patient should not have eaten slowly digesting food like beans, bran or other high-fiber cereals the day before the test is performed.

b. The patient should fast for a minimum of 10 hours, with no food and only water to drink before the test.

c. The patient should not smoke, sleep, nor exercise vigorously for at least ½-hour before, or *at any time during*, the test.

d. Ask the patient about any recent antibiotic therapy and/or recent or current diarrhea. Make the physician aware of such conditions if they have occurred, since they *can* affect the outcome of the test. Antibiotics should not be prescribed for at least two weeks before testing for lactose malabsorption.

Patient Test Protocol

If the patient meets the preconditions for testing as outlined above, proceed with the following protocol:

1. Collect an alveolar sample and analyze it to establish a baseline for breath-H_2 (and for breath-CH_4 if a methane analyzer is available). If the Model SC MicroLyzer is used, correct the values for possible contamination on the basis of the CO_2 analysis. The corrected H_2 baseline is typically less than 10 parts per million (ppm). Methane is commonly not seen, but it is normal to have up to 6-8 ppm in the baseline sample. Higher values for H_2 may indicate incomplete fasting prior to the test, the ingestion of slowly digesting foods the day before, or (if either the H_2 or CH_4 levels exceed 20-30 ppm) the presence of bacterial overgrowth in the small intestine. Discuss this finding with the physician, if possible, before continuing the test since it may indicate the importance of collecting an additional alveolar air sample 15-20 minutes after the lactose ingestion to differentiate malabsorption from possible bacterial overgrowth.

2. Have the patient ingest the designated dose of lactose, usually 1.0g/kg body weight, up to 25g total. Higher doses are used by some physicians, but most of the literature suggests that 1g/kg produces reliable test results without causing uncomfortable cramps, bloating and diarrhea in lactose intolerant patients.

3. For the standard protocol, an alveolar air sample is collected and analyzed every 30 minutes after the ingestion of the lactose (or according to instructions from the physician) until the H_2 in the alveolar air exceeds the lowest preceding value by at least 20 ppm, or for up to three hours if the challenge-dose was lactose dissolved in water. Some laboratories routinely collect the samples at one-hour intervals after the lactose ingestion, which is acceptable if there is no likelihood that the patient has bacterial overgrowth, and if there is no need to complete the test in as short a time as possible. If milk is used as the challenge-dose, an additional hour should be added to the sampling protocol to allow for a possibly longer transit time caused by slower gastric emptying. If food is consumed, the test period should be prolonged to five to six hours.

Data Recording

If CO_2 has been measured in the samples, the H_2 and CH_4 data can be corrected for dead space contamination. Divide 5.0 by the percent CO_2 in the sample, then multiply the sample H_2 and CH_4 analyses by that correction factor. This procedure is done automatically with the

Model SC MicroLyzer, but not all laboratories use that instrument to measure the CO_2. If CO_2 was not measured, the assumption will be made that no samples were contaminated or that all samples were diluted to the same degree in the sampling procedure (which may or may not be true).

Record the information relative to the test on a form such as that reproduced on the next page so the data can be properly interpreted by the physician. Use the available (corrected, if possible) H_2 and CH_4 values to prepare a graph of the trace-gas response. The breath trace-gas response to the lactose challenge will be interpreted according to the standards set by the physician. A guideline to interpreting the lactose malabsorption test is presented in the next section, based on discussions in the literature.

Lactose Malabsorption Analytical Record

Date of Test _____ Technician_____

Patient's Name_____Date of Birth_____

Patient Wt _____Pounds   Lactose Dose _____ gram

Doctor's Name _____ Phone No. _____

Fax No. _____

<u>Sample</u> <u>Time</u>   <u>Clock</u>   <u>ppm H_2</u> <u>ppm CH_4</u>   <u>(f)CO_2</u>

The f(CO_2) is the correction factor, measured or calculated , H_2 and CH_4 values are corrected for sample contamination with the SC MicroLyzer.

No. 1   0 hr   _____   _____   _____   _____

LacTest administered
No. 2   ½ hr   _____   _____   _____   _____

No. 3   1 hr   _____   _____   _____   _____

No. 4   1½ hr   _____   _____   _____   _____

No. 5   2 hr   _____   _____   _____   _____

No. 6   2½ hr   _____   _____   _____   _____

No. 7   3 hr   _____   _____   _____ _____

Additional Samples if Required
No. 8   3½ hr   _____   _____   _____

No. 9   4 hr   _____   _____   _____   _____

Notes and Diagnosis_____

_____

_____

 Signature_____

6. Interpreting Lactose Malabsorption Breath-Tests

For the standard protocol for lactose malabsorption, the dose of lactose prescribed should be large enough so that if the sugar comes in contact with bacteria after it is ingested, the resultant generation of H_2 and CH_4 will be readily detected in the alveolar samples. A dose of 2g/Kg body weight, up to 50 grams, of lactose (equivalent to that in about one quart of milk) was necessary for the blood test to be decisive, and was the recommended dose for the breath-H_2 test when it was first introduced. However, it was soon demonstrated that a smaller dose of 25g (in adults) was sufficient for the breath-test. This lower dose has been generally accepted as the standard challenge-dose for the breath-test because when a 50g dose is given to patients who are intolerant, they experience considerable discomfort following such a large dose of lactose. Furthermore, it is not common for a patient to drink a quart of milk at one sitting, so there is some irrelevance in having a patient drink that much milk for the test.

25g of lactose (equal to that in about a pint of milk) produces an almost equivalent level of breath-H_2 peak height response as that produced by the larger 50g dose, and if symptoms occur they are mild and more tolerable. This surprising observation about the dose-effect relationship probably reflects an increased percentage of the gas being lost in flatus due to greater colonic activity, or represents a limitation in the *rate* of gas production by bacteria in the colon. Some physicians prefer to use the larger dose of lactose in the belief that demonstrating symptoms will impress the patient with evidence that the sugar being tested is, indeed, the culprit causing the problem.

The patient should fast for a minimum of 10 hours before the test is performed in order to have a low resting (basal) level for H_2 and CH_4. Even with this precaution, there may be considerable variability in the resting values due to residual starches and fiber in the colon from food ingested the day before, or from incomplete fasting the night before the test. Therefore, it is important to collect an alveolar air sample at the beginning of the procedure, to be used as a baseline against which the response during the test will be measured.

After the baseline (control) sample has been collected, the physiological dose of lactose is consumed by the patient and a timer is started

so subsequent samples can be collected on a prescribed schedule. If lactose dissolved in water is used as the challenge dose, the test ordinarily needs to be conducted for no more than three hours. If milk is used, an additional hour of sampling should be added since milk slows gastric emptying and will retard the appearance of the sugar in the colon. If the challenge-dose is accompanied by food, it will require five hours to be sure the sampling period extends through the expected intestinal transit time required for all the lactose to reach the colon.

At 30-minute intervals for the minimum period of the test, samples of alveolar air should be collected and analyzed for both H_2 and CH_4. When a positive response is observed, as defined by the criteria described below, the sample collecting procedure can be discontinued, since the test will be defined as positive for malabsorption of that sugar.

Breath Hydrogen

The H_2 concentration in an alveolar air sample from a healthy patient who has fasted for 12 hours is normally less than 10 ppm (parts per million). With patients who have elevated control values (e.g., 10-20 ppm) due to residual fiber in the colon, the H_2 level might fall during the test period as the colon fiber-content is reduced through digestion. If the patient is a lactose malabsorber, the change in trace-gas levels during the test should be related to the lowest point recorded for the H_2 level prior to its increase.

If the patient is a lactose malabsorber, the breath-H_2 concentration will increase by over 20 ppm within the test period following the ingestion of 1g lactose per kg body weight. The positive response results from undigested lactose reaching the colon where bacteria hydrolyze the sugar and produce H_2 as a metabolic product. The gas is equilibrated with the blood serving the colon and is returned to the lungs, where it is exchanged with the alveolar air. In lactose absorbers, the lactose is hydrolyzed to glucose and galactose by *lactase enzyme* and they are absorbed in the small intestine. Thus, there is normally no significant change in the breath-H_2 during the test, but some variation (of a few ppm) in alveolar concentrations is common.

Some patients do not expel H_2 in the alveolar air even though they are malabsorbers. This is presumably because they do not produce H_2, or the H_2 is consumed by methanogenic bacteria. Patients who are strongly suspected of being lactose malabsorbers but do not produce H_2 make up some 5-15% of patients tested. Those patients can be examined further in one of two ways:

(1) Repeat the test with lactulose (which is a disaccharide not hydrolyzed in the intestine and, therefore, is expected to get to the colon) on another day, to determine whether the patient is able or not able to produce measurable amounts of H_2, or,

(2) Measure CH_4 with a Model DP or Model SC MicroLyzer to detect a response in malabsorbers who generate CH_4 instead of, or in addition to, H_2.

Breath Methane

The major reason for including CH_4 in the test for lactose malabsorption is to detect those patients who present a "false-negative" result if only H_2 is measured. Many fasting patients do not have CH_4 in their breath, and may not have a CH_4 response even if they are lactose malabsorbers. Others may have low baseline levels (up to 5-7 ppm), and increase CH_4 by 10-15 ppm if they are malabsorbers. If there is an increase in the CH_4 level by at least 12 ppm within the test period following the ingestion of lactose, the test is considered to be positive without reference to the H_2 response. If both H_2 and CH_4 are increased after a lactose challenge, the two responses should be summed to estimate the patient's degree of malabsorption. Since CH_4 is either generated from the same substrate hydrolyzed by H_2-producing bacteria or produced by converting H_2 to CH_4, any increase in CH_4 is at the expense of H_2. Therefore, a suitable threshold for the test is the requirement that the sum of H_2 and CH_4 increases should equal 15-20 ppm. Considering that the synthesis of one molecule of CH_4 would require two molecules of H_2, this criterion for a positive response is probably conservative.

These guidelines should be used along with the patient history, clinical judgment and other considerations available to the physician. The CH_4 pattern is less dependent on ingested "substrate" than is H_2 (which means that it is not closely identified with the kind of food eaten). How-

ever, the CH_4 response is reliable in lactose malabsorbers who do not produce H_2, or produce only small amounts of H_2. The measured CH_4 response should be added to any H_2 response which is recorded. The pattern of gas production is no doubt related to the type of bacteria resident in the colon, and can not be predicted in advance. Some investigators believe there is a genetic dependency on methane producers, but no explanations for the observations are adequate by themselves.

Breath CO_2

It is widely recognized that a measurement of CO_2 in the sample analyzed for H_2 and CH_4 can be used to increase the accuracy of the measurement. The CO_2 concentration permits a calculation of the degree of sample contamination with dead space air, or the introduction of room air into the sample as the result of poor collection technique or problems with difficult patients, such as babies. This topic is covered in greater detail elsewhere, but it is important to recognize that correcting for sample contamination on the basis of the deviation from the expected normal alveolar CO_2 concentration is a widely accepted procedure. The Model SC MicroLyzer was designed to make this an easy, semi-automatic procedure which will significantly improve the accuracy of trace-gas analyses, especially in pediatrics.

False-Negative H_2-Tests and the Role of CH_4

A small percentage of lactose malabsorbers do not produce H_2 after ingesting lactose, and some of them even excrete no hydrogen after ingesting a nonabsorbable sugar, such as lactulose. The incidence of false-negative tests with the breath-test is well below that seen with the blood test.[1-3] False-negative results are reported in 5-18% of all lactose malabsorbers.[4-7] There are numerous conditions which can lead to false-negative reports (erroneously suggesting no evidence for malabsorption).

Administering a course of antibiotics may sterilize the colon, so the colony-count of bacteria is low or non-existent.[8, 9] However, not all antibiotics have this effect on the breath-test; it was reported that Neomycin may even increase H_2 excretion,[10] presumably due to the selective inhibition of hydrogen-consuming bacteria by the antibiotic.

Laxatives and enemas can decrease the H_2 and CH_4 responses in malabsorbers.[11] The decrease may result from reducing the resident-time for carbohydrate in the colon, leading to reduced time for fermentation. The reduced gas production may also result from a change in the concentration of the bacteria and from a change in the environment, such as increasing the acidity, which could inhibit bacterial action.[12] Inherent rapid transit of the carbohydrate through the colon due to hypermotility will also reduce the exposure time and decrease H_2 production in the colon.

H_2 production is affected by colonic pH. A decrease in stool pH from 7.0 to 5.5 will cause a drop in H_2 generation to ¼ the former rate.[13] The rate is returned promptly by increasing the pH. Thus, severe diarrhea and/or hyperacidic colon contents may inhibit the generation of hydrogen, or cause the generation of methane in addition to,[13, 14] or instead of,[11, 12] hydrogen by colonic bacteria.

The majority of malabsorbers who do not produce H_2 when exposed to lactose will generate CH_4 instead. These patients will be properly diagnosed if CH_4 is measured as part of the routine test. Bjrnecklett et al.[15] found that 44% of 120 healthy subjects were CH_4 producers. Eight of 100 subjects failed to produce significant H_2 after 33g of lactulose, but all eight excreted large amounts of CH_4. Thirteen subjects had responses greater than 5 ppm CH_4. All 13 had lower fasting H_2 and lower H_2 responses to lactulose than did the others. Coryzza, *et al.*[16] reported that out of 13 subjects with a false-negative H_2 test for lactose, 11 more than doubled CH_4 production, and 11 of 32 lactose intolerant patients with negative H_2 tests more than doubled CH_4 following a lactose challenge. Fritz et al,[14] studied H_2 and CH_4 after administering a commercial disaccharide mixture (Palatinit). A linear relationship was found between the amount given and the H_2 produced over a 10-hour period. If CH_4 was formed, the sum of both gases followed a linear dose-effect relationship, indicating an interaction between the two components. Cloarac *et al.*[17] demonstrated an effect of CH_4-production on (a) fasting H_2 baseline values, (b) the area under the curve following lactulose and, (c) orocecal transit time, suggesting that knowledge of CH_4 status is necessary for the proper interpretation of the H_2 breath-test. One dissenting paper exists in the literature. Montes and co-workers[18] reported a high correlation between the H_2 response and the

CH_4 response in malabsorbers and found the percentage of malabsorbers to be the same (51%) regardless of CH_4 production. They concluded that attention to CH_4-producing status was not necessary for the interpretation of the lactose breath-test.

Recent literature has been focused on the interrelationship between H_2 and CH_4 production in the colon. In addition to the reports of Cloarec and co-workers in France,[17] Strocchi and Levitt in Minnesota[19] reviewed the literature about CH_4 production, including the conversion of H_2 to CH_4, and concluded that most of the disposal of intestinal H_2 is through its utilization by H_2-consuming micro-organisms. The main point of their editorial is that the excretion of H_2 is far more complex than previously assumed, and is not simply a function of the quantity of fermentable substrate (e.g., lactose) reaching the colon and the number of H_2-producing bacteria which are resident there.

If methane analysis is not available, an alternate approach is to test suspected false-negative patients with another disaccharide which is known to be carried to the colon. A 10g dose of lactulose will produce a positive breath-hydrogen test in patients *who are capable of producing hydrogen.*[20] That procedure in a patient suspected of being a lactose malabsorbers but who tests "negative" may show that the patient is not able to generate H_2, even if the disaccharide gets to the colon. It does not separate the academic question of whether the patient does not produce gas from that of the patient who converts H_2 to CH_4. That requires the measurement of CH_4. The lactulose test with only H_2 analysis will indicate only that a patient who is suspected of being a lactose malabsorber is or is not capable of producing H_2. It was reported[7] that 6% of patients with symptoms of lactose intolerance were not classified with breath-H_2 and blood glucose together. It would be interesting to know how many would have been found to be positive with breath-CH_4.

Those patients who have been treated with antibiotics before the test will produce neither H_2 nor CH_4. A false-negative test caused by colon hyperacidity will be prevented by pretreatment with $MgSO_4$.[13] Thus, measuring breath CH_4 in addition to H_2 (or if only H_2 measurement is available, either repeating the test with lactulose on another day or reducing colonic acidity before the test) will reduce false-negative results to very acceptable levels.

High Control Values

Elevated control values (above 10 ppm) for H_2 can be observed in normal patients or lactose malabsorbers who have not fasted properly or have eaten slowly-digesting, fiber-containing foods the day before the test. High baseline (control) levels for H_2 (usually above 20 ppm) are also seen with patients who have bacterial overgrowth. In those patients, it may increase modestly from the elevated baseline soon after the ingestion of a sugar such as lactose (within 15-45 minutes), and fall back to near control levels later in the test (perhaps by the three-hour sample). Elevated CH_4 levels can also be seen with bacterial overgrowth,[12] though it may not change as much as H_2 does in response to the challenge dose of sugar.

Large Trace-Gas Responses

In general, lactose malabsorbers who have the greatest rise in breath-H_2 and CH_4 following the challenge dose of lactose will have more severe lactose intolerance (more severe symptoms in response to greater sensitivity to milk products). This is particularly true when the total response is considered to be the sum of the separate H_2 and CH_4 responses.

An increase of 20-40 ppm in the H_2 plus CH_4 response during the test would generally be classified as a mild response. If the increase reached 40-80 ppm, it would be considered moderate, and if it increased by more than 80 ppm it would generally be classified as a severe degree of lactose malabsorption. Responses up to 400-500 ppm are not common, but they are seen. They indicate serious intolerance, usually with severe symptoms following lactose ingestion.

Such a guideline is only semi-quantitative because the proportion of intestinal gases which are absorbed into the blood stream can be quite variable (some of the early reports not withstanding), and the perception of symptom severity is at least partly subjective. When the increases in breath H_2 and CH_4 are mild or moderate, advising the patient to limit the intake of milk products and avoid large amounts of the offending lactose at any one meal may be sufficient to prevent symptoms of lactose intolerance. Use of commercially available *lactase enzyme* preparations may

provide relief if the restrictions are exceeded. It has been demonstrated that milk is tolerated better by lactose malabsorbers if its ingestion is accompanied by food, rather than being consumed alone. When the increase in either H_2 or CH_4 is large, it is likely that the patient must avoid all milk products or the use of the *lactase enzyme* preparations is virtually mandatory when milk products are consumed.

False-Positive Hydrogen Tests

Falsely high H_2-levels in response to the ingestion of lactose (false-positive tests)[10] may occur, but they are almost always the result of improper preparation for the test or improper conduct of the test. Such errors may include:

a. Improper preparation of the patient - The inappropriate choice or incomplete avoidance of food by the patient on the night before the test will provide a high, but gradually falling level of H_2 on which the test will be superimposed. This is because the amount of fiber in the colon will be elevated at the beginning of the test, and will fall during the hours of the measurement. Even if H_2 is produced from the challenge-dose of carbohydrate, it may not exceed the initial baseline level by enough to be classified as a positive test. Breath levels of CH_4 are not as affected by the ingestion of food as are levels of H_2. The reasons for high CH_4 levels when they occur is not completely clear, but may be related to endogenous mucopolysaccharides or other residual material in the colon, though sugars and other carbohydrates, quite obviously can change CH_4 levels.

b. Smoking in the area of the test - Tobacco smoke contains high levels of H_2, so smoking (by the patient or by anyone in the area) will produce high H_2 levels and will cause extreme baseline instability of the instrument, since it uses room air as a standard against which breath samples are measured. Smoking by the patient should be avoided for at least a half hour before any sample is taken, and there should be no smoking at any time in the vicinity of the MicroLyzer.

c. Sleeping - Allowing the patient to sleep during the test will cause an increase in breath-H_2, and probably in CH_4. There are two reasons for such increases. Hypoventilation, which is an inadequate rate of air turnover in the lung, slows down the rate of H_2-removal from the blood. Sleep also decreases intestinal motility, which slows down the movement of carbohydrates through the colon and allows a longer time for

the accumulation of H_2. Thus, intermittent sleeping during the test will interfere, and should not be allowed.

High fasting levels of H_2 (and perhaps of CH_4) at the beginning of the test may suggest that the patient did not follow instructions for the complete avoidance of carbohydrate and fiber the night before; but it also may suggest that the patient has bacterial overgrowth, which is defined as the presence of bacteria in the small intestine. Bacterial overgrowth exposes the complex sugars and other soluble carbohydrates in the small intestine to bacterial fermentation instead of allowing them to be hydrolyzed enzymatically and absorbed in the relatively sterile intestine. If bacterial overgrowth causes a false-positive test, the response will appear early, and though it will plateau, should begin to decrease near the end of the test (if the patient is not a malabsorber).

If the breath-H_2 increases to more than 20 ppm above the baseline reading, it may be interpreted by the physician to indicate lactose malabsorption[2] if other symptoms and history support the diagnosis. The same condition is called *lactose intolerance* when the increase in H_2 is accompanied by acute symptoms of discomfort following dietary exposure to milk or milk products.

False-negative results are commonly reported in about 10% of lactose malabsorbers. Methanogenic bacteria commonly produce CH_4 from the H_2,[4, 5] but false-negatives are seen following antibiotics,[6] severe diarrhea[7] or hyperacidic colon contents.[8]

Problems from Sample Contamination

Diluting the alveolar air sample with "dead space air" (the part of the expired air sample which is not from the alveolar region of the lung) or with room air collected during the sampling process will produce falsely low H_2 levels. This is a much more common occurrence than is usually suspected and will lead to erratic, inconsistent analyses for H_2 and CH_4 in the samples.

The problem of sample contamination can be reduced by using the QuinTron GaSampler device or the QuinTron AlveoSampler for sample collection. It can be corrected by normalizing the sample-H_2 concentration on the basis of the carbon dioxide level in the breath. Any reliable

CO_2 analyzer can be used, but the QuinTron Model SC MicroLyzer was designed specifically for this problem and will provide reliable calculations of "alveolar gas" concentrations. This permits the breath-H_2 test to be used reliably in neonatology and in other areas where patients may not be able to cooperate in sample collection or in research studies where small variations in the measured gas concentrations can affect the results.

If CH_4 increases during the test, the response of H_2 will be blunted or absent.[4] If the *sum* of the response in H_2 plus CH_4 exceeds 15 ppm, the test would ordinarily be considered positive for malabsorption.

If the baseline H_2 level is over 20 ppm and the one-hour sample is elevated even more, there is a strong suspicion that the patient has bacterial overgrowth. Even with overgrowth, a later significant increase in H_2 or H_2 and CH_4 according to the criteria above can be interpreted as a positive test for lactose malabsorption.

References

1.	Fernandes, J, ; Vos, C.E.; Douwes, A.C.; Slotema, E.; Degenhart, H.J. Respiratory hydrogen excretion as a parameter for lactose malabsorption in children. Amer. J. Clin Nutr. 1978 (Apr);31:597-602

2.	Davidson, G.P.; Robb, T.A. Value of breath hydrogen analysis in management of diarrheal illness in childhood: Comparison with duodenal biopsy. J. Ped Gastroenterol Nutr 1985 (Jun); 4(3):381-7

3.	DiPalma, J.A.; Narvaez, R.M. Prediction of lactose malabsorption in referral patients. Dig Dis Sci 1988 (Mar);33:303-7

4.	Douwes, AC, Schaap, C and van der Klei-van Moorsel, JM. Hydrogen breath test in school children. Arch Dis Child. 1985 (Apr);60(4):333-7

5.	Rogerro, P, Offredi, ML, Mosca, F, Perazzani, M, Mangiaterra, V, Ghislanzoni, P, Marenghi, L and Careddu, P. Lactose absorption and malabsorption in healthy Italian children: Do the quantity of malabsorbed sugar and the small bowel transit time play roles in symptom production? J Pediatr Gastroenterol Nutr 1985 (Feb);4:82-6

6.	Filali, A, Ben Hassine, L, Dhouib, H, Matri, S, Ben Ammar, A and Garoui, H. Study of malabsorption of lactose by the hydrogen breath test in a population of 70 Tunisian adults. Gastroenterol Clin Biol. 1987 (Aug);11(8-9):554-7

7.	Hammer, H.F.; Petritsch, W.; Pristautz, H.; Krejs, G.J. Assessment of the influence of hydrogen nonexcretion on the usefulness of the hydrogen breath test and lactose tolerance Wien Klin Wochenschr 1996;108(5):137-41

8.   Metz, G, Jenkins, DL, Peters, TJ, Newman, A and Blendis, LM. Breath hydrogen as a diagnostic method for hypolactasia. Lancet 1975 (May 24);1(7917):1155-7

9.   Gilat, T, Ben Hur, H, Gelman-Malachi, E, Terdiman, R and Peled, Y.   Alterations of the colonic flora and their effect on the hydrogen breath test. Gut 1978 (Jul);19:602-5

10.   Lembke, B.; Honig, M.; Caspary, W.F. Different actions of neomycin and metronidazole on breath hydrogen (H_2) exhalation. Z Gastroenterol. 1980 (Mar); 18(3):155-60

11.   Solomons, N.W.; Garcia, R.; Schneider, R.; Viteri, F.E.; von Kaenel, V.A. H_2 breath tests during diarrhea. Acta Paediatr Scand. 1979 (Mar);88:171-2

12.   Perman, J.A.; Modler, S.; Olson, A.C. Role of pH in production of hydrogen from carbohydrates by colonic bacterial flora: Studies in vivo and in vitro. J. Clin Investig 1981 67(3):643-50

13.   Vogelsang, H.; Ferenci, P.; Frotz, S.; Meryn, S.; Gangl, A. Acidic colonic microclimate—possible reason for false negative hydrogen breath tests. Gut. 1988 (Jan);29(1):21-6

14.   Fritz, M.; Sievert, G.; Kasper, H. Dose dependence of breath hydrogen and methane in healthy volunteers after ingestion of a commercial disaccharide mixture, Palatinit. Br J Nutr. 1985 (Sep);54(2):389-400

15.   Bjrneklett, A.; Jenssen, E. Relationships between hydrogen (H_2) and methane (CH_4) production in man. Scand J Gastroenterol. 1982 (Nov);17(8):985-92

16.   Corazza, G.R.; Benati, G.; Strocchi, A.; Malservisi, S.; Gasparrini, G. The possible role of breath methane measurement in detecting carbohydrate malabsorption. J Lab Clin Med. 1994 (Nov); 124(5):695-700

17.   Cloarac, D.; Bornet, F.; Gouilloud, S.; Barry, J.L.; Salim, B.; Galmiche, J.P. Breath hydrogen response to lactulose in healthy subjects: relationship to methane producing status. Gut 1990 (Mar);31(3):300-4

18.   Montes, R.G.; Saavedra, R.G.; Perman, J.A. Relationship between methane production and breath hydrogen excretion in lactose-malabsorbing individuals. Dig Dis Sci 1993; 38(3):445-8

19.   Strocchi, A and Levitt, MD. Maintaining intestinal H2 balance: Credit the colonic bacteria. Gastroenterol. 1992; 102:1424-6

20.   Robb, TA, Goodwin, DA and Davidson, GP. Faecal hydrogen production in vitro as an indicator for in vivo hydrogen producing capability in the breath hydrogen test. Acta Paediatr Scand. 1985 (Nov);74(6):942-4

7. Bacterial Overgrowth

Because its complications have been recognized for many years, bacterial overgrowth was studied soon after breath-testing methods were described, and efforts have continued since that time to develop the most reliable diagnostic techniques.[1-7] Looking at the combination of an elevated control (fasting) level for H_2 and an elevation in expired H_2 following lactulose or glucose administration, has improved the reliability of the breath test for this application.[8-10] It is fair to point out, however, that some disagreement exists in the literature about how sensitive and how specific the breath-tests are for bacterial over-growth[11-13] if that combination is not used for the test.

The harsh acidic environment of the stomach kills most bacteria when they are ingested, so there is ordinarily a low bacterial count in the proximal part of the intestine (the duodenum and the jejunum). However, in patients with achlorhydria (lack of acid production in the stomach), the concentration of bacteria increases[14] and they may pass into the small intestine and colonize there. In addition, conditions of intestinal hypomotility caused by hypothyroidism[15] or other causes of "stasis"[16] permit bacteria to invade the small intestine from the colon. These conditions permit an increase in bacterial count to over 10^5 (100, 000) colonies per ml of intestinal contents, which defines the condition called "bacterial overgrowth." It leads to symptoms similar to those for disaccharide malabsorption. In addition, it also destroys some vitamins, interferes with the absorption of fatty acids and competes for sugars and other foodstuff ordinarily absorbed in the jejunum. Thus, it is a serious digestive disturbance which can be treated effectively, but only if it is diagnosed. Contrary to expectation, several reports have shown that the administration of agents which inhibit gastric acid production do not lead to bacterial overgrowth.[17-19]

Two methods have been proposed for the detection of bacterial overgrowth by breath-tests. The first method uses glucose,[1] which is ordinarily absorbed in the small intestine without exposure to bacteria, since it is absorbed before it gets to the colon. The second method utilizes lactulose,[3, 4] which is ordinarily not hydrolyzed until it gets to the colon. Thus, if a response appears soon after lactulose ingestion, and is followed by a larger response after a time delay to allow for the arrival of lactulose in the colon, the diagnosis of bacterial overgrowth is sug-

gested. D-xylose was recommended by Casselas and co-workers,[5] who reported a sensitivity index of 0.86, meaning that 86% of patients with bacterial overgrowth were detected. However, most clinicians have continued to use either glucose or lactulose as a substrate for the test. No consensus has been reached on a standard protocol for choosing one over the other, but Kristensen and Hoeck[20] compared the results of the glucose method and the lactulose technique with results of intestinal culture. In their limited study of 12 patients, 10 patients with overgrowth were positive with glucose (with one false-positive), while only four patients had an abnormal lactulose breath-test.

Glucose for Bacterial Overgrowth

Bacterial overgrowth is diagnosed by measuring the *early* appearance of hydrogen following the administration of a challenge-dose of carbohydrate. The simplest challenge-dose is glucose. The test consists of administering a 60-75g dose of glucose (dissolved in water) following a 12-hour fast. Breath samples are measured for H_2 (and CH_4 if instrumentation is available) every 20 minutes for at least a two- hour period of time or until a positive response is seen. If bacteria exist in the small intestine, they will compete with the natural digestive process and metabolize the glucose before it can be absorbed. Thus, an increase of at least 12 ppm within a two-hour period is indicative of bacterial overgrowth. In several reports, it has been demonstrated that when only H_2 is measured, this test has a sensitivity of slightly more than 75%[3, 9] (meaning that it detects at least three of four patients with bacterial overgrowth) and a specificity of about 90%[9] (reporting a false-positive in only one out of 10 patients). The specificity can be increased even further (to reduce the false-positive reports) by combining the observation of an elevated fasting breath-H_2 level (>20 ppm) with a positive hydrogen-response (greater than 12-15 ppm)[7-9] to the challenge-dose of glucose. The added measurement of CH_4 may reduce the incidence of false-negatives seen when only H_2 is measured. It should be acknowledged here that a review of the literature shows that many investigators do not report similarly high levels of specificity for either of the sugars commonly used for the diagnosis of bacterial overgrowth.

Lactulose for Bacterial Overgrowth

Disaccharides and other sugars have been examined for their specificity in detecting bacterial overgrowth. Some investigators prefer to use a 10g dose of lactulose as the challenge material. A positive test is defined as an unexpectedly early response in H_2. It is distinguished from the arrival of the lactulose in the colon by a slight decrease in the H_2 peak prior to the colonic peak of H_2. Unfortunately, a merging of the two peaks is not uncommon, with the pattern frequently showing more of an early plateau than a bimodal peak. The theoretical advantage of using lactulose is that the challenge-dose is carried further down the jejunum, so using lactulose should increase the sensitivity of the test. Another proposed advantage of using lactulose is that it provides an internal check on the method, in that lactulose may not produce H_2 after it reaches the colon if H_2-producing bacteria are not present. However, aerobic flora can exist in the small intestine, while anaerobic bacteria are mostly limited to the colon. Therefore, there may not be a relationship between the responses for the first and second peaks. On the other hand, if the patient has received antibiotics, there could be a reduction in the bacteria count in the entire intestinal tract. If a late peak is not seen following lactulose ingestion, it would be important to pursue the question further, at least with reference to the previous use of antibiotics.

Rice Flour for Bacterial Overgrowth

An attempt to increase the sensitivity of the H_2 breath-test for bacterial overgrowth was made by using rice flour cakes as the challenge-dose because rice is readily digested and absorbed, so no residual fiber ordinarily reaches the colon, with no H_2 to be generated. It was hypothesized that the rice flour, like lactulose, would pass further down the jejunum, thus being exposed to bacteria closer to the colon. Kerlin and co-workers[21] reported that breath-H_2 in patients with bacterial overgrowth was markedly increased (to over 70 ppm) after the consumption of 100g of carbohydrate in the form of rice pancakes, while normal controls did not exceed 7 ± 1.5 ppm H_2.

Unfortunately, their early reports of using rice flour instead of glucose or lactulose were not supported by their later studies,[9] which reported decreased specificity of the test, so it has not gained wide acceptance.

Methane Measurements for Bacterial Overgrowth

The lack of an H_2 response following lactulose ingestion warrants a check on the presence of methanogenic bacteria by measuring CH_4 production during the period of test. If neither gas is produced in the colon, review the history of the patient to be sure they have not taken antibiotics within the past two weeks.

It has been suggested that patients with bacterial overgrowth would have an increased <u>fasting</u> CH_4-level. This is related to the fact that the basal level of CH_4 is likely affected by glycoproteins, which are increased by bacterial overgrowth.[22] It has been reported that the alveolar breath-H_2 level is suppressed by methanogenic bacteria, so this criterion might be blunted when CH_4 is produced.

Thus, evaluating both H_2 and CH_4 in the control sample should add credence to the test. Having the response limited to H_2 in the presence of elevated CH_4 in the control sample would strongly support a diagnosis of bacterial overgrowth, according to the current theories about the relationship between the two gases.

As with tests for carbohydrate malabsorption, in the past the emphasis in tests for bacterial overgrowth was on H_2, but the application of breath-CH_4 studies to bacterial overgrowth is overdue, and may be a fruitful area of research. The availability of the Model SC MicroLyzer, which measures both H_2 and CH_4, and corrects for any inadvertent contamination of the sample may encourage such research to be done.

References

1.   Metz, G.; Gassull, M. A.; Drasar, B. S.; Jenkins, D. J.; Blendis, L. M. Breath-hydrogen test for small-intestinal bacterial colonization. Lancet. 1976 Mar 27; 1(7961):668-9

2.   Adlung, J. Breath analysis tests in gastrointestinal disorders. Z Gastroenterol. 1978 Mar; 16(3):179-87

3.   Rhodes, J.M.; Middleton, P.; Jewell, D.P. The lactulose hydrogen breath test as a diagnostic test for small-bowel bacterial overgrowth. Scand J Gastroenterol. 1979; 14:333-6

4.   Wildgrube, H.J.; Classen, M. Hydrogen (H2) breath tests in the diagnosis of small intestine diseases. Z Gastroenterol. 1983 (Nov); 21(11): 628-36.

5. Casellas, F.; Chicharro, L.; Malagelada, J. R. Potential usefulness of hydrogen breath test with D-xylose in clinical management of intestinal malabsorption. Dig Dis Sci. 1993 (Feb); 38(2):321-7

6. de Boissieu, D.; Chaussain, M.; Badoual, J.; Raymond, J.; Dupont, C. Small-bowel bacterial overgrowth in children with chronic diarrhea, abdominal pain, or both. J Pediatr. 1996 (Feb);128(2):203-7

7. Davidson, G. P.; Robb, T. A.; Kirubakaran, C. P. Bacterial contamination of the small intestine as an important cause of chronic diarrhea and abdominal pain: diagnosis by breath hydrogen test. Pediatrics. 1984 (Aug); 74(2):229-35

8. Corazza, G. R.; Strocchi, A.; Gasbarrini, G. Fasting breath hydrogen in celiac disease. Gastroenterol. 1987 (Jul); 93(1)53-8

9. Kerlin, P.; Wong, L. Breath hydrogen testing in bacterial overgrowth of the small intestine. Gastroenterol. 1988 (Oct); 95(4):982-8

10. Perman, J. A.; Modler, S.; Barr, R. G.; Rosenthal, P. Fasting breath hydrogen concentration: normal values and clinical application. Gastroenterol. 1984 (Dec); 87(6):1358-63

11. Corazza, G. R.; Menozzi, M. G.; Strocchi, A.; Rasciti, L.; Vaira, D.; Lecchini, R.; Avanzini, P.; Chezzi, C.; Gasbarrini, G. The diagnosis of small bowel bacterial overgrowth. Reliability of jejunal culture and inadequacy of breath hydrogen testing [see comments]. Gastroenterol. 1990 (Feb); 98(2)302-9

12. Riordan, S.M.; McIver, C.J.; Walker, B.M.; Duncombe, V.M.; Bolin, T.D.; Thomas, M.C. The lactulose breath hydrogen test and small intestinal bacterial overgrowth. Am J Gastroenterol. 1996 (Sep);91(9):1795-803

13. King, C. E.; Toskes, P. P. Comparison of the 1-gram [14C]xylose, 10-gram lactulose-H2, and 80-gram glucose-H2 breath tests in patients with small intestine bacterial overgrowth. Gastroenterol. 1986 (Dec); 91(6):1447-51

14. Armbrecht, U.; Bosaeus, I.; Gillberg, R.; Seeberg, S.; Stockbruegger, R. Hydrogen (H2) breath test and gastric bacteria in acid-secreting subjects and in achlorhydric and postgastrec-tomy patients before and after antimicrobial treatment. Scand J Gastroenterol; 1985 (Sep); 20(7): 805-13.

15. Goldin, E.; Wengrower, D. Diarrhea in hypothyroidism: bacterial overgrowth as a possible etiology. J Clin Gastroenterol; 1990 (Feb); 12(1): 98-9.

16. Cook, G.C. Delayed small intestine transit time in tropical malabsorption. Br Med J 1978 (Jul);22:2(6132):238-40

17. Bourne, J. T.; Mountford, R. A.; Barry, R. E. Twice-daily cimetidine does not increase gastric bacterial flora. Postgrad Med J. 1984 (Jul); 60(705):464-6

18. Nelis, A.U.; Nelis, G.F.; Engelage, A.H.; Samson, G. Does long-term inhibition of gastric acid secretion with omeprazole lead to small intestinal bacterial overgrowth? Neth J Med 1994 (Sep);45(3):93-100

19. Hutchinson, S.; Logan, R. The effect of long-term omeprazole on the glucose-hydrogen breath test in elderly patients. Age Ageing. 1997 (Mar);26(2):87-9

20.   Kristensen, M.; Hoeck, H.C. Abnormal flora in the small intestine. Diagnostic evaluation of the H2 breath test.   Ugeskr Laeger. 1994 (Dec 12);156(50):7530-3

21. Kerlin, P.; Wong, L.; Harris, B.; Capra, S. Rice flour, breath hydrogen, and malabsorption. Gastroenterol. 1984 (Sep); 87(3):578-85

22.   Perman, J.A.; Modler, S. Glycoproteins as substrates for production of hydrogen and methane by colonic bacterial flora. Gastroenterol 1982 (Aug); 83(2):388-93

8. Protocol for Bacterial Overgrowth

Patient Instructions and Preconditions

1. The patient should fast for 12 hours, with no food and only water to drink before the test. Avoid slowly digesting foods, like beans and some other vegetables, the day before the test.

2. Advise the patient that he/she SHOULD NOT smoke, sleep nor exercise vigorously for at least ½-hour before or at any time during the test.

3. Ask the patient about any recent antibiotic therapy. Be sure the physician is aware of such treatment, since it can affect the test.

Patient Test Protocol

If the patient meets the preconditions for testing as outlined above, proceed with the following protocol:

1. Collect an alveolar sample and analyze it to establish a baseline value for breath-H_2 (and for CH_4 if your instrument has such capability). The baseline sample typically has less than 10 parts per million (ppm) of H_2, and may be from 0 to under 7 ppm for CH_4. Ordinarily, values over 20 ppm H_2 are suspicious for bacterial overgrowth, and values between 10 and 20 (or so) suggest incomplete fasting before the test or the ingestion of slowly digesting foods the day before the test, with the colon being the source of the elevated levels.

2. Administer the challenge-dose of sugar according to Protocol A or Protocol B.

Protocol A: Glucose Ingestion
Have the patient ingest 70-100g glucose dissolved in 8 oz. water. A convenient form is Koladex, used for the glucose tolerance test. For children, administer 1g per kg body weight.

Protocol B: Lactulose Ingestion
Administer 10g lactulose in 6-8 oz. water. Lactulose syrup is a convenient form of lactulose. For children, administer 0.5g/kg body weight, up to 10g total.

3. Collect and measure alveolar samples for H_2 (and for CH_4) every 20 minutes for at least two hours after the challenge-dose of the sugar has been ingested or until a positive increase of 12-20 ppm has been recorded. If lactulose is used (Protocol B), it may be desirable to extend the test to three or three and a half hours in order to record the colonic response to the lactulose, which might aid in the interpretation.

Interpretation of the Breath-Test for Bacterial Overgrowth

Protocol A. If *glucose* was used for the challenge-dose, an increase of at least 12 ppm in H_2 levels over the base line value is indicative of bacterial overgrowth. It is unlikely that CH_4 will share in the response, even though it might be present in the control value. The test can be discontinued when a positive response is recorded. Without bacterial overgrowth, the sugar is not exposed to bacteria before it is absorbed, so no response is seen. If bacteria are present in the upper part of the small intestine, H_2 will be generated and will ordinarily appear in the alveolar air within 20-60 minutes. Measuring the H_2 for at least two hours if it is negative assures that the negative result did not result from retarded gastric emptying.

Protocol B. If lactulose was used for the challenge-dose, bacterial overgrowth will ordinarily cause a biphasic pattern in breath-H_2. There will be an early response of H_2, with an increase of 12 ppm over the baseline value, followed by a second response which will be very much larger (perhaps accompanied by or replaced by CH_4) when the sugar reaches the colon. Unfortunately, it is not uncommon for the two peaks to merge, with the pattern showing more of an early plateau than a bimodal peak. The theoretical advantage of using lactulose is that the challenge dose is carried further down the jejunum and into the ileum, so using lactulose should increase the sensitivity of the test. The second (later) peak which will appear about one hour or so after lactulose ad-ministration will be expected in all patients, with or without bacterial overgrowth.

The lack of both an H_2 and a CH_4 peak following lactulose admin-istration suggests that the patient may have received antibiotics recently, or may have abnormally acidic colon contents which inhibit colonic bac-terial action. An acidic colon will not necessarily affect the diagnostic

accuracy of lactulose for bacterial overgrowth in the small intestine since the media are different in the two sites.

A single early response in H_2 with no secondary H_2 peak and no CH_4 response could be interpreted as bacterial overgrowth with an accompanying acidic colon.

As discussed earlier, patients with bacterial overgrowth may have an elevated fasting CH_4 level. Perman proposed that the substrate consists of glycoproteins from the intestinal wall, which are increased in bacterial overgrowth, and the site of CH_4 production is probably the colon. However, no definitive studies of the CH_4-pattern following sugar ingestion in bacterial overgrowth have been reported, to our knowledge.

4. Plot the results on a graph to present the data for interpretation by the physician.

9. Intestinal Transit Time

Breath-tests for H_2 have been adapted to the measurement of the intestinal transit time (ITT), referred to interchangeably as ITT or orocecal transit time (OCTT). Earlier chapters in this monograph will refresh your memory about how breath-H_2 is generated and the conditions under which the ingestion of some sugars results in malabsorption.

Since there is no intestinal enzyme which hydrolyzes lactulose, it is a sugar which is normally carried intact to the colon, and from which bacteria there produce H_2 and CH_4. Many reports support the use of lactulose for the measurement of intestinal transit time. The test is based on the appearance of H_2[1-3] (and sometimes CH_4)[4] in the breath after the ingestion of a small dose of lactulose. Ordinarily, a 10g dose of lactulose is ingested and breath-samples are measured at 10-minute intervals (beginning 30 minutes after the dose of lactulose) for the first two hours and at 20-minute intervals after two hours, or until a response is seen. The time for the first sustained increase in trace gas concentration in alveolar air is identified as the ITT. The mean normal ITT is reported to be from slightly over 60 minutes to about 110 minutes,[5-22] and was shorter in women than in men.[23] If a response has not been seen within a five-hour period, the test can be discontinued and a false-negative report made on the basis of the inability of the colon to produce H_2 or CH_4.

Considerable variation has been reported for the intra-subject variability of the breath-H_2 response to lactulose.[24-27] However, Read and co-workers[28] and Camboni *et al.*[15] reported satisfactory repeatability, with the scatter of differences being greater when the mean gastrointestinal transit time was increased. It was reported that exercise increases[29] or reduces[30] intestinal transit time, but Scott and Scott[31] showed that moderate exercise taken before food intake did not interfere with transit time.

Modification of ITT by Lactulose

It is recognized that lactulose has the effect of shortening normal transit time through the small intestine,[32-34] and others, because it retains water through an osmotic effect and it directly increases intestinal motility. The consequence is that the measured ITT is affected by the dose

of lactulose used for the test,[28, 35, 36] so it is important to use a small dose.[37] Even more important, the same dose of lactulose should be used with all patients in order to compare them with the standardized ITT. Staniforth and Rose[38] presented evidence that the variability is reduced if the lactulose is administered with a meal, and that adopting a semi-recumbent position further reduced variability. However, Caride et al.,[39] reported that the small intestinal transit time was similar with the scintigraphic method and with the hydrogen breath-test, having mean values of 73 and 75 minutes, with similar SEM (standard errors of the means). DeVries and co-workers[35] used the complex carbohydrates in barley groats to determine mouth-to-cecum transit time, and showed a transit time of several hours, which was not affected by particle size or dose of the carbohydrate (0.75, 1.0 or 1.5 g/kg body weight).

Factors Affecting ITT

It has been demonstrated that agents which modify intestinal motility reflect an appropriate change in intestinal transit time; those which prolong transit time may reduce diarrhea,[11, 12, 40, 41] while those which shorten a prolonged transit time may correct constipation.[42] Cigarette smoking has been shown to prolong ITT in males, while nicotine had a similar effect on both males and females.[13] Transit time has been shown to be prolonged in cystic fibrosis,[43-45] Crohn's disease[46] and obesity.[47] Many reports show that transit time is prolonged in diabetes mellitus,[10, 19, 48, 49] while some studies showed a shortened transit time as well in some patients.[50] It has also been shown that hormones affect intestinal transit time in late pregnancy,[9, 51, 52] but that the normal menstrual cycle does not alter the transit time.[2, 53] Psychological mood,[54] anorexia nervosa,[55] and hypnosis[14] also affect intestinal motor function, as measured by changes in ITT . Thyroid disorders affect transit time, whereby hyperthyroidism shortens it.[56-58] Chronic alcoholism and cirrhosis increased ITT, explained on the basis of an alcoholic autonomic neuropathy,[18] though Keshavarzian and co-workers[59] reported a shortened transit time, with an increased sensitivity to osmotic loads. Pfeiffer, Hogl and Kaess[60] reported that beer and wine accelerated gastric emptying in comparison with ethanol, while the gastrocecal (small intestine only) transit time was shorter with beer compared with ethanol and water, though the constituents responsible for the observations remain to be found. The increased orocecal transit time produced by red pepper was attributed to the known effects of capsaicin as a stimulator of many

biologically active peptides.[22] However, Horowitz and co-workers[61] found that 20g chili powder slowed gastric emptying but had no effect on orocecal transit. Suggestions that age affects transit time were not supported from studies by Kupfer and co-workers[62] and by Piccione, *et al.,*[20] which showed no differences with younger adult subjects, but ITT was longer in geriatric females than in males.

Beaugerie and co-workers reported that malabsorption of sorbitol was reduced from 98% to about 70% when accompanied by the ingestion of glucose or lipids,[63] but the transit time was not affected, giving support to the recommendation by Wursch, *et al.,*[64] and adopted by Yuan and co-workers,[65] that sorbitol be used to replace the more expensive lactulose used for measuring intestinal transit time. In addition to Wursch, other investigators have shown that the measurement of transit time was not different when sorbitol or lactitol was substituted for lactulose.[63, 66, 67] Jain, Patel and Pitchumoni[6] proposed that the frequently observed sorbitol intolerance was a result of the short transit time for the sugar. Kondo and co-workers[8] suggested that milk is a suitable test meal for measuring ITT. The study originated in Japan, where 90% of the adults are lactose malabsorbers, according to the authors, so it would be less suitable in most other geographic areas. Variable sensitivity to milk would reduce its usefulness, even in Japan, but it could be convenient for the serial follow-up of disease or drugs which affect small intestinal transit time in lactose malabsorbers.

Methane and ITT

It is possible to find H_2 non-producers when ITT is measured with lactulose. Several reports have indicated that 8-15% of patients did not produce H_2 following lactulose ingestion.[20, 68, 69] In fact, a separate challenge-test using lactulose has been described to verify that questionable results for the disaccharide malabsorption test may signal a falsenegative test for that sugar during malabsorption tests. If patients do not produce H_2 when lactulose is given, they will also be H_2 non-producers for that sugar, and breath-CH_4 should be checked. Fiedorek and co-workers[4] found an increase in CH_4 production in children with constipation and encopresis, which was reduced by treatment. Other papers have not been published which indicate that CH_4 should be used routinely in addition to H_2 for the measurement of ITT in patients who do not produce H_2 during the measurement of ITT, but it is a likely alterna-

tive, and someone should demonstrate the feasibility of the procedure. It has been reported that from 5-15% of false- negative tests are found in lactose malabsorption tests, and there is no reason to expect otherwise for patients in need of the transit time measurement. It was reported by Cloarac[70] that intestinal transit time measured with lactulose and breath-H_2 testing was different in CH_4-producers than in CH_4-non-producers. The meaning of this is not clear, but may reflect a slower accumulation of H_2 in the colon when it is converted to CH_4.

References

1.   Jorge, J.M.; Wexner, S.D.; Ehrenpreis, E.D. The lactulose hydrogen breath test as a measure of orocaecal transit time. Eur J Surg 1994 (Aug);160(8):409-16

2.   Bovo, P.; Paola Brunori, M.; di Francesco, V.; Frulloni, L.; Montesi, G.; Cavallini, G. The menstrual cycle has no effect on gastrointestinal transit time. Evaluation by means of the lactulose H_2 breath test. Ital J Gastroenterol 1992 (Oct);24(8):449-51

3.   Peters, H.P.; Schep, G.; Koster, D.J.; Douwes, A.C.; de Vries, W.R. Hydrogen breath test as a simple noninvasive method for evaluation of carbohydrate malabsorption during exercise. Eur J Appl Physiol 1994;68(5):435-40

4.   Fiedorek, S. C.; Pumphrey, C. L.; Casteel, H. B. Breath methane production in children with constipation and encopresis. J Pediatr Gastroenterol Nutr 1990 (May); 10(4):473-7

5.   Badiga, M.S.; Jain, N.K.; Casanova, C.; Pitchumoni, C.S. Diarrhea in diabetics; the role of sorbitol. J Am Coll Nutr 1990 (Dec); 9(6):578-82

6.   Jain, N. K.; Patel, V. P.; Pitchumoni, C. S. Sorbitol intolerance in adults. Prevalence and pathogenesis on two continents. J Clin Gastroenterol 1987 (Jun); 9(3):317-9

7.   Szilagyi, A.; Salomon, R.; Smith, B.E.; Martin, M.; Seidman, E. Determinants of prolonged oral cecal transit time during late phase pregnancy: Clin Invest Med 1996 (Feb); 19(1):20-7

8.   Kondo, T.; Liu, F.; Toda, Y. Milk is a useful test meal for measurement of small bowel transit time. J Gastroenterol 1994 Dec;29(6):715-20

9.   Huang, Y. X.; Xu, C. F.; Zhao, G. N. [Small bowel transit time measured with native lactulose breath hydrogen test]. Chung Hua Nei Ko Tsa Chih 1991 (Dec); 30(12):761-3

10.   Sarno, S.; Erasmas, L.P.; Haslbeck, M.; Holzl, R. Orocaecal transit, bacterial overgrowth and hydrogen production in diabetes mellitus. Ital J Gastroenterol 1993 (Nov-Dec);25(9):490-6

11.   Chiarioni, G.; Scattolini, C.; Bonfante, F.; Brentegani, M.T.; Vantini, I. Effect of nifedipine on mouth-to-cecum transit of liquid meal in normal subjects. Dig Dis Sci 1993 (Jun);38(6):1022-5

12.   Baumer, P.; Danays, T.; Lion, L.; Cosnes, J.; Gendre, J. P.; Le Quintrec, Y. Effect of clonidine on oro-cecal transit time in normal man. Ann Gastroenterol Hepatol (Paris) 1989 (Jan); 25(1):5-9

13.   Scott, A.M.; Kellow, J.E.; Eckersley, G.M.; Nolan, J.M.; Jones, M.P. Cigarette smoking and nicotine delay postprandial mouth-cecum transit time. Dig Dis Sci 1992 (Oct);37(10):1544-7

14.   Beaugerie, L.; Burger, A. J.; Cadranel, J. F.; Lamy, P.; Gendre, J. P.; Le Quintrec, Y. Modulation of orocaecal transit time by hypnosis. Gut 1991 Apr; 32(4):393-4

15.   Camboni, G.; Basilisco, G.; Bozzani, A.; Bianchi, P. A. Repeatability of lactulose hydrogen breath test in subjects with normal or prolonged orocecal transit. Dig Dis Sci 1988 (Dec); 33(12):1525-7

16.   Corbett, C. L.; Thomas, S.; Read, N. W.; Hobson, N.; Bergman, I.; Holdsworth, C. D. Electrochemical detector for breath hydrogen determination: measurement of small bowel transit time in normal subjects and patients with the irritable bowel syndrome. Gut 1981 (Oct); 22(10):836-40

17.   Herszenyi, L.; Miskolczi, K.; Tolnay, E.; Szalay, L.; Feher, J. Experience with hydrogen (H2) breath test. Orv Hetil 1992 (Sep 27); 133(39):2483-7

18.   Huppe, D.; Tonissen, R.; Hofius, M.; Kuntz, H. D.; May, B. Effect of chronic alcohol drinking and liver cirrhosis on oro-cecal transit time (H_2 breath test)]. Z Gastroenterol 1989 (Oct); 27(10):624-8

19.   Lautenbacher, S.; Galfe, G.; Haslbeck, M.; H:olzl, R.; Strian, F. Studies on orocecal transit in diabetes mellitus. Med Klin 1990 (May 15); 85(5):297-301

20.   Piccione, P. R.; Holt, P. R.; Culpepper-Morgan, J. A.; Paris, P.; O'Bryan, L.; Ferdinands, L.; Kreek, M. J. Intestinal dysmotility syndromes in the elderly: measurement of orocecal transit time. Am J Gastroenterol 1990 (Feb); 85(2):161-4

21.   Rubinoff, M. J.; Piccione, P. R.; Holt, P. R. Clonidine prolongs human small intestine transit time: use of the lactulose-breath hydrogen test. Am J Gastroenterol 1989 (Apr); 84(4):372-4

22.   Vazquez-Olivencia, W.; Shah, P.; Pitchumoni, C. S. The effect of red and black pepper on orocecal transit time. J Am Coll Nutr 1992 (Apr); 11(2):228-31

23.   Jorge, J.M.; Wexner, S.D.; Ehrenpreis, E.D. The lactulose hydrogen breath test as a measure of orocaecal transit time. Eur J Surg 1994 (Aug); 160(8):409-16

24.   Turberg, Y.; Dederding, J.P. Evaluation of small intestinal motility. Schweiz Med Wochenschr Suppl 1993;54:26-31

25.   La Brooy, S. J.; Male, P. J.; Beavis, A. K.; Misiewicz, J. J. Assessment of the reproducibility of the lactulose H2 breath test as a measure of mouth to caecum transit time. Gut 1983 (Oct);24(10):893-6.

26.   Gelissen, I.C.; Allgood, G.S.; Eastwood, M.A. Reproducibility of the breath hydrogen measurement after a low and high fibre meal. Eur J Clin Nutr 1994 (Apr);48(4):266-72

27.   Diggory, R. T.; Cuschieri, A. The effect of dose and osmolality of lactulose on the oral-caecal transit time determined by the hydrogen breath test and the reproducibility of the test in normal subjects. Ann Clin Res 1985; 17(6):331-3

28.   Read, N. W.; Miles, C. A.; Fisher, D.; Holgate, A. M.; Kime, N. D.; Mitchell, M. A.; Reeve, A. M.; Roche, T. B.; Walker, M. Transit of a meal through the stomach, small intestine, and colon in normal subjects and its role in the pathogenesis of diarrhea. Gastroenterol 1980 Dec; 79(6):1276-82

29.   Meshkinpour, H.; Kemp, C.; Fairshter, R. Effect of aerobic exercise on mouth-to-cecum transit time [see comments]. Gastroenterol 1989 (Mar); 96(3):938-41

30.   Fujisawa, T.; Mulligan, K.; Wada, L.; Schumacher, L.; Riby, J.; Kretchmer, N. The effect of exercise on fructose absorption. Am J Clin Nutr 1993 (Jul); 58(1):75-9

31.   Scott, D.; Scott, B. Should an athlete eat straight after training?—A study of intestinal transit time and its relationship to prior exercise. Br J Sports Med 1994 (Mar);28(1):22-4

32.   Ladas, S. D.; Latoufis, C.; Giannopoulou, H.; Hatziioannou, J.; Raptis, S. A Reproducible lactulose hydrogen breath test as a measure of mouth-to-cecum transit time. Dig Dis Sci 1989 (Jun); 34(6):919-24

33.   Wutzke, K.D.; Heine, W.E.; Plath C.; Leitzmann, P.; Radke, M.; Mohr, C.; Richter, I.; Gulzow, H.U.; Hobusch, D. Evaluation of oro-coecal transit time: a comparison of the lactose-[13C, 15N]ureide 13CO2- and the lactulose H2-breath test in humans. Eur J Clin Nutr 1997 (Jan);51(1):11-9

34.   Miller, M.A.; Parkman, H.P.; Urbain, J.L.; Brown, K.L.; Donahue, D.J.; Knight, L.C.; Maurer, A.H.; Fisher, R.S. Comparison of scintigraphy and lactulose breath hydrogen test for assessment of orocecal transit: lactulose accelerates small bowel transit. Dig Dis Sci 1997 (Jan);42(1):10-8

35.   De Vries, J. J.; Collin, T.; Bijleveld, C. M.; Kleibeuker, J. H.; Vonk, R. J. The use of complex carbohydrates in barley groats for determination of the mouth-to-caecum transit time. Scand J Gastroenterol 1988 (Oct); 23(8):905-12

36.   Wilberg, S.; Pieramico, O.; Malfertheiner, P. The H2-lactulose breath test in the diagnosis of intestinal transit time. Leber Magen Darm 1990 (May); 20(3):129-37

37.   Murphy, M. S.; Nelson, R.; Eastham, E. J. Measurement of small intestinal transit time in children. Acta Paediatr Scand 1988 (Nov); 77(6):802-6

38.   Staniforth, D. H.; Rose, D. Statistical analysis of the lactulose/breath hydrogen test in the measurement of orocaecal transit: its variability and predictive value in assessing drug action. Gut 1989 (Feb); 30(2):171-5

39.   Caride, V. J.; Prokop, E. K.; Troncale, F. J.; Buddoura, W.; Winchenbach, K.; McCallum, R. W. Scintigraphic determination of small intestinal transit time: comparison with the hydrogen breath technique. Gastroenterol 1984 (Apr); 86(4):714-20

40.   Szilagyi, A.; Salomon, R.; Seidman, E. Influence of loperamide on lactose handling and oral-caecal transit time. Alliment Pharmacol Ther 1996 (Oct);10(5);765-70

41.   Chiarioni, G.; Scattolini, C.; Bonfante, F.; Brentegani, M. T.; Vantini, I. Effect of nifedipine on mouth-to-cecum transit of liquid meal in normal subjects. Dig Dis Sci 1993 (Jun); 38(6):1022-5

42.   Marzio, L.; Del Bianco, R.; Donne, M. D.; Pieramico, O.; Cuccurullo, F. Mouth-to-cecum transit time in patients affected by chronic constipation: effect of glucomannan. Am J Gastroenterol 1989 (Aug); 84(8):888-91

43.   Bali, A.; Stableforth, D. E.; Asquith, P. Prolonged small-intestinal transit time in cystic fibrosis. Br Med J [Clin Res] 1983 (Oct 8); 287(6398):1011-3

44.   Dalzell, A. M.; Freestone, N. S.; Billington, D.; Heaf, D. P. Small intestinal permeability and orocaecal transit time in cystic fibrosis. Arch Dis Child 1990 (Jun); 65(6):585-8

45.   Escobar, H.; Perdomo, M.; Vasconez, F.; Camarero, C.; del Olmo, M. T.; Suarez, L. Intestinal permeability to 51Cr-EDTA and orocecal transit time in cystic fibrosis. J Pediatr Gastroenterol Nutr 1992 (Feb); 14(2):204-7

46.   Gotze, H.; Ptok, A. Orocaecal transit time in patients with Crohn disease. Eur J Pediatr 1993 (Mar); 152(3):193-6

47.   Basilisco, G.; Camboni, G.; BozzanY; Vita, P.; Doldi, S.; Bianchi, P. A. Orocecal transit delay in obese patients. Dig Dis Sci 1989 (Apr); 34(4):509-12

48.   Minocha, A.; Katragadda, R.; Rahal, P.S.; Ries, A. Erythromycin shortens orocaecal transit time in diabetic male subjects: a double-blind placebo-controlled study.   Aliment Pharmacol Ther 1995 (Oct);9(5):529-33

49.   Pelli, M.A.; Bassotti, G.; Gattucci, M.; Scionti, L.; Santeusanio, F.; Morelli, A. Hydrogen breath test and scintigraphic gastrocolic transit time in diabetics with autonomic neuropathy. Recenti Prog Med 1993 (Jan);84(1):27-33

50.   Keshavarzian, A.; Iber, F. L. Intestinal transit in insulin-requiring diabetics. Am J Gastroenterol 1986 (Apr); 81(4):257-60

51.   Szilagyi, A.; Salomon, R.; Martin, M.; Fokeeff, K.; Seidman, E. Lactose handling by women with lactose malabsorption is improved during pregnancy. Clin Invest Med 1996 (Dec);19(6):416-26

52.   Lawson, M.; Kern, F. Jr; Everson, G. T. Gastrointestinal transit time in human pregnancy: prolongation in the second and third trimesters followed by postpartum normalization. Gastroenterol 1985 (Nov); 89(5):996-9

53.   Bovo, P.; Paola Brunori, M.; di Francesco, V.; Frulloni, L.; Cavallini, G. The menstrual cycle has no effect on gastrointestinal transit time. Evaluation by means of the lactulose H2 breath test. Ital J Gastroenterol 1992 (Oct); 24(8):449-51

54.   Gorard, D.A.; Gomborone, J.E.; Libby, G.W.; Farthing, M.J. Intestinal transit in anxiety and depression. Gut 1996 Oct;39(4):551-5

55.   Hirakawa, M.; Okada, T.; Iida, M.; Tamai, H.; Kobayashi, N.; Nakagawa, T.; Fujishima, M. Small bowel transit time measured by hydrogen breath test in patients with anorexia nervosa. Dig Dis Sci 1990 (Jun); 35(6):733-6

56.   Bozzani, A.; Camboni, M. G.; Tidone, L.; Cesari, P.; Della Mussia, F.; Quatrini, M.; Ghilardi, G.; Ferrar, L.; Bianchi, P. A. Gastrointestinal transit in hyperthyroid patients before and after propranolol treatment. Am J Gastroenterol 1985 (Jul); 80(7):550-2

57   Schlesinger, D. P.; Rubin, S. I.; Papich, M. G.; Hamilton, D. L. Use of breath hydrogen measurement to evaluate orocecal transit time in cats before and after treatment for hyperthyroidism. Can J Vet Res 1993 (Apr); 57(2):89-94

58.   Wegener, M.; Wedmann, B.; Langhoff, T.; Schaffstein, J.; Adamek, R. Effect of hyperthyroidism on the transit of a caloric solid-liquid meal through the stomach, the small intestine, and the colon in man. J Clin Endocrinol Metab 1992 (Sep); 75(3):745-9

59.   Keshavarzian, A.; Iber, F. L.; Dangleis, M. D.; Cornish, R. Intestinal-transit and lactose intolerance in chronic alcoholics. Am J Clin Nutr 1986 (Jul); 44(1):70-6

60.   Pfeiffer, A.; Hogl, B.; Kaess, H. Effect of ethanol and commonly ingested alcoholic beverages on gastric emptying and gastrointestinal transit. Clin Investig 1992 (Jun); 70(6):487-91

61.   Horowitz, M.; Wishart, J.; Maddox, A.; Russo, A. The effect of chilli on gastrointestinal transit. J Gastroenterol Hepatol 1992 (Jan); 7(1):52-6

62.   Kupfer, R. M.; Heppell, M.; Haggith, J. W.; Bateman, D. N. Gastric emptying and small-bowel transit rate in the elderly.   J Am Geriatr Soc 1985 (May); 33(5):340-3

63.   Beaugerie, L.; Flourie, B.; Lemann, M.; Achour, L.; Franchisseur, C.; Rambaud, J.C. Sorbitol absorption in the healthy human small intestine is increased by the concomitant ingestion of glucose or lipids. Eur J Gastroenterol Hepatol 1995 Feb);7(2):125-8

64.   Wursch, P.; Koellreutter, B.; Schweizer, T.F. Hydrogen excretion after ingestion of five different sugar alcohols and lactulose. Eur J Clin Nutr 1989 (Dec); 43(12):819-25

65.   Yuan, J.; Shen, X.Z.; Zhu, X.S. Effect of berberine on transit time of human small intestine. Chung Kuo Chung Hsi I Chieh Ho Tsa Chih 1994 (Dec); 1412(12):718-20

66.   Cloarac, D.; Bruly Des Varannes, S.; Bizais, Y.; Lehjur, P.A.; Gaimiche, J.P. Reproducability of orocecal transit time and hydrogen production measured by breath tests. Gastroenterol Clin Biol 1992; 16(5):388-94

67.   Pitzalis, G.; Deganello, F.; Mariani, P.; Chiarini-Testa, M.B.; Virgilii, F.; Gasparri, R.; Calvani, L.; Bonamico, M. Lactitol in chronic idiopathic constipation in children. Pediatr Med Chjr 1995 (May-Jun); 17(3):223-6

68.   Bj:rneklett A; Jenssen E. Relationships between hydrogen (H2) and methane (CH4) production in man. Scand J Gastroenterol 1982 (Nov); 17(8): 985-92

69.   Douwes, A.C.; Schaap, C.; van der Klei-wsan Moorsel, J.M. Hydrogen breath test in school children. Arch dis child 1985 (Apr);60:333-7

70.   Cloarac, D.; Bornet, F.; Gouilloud, S.; Barry, J. L.; Salim, B.: Galmiche, J.P. Breath hydrogen response to lactulose in healthy subjects; relationship to methane producing status. Gut 1990 (Mar); 31(3):300-4

10. Protocol for Intestinal Transit Time

Patient Instructions and Preconditions

   a. The patient (subject) should be instructed to not eat high-fiber cereals or other hard-to-digest carbohydrate foods (like beans or coarse-grain bread) the day before the test.

   b. The patient should fast, with only water to drink, for 12 hours before the test is performed.

   c. The patient should not smoke for at least one hour prior to the test, and should not smoke or sleep during the test.

   d. Inquire about any recent antibiotic therapy and/or any recent severe diarrhea, since these condition <u>can</u> prevent detectable changes in breath-H_2 levels during the test and can modify the time-schedule recommended for breath sampling.

Patient Test Protocol

   1. Collect an alveolar air sample and measure the baseline H_2 and CH_4 levels. A fasting baseline value is normally less than 10 ppm H_2 and the normal value for CH_4 is below 6-8 ppm.

   2. Have the patient ingest 0.5g lactulose per kg body weight, up to a maximum dose of 10g lactulose syrup (also known as *Cephulac*, it is a convenient form of lactulose which is available through pharmacies or from QuinTron). Some reports have used 20g in adults, but there is no evidence that the higher dose is significantly more reliable, and it will cause discomfort and severe diarrhea in many patients. It will also hasten transit through the small intestine and shorten ITT.

   3. Beginning 30 minutes after administering the lactulose, collect an alveolar sample and measure the breath-H_2 and breath-CH_4 at 10-minute intervals until either component rises to a level at least 3 ppm higher than the immediately <u>previous</u> level, and meets that criterion for at least three successive time-periods. The test can be terminated after that pattern has been shown, or can be reduced in frequency to a breath-sample every 20 minutes after two hours. The time for the first sustained increase in alveolar H_2 is considered to be the mouth-to-cecum transit time. Ordinarily, the test can be discontinued and considered to be invalid (no H_2 will be produced) if an increase in H_2 is not registered

within five hours in a fasting patient. If there is reason to believe the transit time might be extended beyond that time, the sampling protocol should be modified earlier in the test. These invalid tests can usually be avoided if CH_4 is measured during the test to eliminate those patients who are only CH_4 producers.

Interpretation of Transit Time Measurement

The normal lactulose transit time is considered to be about 75 minutes,[1, 2] though a considerably wide range has been reported by various investigators (see the preceding chapter).

The test will be invalid (with no H_2 response) in those patients or subjects who do not have bacteria in their colon to metabolize the lactulose and form H_2 gas (or in whom the H_2 is transformed into CH_4). These patients will usually have little or no H_2 in the control samples, as well as in the post-lactulose samples. That condition will ordinarily be seen in 5-10% of patients,[3] though higher frequencies have also been reported, as discussed previously. False-negative results based on H_2 can ordinarily be explained on the basis of having no bacteria to produce H_2 or because of the bacterial conversion of the H_2 to CH_4.[4] Use of the Model DP or the Model SC MicroLyzers will detect those patients.

Other factors present may prevent the generation of H_2, including:
a. Recent use of antibiotics which have effectively sterilized the colon;[5]
b. Severe diarrhea which reduces the bacterial density in the colon or rushes the lactulose through with insufficient time for its hydrolysis;[6]
c. An abnormally acid colon which inhibits bacterial production of H_2;[7] and,
d. A metabolic pathway for lactulose digestion which does not include H_2-producing bacteria, e.g., methanogenic[8] or acetogenic or sulfate-reducing bacteria.[9]

An abnormally early appearance of H_2 can be seen if there is bacterial overgrowth, in which bacteria reside in the ileum or jejunum. This will produce an abnormally early peak, followed by a return *toward* the control level (depending on the location of the intestinal invasion) and then a higher, more sustained increase in breath-H_2. The second peak

will be sustained, and will signal the mouth-cecum transit time. Even in the presence of bacterial overgrowth, the measurement of ITT can be made on the basis of the second rise in the H_2 or CH_4 levels.

References

1.   Caride VJ; Prokop EK; Troncale FJ; Buddoura W; Winchenbach K; McCallum RW. Scintigraphic determination of small intestinal transit time: comparison with the hydrogen breath technique. Gastroenterol 1984 (Apr); 86(4): 714-20

2.   Lawson M; Kern F Jr; Everson GT. Gastrointestinal transit time in human pregnancy: prolongation in the second and third trimesters followed by postpartum normalization. Gastroenterol 1985 (Nov); 89(5): 996-9

3.   Bj:rneklett A; Jenssen E. Relationships between hydrogen (H2) and methane (CH4) production in man. Scand J Gastroenterol; 1982 (Nov); 17(8): 985-92

4.   Douwes, A.C.; Schaap, C.; van der Klei-wsan Moorsel, J.M. Hydrogen breath test in school children. Arch dis child 1985 (Apr);60:333-7

5.   Gilat , T.; Ben Hurrr, H.; Gelman-Malachi, E.; Terdiman, R.; Peled, Y. Alterations of the colonic flora and their effect on the hydrogen breath test. Gut 1978 (Jul); 19(7): 602-5

6.   Solomons, N.W.; Garc:ia, R.; Schneider, R.; Viteri, F.E.; von Kaenel, V.A. H2 breath tests during diarrhea. Acta Paediatr Scand 1979 (Mar); 68(2): 171-2

7.   Vogelsang, H.; Ferenci, P.; Frotz, S.; Meryn, S.; Gangl, A. Acidic colonic microclimate—possible reason for false negative hydrogen breath tests. Gut 1988 (Jan); 29(1): 21-6

8.   Strocchi, A.; Levitt, M. D. Factors affecting hydrogen production and consumption by human fecal flora. The critical roles of hydrogen tension and methanogenesis. J Clin Invest 1992 (Apr); 89(4):1304-11

9.   Christl, S. U.; Murgatroyd, P. R.; Gibson, G. R.; Cummings, J. H. Production, metabolism, and excretion of hydrogen in the large intestine. Comment in: Gastroenterol 1992 (Apr);102(4 Pt 1):1424-6

11.   Breath-Tests For Other Sugars

All disaccharides must be hydrolyzed to monosaccharides before they can be absorbed at all. Specific enzymes are required for the hydrolysis of each kind of disaccharide. As you have learned, *lactase* is required for the hydrolysis of lactose to glucose and galactose. Maltose (present in sprouting barley) requires *maltase* for hydrolysis to two glucose molecules before they are absorbed, and *sucrase* (or invertase) splits sucrose (common table sugar) into glucose and fructose. (It is the fructose fragment which gives sucrose its sweeter taste.) Lactulose is used in tests for intestinal transit time and bacterial overgrowth presented earlier because there is no naturally occurring "*lactulase*" to hydrolyze that molecule.

A deficiency of any of the normally occurring enzymes can be demonstrated by applying the same principle as that used for lactase deficiency; detecting the presence of H_2 and/or CH_4 in alveolar air after they are produced by bacterial action in the colon if the sugar avoids hydrolysis and absorption in the small intestine.

The standard test for malabsorption is the same for all disaccharides. In addition, the same principle is used for other sugars, including fructose (a monosaccharide referred to as fruit sugar when it exists alone, and which is a hydrolysis product of sucrose), sorbitol (a sugar alcohol frequently used as a sugar substitute), and d-xylose (a pentose, commonly used to demonstrate a transport deficiency due to mucosal damage).

Monosaccharide malabsorption is caused by problems with the transport mechanism rather than with hydrolytic enzymes. Congenital glucose malabsorption is an uncommon malabsorption syndrome which can also be demonstrated with the same kind of protocol. Other monosaccharides, even though they are simple sugars, may be poorly absorbed. However, tests for their limited absorption capability can be applied in the same way as for disaccharides.

Sucrose Malabsorption

Sucrose is a disaccharide composed of glucose and fructose. It is called cane sugar (or in some areas, beet sugar), reflecting the world-

wide commercial source of the sugar. It is hydrolyzed by the enzyme *sucrase*, an a-glucosidehydrolase which is naturally occurring in the small intestine.

Caspary in 1978[1] demonstrated that an a-glucoside-hydrolase inhibitor completely blocked the postprandial blood glucose rise and increased the breath-H_2 level following the ingestion of sucrose, indicating the production of a drug-induced sucrose malabsorption. These findings were supported by Cauderay, *et al.*, in 1986,[2] who showed that the inhibitors BAY o1248 and Bay m1099 reduced the plasma glucose and fructose responses during the first two hours, and increased late breath-H_2, reflecting the arrival of the non-hydrolyzed sugar in the colon.

A study by Harms, et al.,[3] with children who had inherited sucrase-isomaltase deficiency showed that enzyme substitution with the yeast *Saccharomyces cerevisiae* induced a 70% reduction in H_2 production and reduced or completely prevented symptoms (cramps, diarrhea, bloating, etc.) in those patients. Thus, enzyme-deficient children who accidentally or intentionally ingest sucrose can ameliorate the symptoms by subsequently ingesting a small amount of viable yeast cells.

Ford and Barnes[4] reported that patients with sucrase deficiency had increased symptoms and greater H_2 production when they ingested larger sucrose loads. Perman and co-workers[5] also showed that breath-H_2 tests were valid for the detection of sucrose malabsorption in children. Likewise, Douwes and co-workers[6] reported that sucrase deficiency was detected with breath-H_2 tests, but their finding of only three such patients in a population of 103 children suggested that (contrary to lactase deficiency) the incidence was not high enough to justify the test for a screening test in children.

The Australians, Davidson, Robb and co-workers [7, 8] have demonstrated that breath-H_2 tests detected primary sucrose malabsorption (due to sucrase deficiency) in addition to secondary sucrose malabsorption (due to enteritis or small intestinal bacterial overgrowth). All patients with sucrase deficiency responded to sucrose restriction, and those with bacterial overgrowth were effectively treated with antibiotics. The breath-H_2 test was the most reliable test (better than duodenal biopsy) for predicting clinical response to treatment, with a predictive accuracy of

96%, according to the investigators. Biopsy results gave misleading results in 23% of the cases. False-negative breath-H_2 tests were recorded in 4% of the cases, but were mostly related to recent antimicrobial therapy or deficient mechanics of the test, such as vomiting, improper sampling, etc.

Lembcke and co-workers[9] induced sucrose malabsorption by the administration of the a-glucosidase inhibitor *acarbose* and then demonstrated that metronidazole reduced flatulence and breath-H_2 production after the ingestion of sucrose. The observation suggested that anaerobic bacteria (largely confined to the colon) mediate the production of H_2 seen with sucrose malabsorption.

Yolken, et al.,[10] used breath-H_2 testing to find a strong relationship between disaccharide malabsorption (including sucrose malabsorption) and persistent diarrhea in children infected with HIV. The observation did not find a relationship to microbiologic findings, so the increased incidence of bacterial overgrowth in AIDS patients was probably not responsible, according to these investigators. The genetic background and the ages of the patients were not presented, so judgement about the expected incidence of disaccharide malabsorption was not available.

Sorbitol Malabsorption

Sorbitol is an alcohol-sugar which is used for two kinds of breath-tests. The most common use is to check for sorbitol-induced digestive distress by measuring H_2 and CH_4 in the alveolar air after a challenge-dose of the sugar. The second use of sorbitol is, for economic reasons, as a substitute for lactulose in the measurement of intestinal transit time by breath trace-gas measurements.

It is recognized that diabetic patients frequently consume food with sorbitol as the sweetener. Ingesting as little as 10g of sorbitol will produce osmotic diarrhea in about 80%, and abdominal symptoms in slightly over half, of both normals and diabetics.[11] Jain and Patel[12] reported similar results, with abdominal symptoms found after 10g sorbitol in 30-40% of healthy adults in the US and in India. Most patients who were asked were unaware of the presence of sorbitol as a non-sugar sweetener in food, and very few knew about sorbitol's effect on gastric symptoms. Vernia and co-workers[13] indicated that sorbitol-induced diarrhea

was not a problem with most diabetics until the dose reached 20g, and a moderate dose of 10g of sorbitol was not contraindicated in Type II diabetes.

Sorbitol produced osmotic diarrhea and a considerable increase in breath H_2 and CH_4 when given alone, but did not cause osmotic diarrhea when it was combined with a mixed meal.[14] Beaugeri, *et al.*,[15] reported that sorbitol malabsorption was reduced when it was given with glucose or lipids, but no explanation was offered for the observation. Corazza, *et al.*,[16] evaluated sorbitol ingestion in normal volunteers and patients with coeliac disease. Normals could tolerate low doses of sorbitol, but patients with coeliac disease suffered symptoms from even a 5g dose of sorbitol in a 2% solution. Patients on chronic hemodialysis have an increased incidence of sorbitol malabsorption.[17]

Foods containing sorbitol are popular among diabetics and "weight-watchers." Without careful history-taking and breath-testing for sorbitol malabsorption, these patients could generate an extensive work-up and an erroneous lifelong diagnosis of irritable bowel syndrome.

It would be proper for physicians to point out to patients who use diabetic candy (sweetened with sorbitol) for weight control that their intention of avoiding calories is misguided because, though they avoid glucose, they do not reduce their caloric intake. The break-down products of sorbitol are absorbed from the colon and provide energy similar to that received from an equivalent amount of sucrose.[18]

Excess breath-H_2 was found in virtually all healthy children following the ingestion of pear juice (which contains about 2% sorbitol), in half of the children following apple juice (0.5% sorbitol) and in 25% of those ingesting grape juice (no sorbitol).[19] About half of the children with elevated breath H_2 experienced abdominal cramping and diarrhea. Kneepkens et al.,[20] demonstrated that about 20% of the malabsorption from apple juice was due to sorbitol, the remaining 80% was from fructose. Inquiries about fruit and fruit juice consumption should be a part of the history of all babies and children with abdominal symptoms.

An interaction between the absorption of fructose and sorbitol in patients with functional bowel disease has been reported.[21-23] Giving the sugars separately, or substituting sucrose for fructose eliminated the

malabsorption problem. The investigators reported that the interaction was seen with similar frequency in healthy individuals and in patients with functional bowel disease.

Sorbitol has been recommended as a replacement for the more expensive lactulose used to measure intestinal transit time. Wursch, *et al.*,[24] and Yuan and co-workers[25] demonstrated that the measurement of transit time was not different when sorbitol was substituted for lactulose in the measurement.

References

1.   Caspary, W.F. Sucrose malabsorption in man after ingestion of alphaglucosidehydrolase inhibitor. Lancet; 1978 (Jun) 10; 1(8076): 1231-3

2.   Cauderay, M.; Tappy, L.; Temler, E.; J:equier, E.; Hillebrand, .I; Felberm, J.P. Effect of alphaglycohydrolase inhibitors (Bay m1099 and Bay- o1248) on sucrose metabolism in normal men. Metabolism; 1986 (May); 35(5): 472-7

3.   Harms, H.K.; Bertele Harms, R.M.; Bruer-Kleis, D. Enzyme substitution therapy with the yeast Saccharomyces cerevisiae in congenital sucrase isomaltase deficiency. N Engl J Med; 1987 (May 21); 316(21): 1306-9

4.   Ford, R.P.; Barnes, G.L. Breath hydrogen test and sucrase isomaltase deficiency. Arch Dis Child; 1983 (Aug); 58(8): 595-597

5.   Perman, J.A.; Barr, R.G.; Watkins, J.B. Sucrose malabsorption in children: noninvasive diagnosis by interval breath hydrogen determination. J. Pediatr; 1978 (Jul); 93(1): 17-22

6.   Douwes, A.C.; Fernandes, J.; Jongbloed, A.A. Diagnostic value of sucrose tolerance test in children evaluated by breath hydrogen measurement. Acta Paediatr Scand; 1980 (Jan); 69(1): 79-82

7.   Davidson GP; Robb TA. Detection of primary and secondary sucrose malabsorption in children by means of the breath hydrogen technique. Med J Aust; 1983 (Jul 9);2(1): 29-32

8.   Davidson, G.P.; Robb, T.A.; Kirubakaran, C.P. Bacterial contamination of the small intestine as an important cause of chronic diarrhea and abdominal pain: diagnosis by breath hydrogen test. Pediatrics; 1984 (Aug); 74(2): 229-35

9.   Lembcke, B.; F:olsch, U.R.; Caspary, W.F.; Ebert, R.; Creutzfeldt, W. Influence of metronidazole on the breath hydrogen response and symptoms in acarbose-induced malabsorption of sucrose. Digestion; 1982; 25(3): 186-93

10. Yolken, R.H.; Hart, W.; Oung, I.; Shiff, C.; Greenson, J.; Perman, J.A. Gastrointestinal dysfunction and disaccharide intolerance in children infected with human immunodeficiency virus. J Pediatr; 1991 (Mar); 118(3): 359-63

11. Badiga, M.S.; Jain, N.K.; Casanova, C.; Pitchumoni, C.S. Diarrhea in diabetics: the role of sorbitol. J Am Coll Nutr; 1990 (Dec); 9(6): 578-82

12. Jain, N.K.; Patel, V.P.; Pitchumoni, C.S. Sorbitol intolerance in adults. Prevalence and pathogenesis on two continents. J Clin Gastroenterol; 1987 (Jun); 9(3): 317-9

13. Vernia, P.; Frandina, C.; Bilotta, T.; Ricciardi, M.R.; Villotti, G.; Fallucca, F. Sorbitol malabsorption and nonspecific abdominal symnptoms in type II diabetes. Metabolism. 1995(Jun); 44(6):796-9

14. Vaaler, S.; Bj:rneklett, A.; Jelling, I.; Skrede, G.; Hanssen, K.F.; Fausa, O, ; Aagenaes, O. Sorbitol as a sweetener in the diet of insulin-dependent diabetes. Acta Med Scand; 987; 221(2): 165-70

15. Beaugeri, L.; Flourie, B.; Lemann, M.; Achour, L.; Franchisseur, C.; Rambaud, J.C. Sorbitol absorption in the healthy human small intestine is increased by the concomitant ingestion of glucose or lipids. Eur J Gasgroenterol Hepatol. 1995 (Feb);7(2):125-8

16. Corazza, G.R.; Strocchi, A.; Rossim R.; Sirolam D.; Gasbarrini, G. Sorbitol malabsorption in normal volunteers and in patients with coeliac disease. Gut; 1988 (Jan); 29(1): 44-8

17. Coyne, M.J.; Rodriguez, H. Carbohydrate malabsorption in black and Hispanic dialysis patients. Am J Gastroenterol; 1986 (Aug); 81(8): 662-5

18. Beaugerie, L.; Flourie, B.; Marteau, P.; Pellier, P.; Franchisseur, C.; Rambaud, J.C. Digestion and absorption in the human intestine of three sugar alcohols. Gastroenterol 1990 (Sep);00(3):717-23

19. Hyams, J.S.; Etienne, N.L.; Leichtner, A.M.; Theuer, R.C. Carbohydrate malabsorption following fruit juice ingestion in young children. Pediatrics; 1988 (Jul); 82(1): 64-8

20. Kneepkens, C.M.; Jakobs, C.; Douwes, A.C. Apple juice, fructose, and chronic nonspecific diarrhoea. Eur J Pediatr; 1989 (Apr); 148(6): 571-3

21. Rumessen, J.J.; Gudmand-H:yer, E. Malabsorption of fructose-sorbitol mixtures. Interactions causing abdominal distress. Scand J Gastroenterol; 1987; 22(4): 431-6

22. Rumessen, J.J.; Gudmand-H:yer, E. Functional bowel disease: malabsorption and abdominal distress after ingestion of fructose, sorbitol, and fructose-sorbitol mixtures. Gastroenterology; 1988 (Sep); 95(3): 694-700

23. Nelis, G.F.; Vermeeren, M.A.; Jansen, W. Role of fructose-sorbitol malabsorption in the irritable bowel syndrome. Gastroenterology; 1990 (Oct); 99(4): 1016-20

24. Wursch, P; Koellreutter B; Schweizer TF. Hydrogen excretion after ingestion of five different sugar alcohols and lactulose. Eur J Clin Nutr; 1989 (Dec).; 43(12):819-25

25. Yuan. J.; Shen, X.Z.; Zhu, X.S. Effect of berberine on transit time of human small intestine. Chung Kuo Chung His I Chieh Ho Tsa Chih. 1994 (Dec); 14(12:718-20

26. Douwes, A.C.; van Caillie, M.; Fernandes, J.; Bijleveld, C.M.; Desjeux, J, F. Interval breath hydrogen test in glucose-galactose malabsorption. Eur J Pediatr; 1981 (Nov); 137(3): 273-6

27. Grasset, E.; Evans, L.A.; Dumontier, A.M.; Heyman, M.; Faverge, B.; Beau, J.P.; Desjeux, J.F. [Intestinal malabsorption of glucose and galactose. Study of a family]. Arch Fr Pediatr; 1982 (Dec); 39 Suppl 2: 729-33

28. Kneepkens, C.M.; Vonk, R.J.; Fernandes, J. Incomplete intestinal absorption of fructose. Arch Dis Child; 1984 (Aug); 59(8): 735-738

29. Kneepkens, C.M.; Jakobs, C.; Douwes, A.C. Apple juice, fructose, and chronic nonspecific diarrhoea. Eur J Pediatr; 1989 (Apr); 148(6): 571-3

30. Rumessen, J.J.; Gudmand-H:yer, E. Absorption capacity of fructose in healthy adults. Comparison with sucrose and its constituent monosaccharides. Gut; 1986 (Oct); 27(10): 1161-8

31. Truswell, A.S.; Seach, J.M.; Thorburn, A.W. Incomplete absorption of pure fructose in healthy subjects and the facilitating effect of glucose. Am J Clin Nutr; 1988 (Dec); 48(6): 1424-30

32. Rumessen, J.J. Fructose and related foodcarbohydratesl Sources, intake, absorption and clinical implications. Scand J Gastroenterol. 1992 (Oct); 27(10)819-28

33. Ravich, W.J.; Bayless, T.M.; Thomas, M. Fructose: incomplete intestinal absorption in humans. Gastroenterology; 1983 (Jan); 84(1): 26-9

34. Ladas, S.D.; Haritos, D.N.; Raptis, S.A. Honey may have a laxative effect on normal subjects because of incomplete fructose absorption. Am J Clin Nutr 1995 (Dec);62(6):1212-5

35. Rumessen, J.J.; Gudmand-H:yer, E. Functional bowel disease: malabsorption and abdominal distress after ingestion of fructose, sorbitol, and fructose-sorbitol mixtures. Gastroenterology; 1988 (Sep); 95(3): 694-700

36. Rumessen, J.J.; Gudmand-H:yer, E. Malabsorption of fructose-sorbitol mixtures. Interactions causing abdominal distress. Scand J Gastroenterol; 1987 (May); 22(4): 431-6

37. Lembcke, B.; Bornholdt, C.; Kirchhoff, S.; Lankisch, P.G. Clinical evaluation of a 25 g D-xylose hydrogen (H2) breath test. Z Gastroenterol; 1990 (Oct); 28(10): 555-60

38. Levine, J.J.; Seidman, E.; Walker, W.A. Screening tests for enteropathy in children. Am J Dis Child; 1987 (Apr); 141(4): 435-8

39. Akesson, B.; Flor:en, C.H.; Olsson, S.A. Intestinal xylose degradation and bile acid deconjugation in patients with continent or conventional ileostomy. Acta Chir Scand; 1987 (Mar); 153(3): 221-3

40. King, C.E.; Toskes, P.P. Comparison of the 1-gram [14C]xylose, 10-gram lactulose-H2, and 80-gram glucose-H2 breath tests in patients with small intestine bacterial overgrowth. Gastroenterology; 1986 (Dec); 91(6): 1447-51

41. McKay, L.F.; Brydon, W.G.; Eastwood, M.A.; Smith, J.H. The influence of pentose on breath methane. Am J Clin Nutr; 1981 (Dec); 34(12): 2728-33

42. Bjrnecklett, A.; Jenssen, E. Relationships between hydrogen (H_2) and methane (CH_4) production in man. Scand J Gastroenterol. 1982 (Nov); 17(8):985-92

12.   Monosaccharide Malabsorption

The malabsorption of monosaccharides is not common. Although malabsorption can result from a failure to make the sugar "absorbable," that does not apply to monosaccharides. Monosaccharide malabsorption results from a failure in the system which transports the sugar across the cell membranes and out of the small intestine. It is included in this monograph because the defect is demonstrated with the breath-H_2 test in the same manner as for disaccharides.

Glucose Malabsorption

A congenital defect in glucose-galactose transport is uncommon but not rare. Dowes and co-workers[26] demonstrated an increase in breath-H_2 in an infant with severe diarrhea after breast-feeding. Grasset, et al.,[27] also demonstrated elevated breath-H_2 after the administration of a small glucose-galactose dose (0.5g/kg body weight) in a girl with congenital malabsorption. In both cases, no H_2 was generated after the ingestion of up to 2g/kg of another monosaccharide, fructose, which indicated that the problem was related specifically to the transport of glucose. Galactose is transported by the same pathway, which explains why the two monosaccharides were involved in the absorption defect.

Fructose Malabsorption

Studies have shown that children have a limited absorptive capacity for fructose, even though they are encouraged to include many fruits in their diets. This is due to a limitation in the fructose carrier system which transports that sugar across the cell membranes. It has been demonstrated that the simultaneous administration of glucose or galactose with fructose *decreased* the production of H_2,[28, 29] which is interpreted to mean that the two monosaccharides enhanced fructose absorption, presumably by activating the fructose carrier. Apple juice contains fructose in excess of its glucose concentration,[29] which may explain the abdominal symptoms in children who are more sensitive than average to apples or apple juice. Sucrose is a disaccharide composed of glucose and fructose. Rumessen and Gudmand-Hyer[30] and Truswell and co-workers[31] showed that when the fructose was given in the form of sucrose, its absorption capacity was increased.

Rumessen[32] pointed out that studies of the absorption of fructose in animals are inconsistent, with the mechanism being species-dependent. In most species, fructose uptake takes place by a specific carrier through facilitated transport. There is no evidence for active transport of fructose in the human intestine. This area of research should be extended with more studies of the interaction between fructose and other sugars.

Fructose is an increasingly more common commercial sweetener, even though it is commonly malabsorbed in adults, and even though they completely absorb larger amounts of sucrose (of which fructose is a major component released by hydrolysis) without a problem.[33] This trend of using more fructose in commercial foods may be related to being able to label products as "sugar free," or "no sugar added." Symptoms of malabsorption are common in adults after an ordinary dose of honey,[34] which contains fructose in excess of glucose.

In a study of 25 patients with functional bowel disease (a transport problem rather than a hydrolysis problem), Rumessen and Gudmand-Hyer[35] found one patient who was not an H_2-producer. Thirteen of the patients increased H_2 production after fructose, sorbitol or a fructose-sorbitol mixture. No patient produced H_2 after 50g sucrose. A mixture of 5g sorbitol with 25g fructose caused significantly increased symptoms, and more than additive malabsorption was found.[36] The study suggested that separate entities may be defined for functional bowel disease, and breath-tests may assist in the dietary guidance given to the patients.

Xylose (Pentose) Malabsorption

The 5-carbon sugar, xylose, is used in a screening test for enteropathy, in which its malabsorption indicates a deficit in intestinal transport capability. If xylose is not absorbed, it will move to the colon and the production of H_2 may signal its malabsorption. The failure to produce H_2 in response to xylose administration in 12% of the subjects was considered to be a deficiency of the test.[37] Levine, *et al.*,[38] drew a similar conclusion about the reliability of the breath-H_2 test based on xylose.

Xylose is also used in a ^{14}C test for bacterial overgrowth, in which the labeled sugar is metabolized by bacteria which invade the small intestine[39] to release the labeled CO_2. King and Toskes[40] indicated that the ^{14}C xylose test was more reliable than the H_2 breath-test. **These studies should be repeated with CH_4 included, since McKay and co-workers[41] reported that xylose led to an increase in breath CH_4 in methane producers, though the frequency with which such methane producers were found in the population was not reported. It is reported that 8-15% of subjects produce CH_4 instead of H_2 when sugars reach the colon, as referenced earlier, but most of them produce CH_4 instead.[42]** This incidence is high enough to account for the reported non-specificity of the test.

General Guidelines for Protocols

Prepare the challenge-dose of sugar. Ordinarily, 1g per kg body weight of the patient is dissolved in 6-8 oz. water. The maximal dose is usually 25g or 50g for lactose and 10g or 20g for lactulose. Other sugars may require a slightly different dose for the standard test. A smaller dose of 0.25-0.50g/kg body weight is standard to establish the presence of congenital glucose malabsorption or to demonstrate sorbitol malabsorption, while 2g/kg is more commonly used as a challenge-dose for sucrose.

For any of the tests, the standard sugar-dose should be dissolved in at least 6-8 oz. of water, to be sure the volume is large enough to stimulate emptying of the stomach when the solution is ingested. A *proportionately* larger volume of water (perhaps twice as much) should be used with neonates, to provide greater dilution of the sugar, because a higher concentration of sugar in the intestines may cause an osmotic shift of water, resulting in a reaction similar to the "dumping syndrome."

Heating the water to make the sugar dissolve more easily is a standard part of most test procedures. Disaccharides are not easily dissolved in cold water but are readily dissolved in warm or hot (but not boiling) water. Cool the solution in a refrigerator before giving it to the patient.

At the beginning of any breath-test, measure baseline H_2 and/or CH_4 levels in an alveolar air sample from the patient after an over-night

fast. The change in H_2 and/or CH_4 levels from the baseline is used to interpret the response. In some patients, the values may drop slightly after the baseline values are measured. In those cases, compare the response to the lowest value registered before the concentrations begin to rise. The simplest way to obtain a reliable alveolar sample is to use a QuinTron GaSampler or AlveoSampler system for adults and older children, or one of the collecting systems developed especially for neonates to collect a valid breath sample from babies or others who can not follow instructions.

When the prepared sugar solution is ingested by the patient, start a timer. To measure malabsorption based on passage of the non-hydrolyzed sugar to the colon, analyze air samples at 30-minute intervals for up to three hours after ingesting the challenge-dose. For bacterial overgrowth, samples should be collected every 20 minutes; for intestinal transit time sampling at 10- or 15-minute intervals (after a 30-minute wait at the beginning) will provide a more reliable measure of the appearance time for H_2 in the sample.

If a Model SC MicroLyzer is used so CO_2 is measured, the collection procedure is not critical because a correction factor based on the dilution of alveolar CO_2 is calculated and applied to the measured H_2 and CH_4 values (refer to the section on normalizing breath-gas measurement for a more complete explanation). Collect an expired or alveolar air sample by any available technique and apply the CO_2 correction factor calculated by the instrument. Use the same procedure for calculating the correction factor for all samples analyzed in the test.

Interpreting the Gas Analysis Results

For disaccharide malabsorption, an increase of at least 20 ppm H_2 from its lowest value will be interpreted as malabsorption. If CH_4 participates in the response, when the value for combined H_2 and CH_4 changes increase by 15 or more ppm from the lowest value measured, the test can generally be considered to be positive - the sugar has been malabsorbed. Under some conditions, primarily guided by the signs and symptoms given by the patient, slightly less but clearly significant increases can be interpreted as positive. In general, the greater the increase in the sum of the gas concentrations, the more severe is the malabsorption, but that relationship is only generally semi-quantitative.

Do not rely on absolute values alone, but use the analytical data along with the physical history presented by the patient, a dietary review and symptoms exhibited by the patient during the test procedure.

References

1.   Caspary, W.F. Sucrose malabsorption in man after ingestion of alphaglucosidehydrolase inhibitor. Lancet; 1978 (Jun) 10; 1(8076): 1231-3

2.   Cauderay, M.; Tappy, L.; Temler, E.; J:equier, E.; Hillebrand, .I; Felberm, J.P. Effect of alphaglycohydrolase inhibitors (Bay m1099 and Bay- o1248) on sucrose metabolism in normal men. Metabolism; 1986 (May); 35(5): 472-7

3.   Harms, H.K.; Bertele Harms, R.M.; Bruer-Kleis, D. Enzyme substitution therapy with the yeast Saccharomyces cerevisiae in congenital sucrase isomaltase deficiency. N Engl J Med; 1987 (May 21); 316(21): 1306-9

4.   Ford, R.P.; Barnes, G.L. Breath hydrogen test and sucrase isomaltase deficiency. Arch Dis Child; 1983 (Aug); 58(8): 595-597

5.   Perman, J.A.; Barr, R.G.; Watkins, J.B. Sucrose malabsorption in children: noninvasive diagnosis by interval breath hydrogen determination. J. Pediatr; 1978 (Jul); 93(1): 17-22

6.   Douwes, A.C.; Fernandes, J.; Jongbloed, A.A. Diagnostic value of sucrose tolerance test in children evaluated by breath hydrogen measurement. Acta Paediatr Scand; 1980 (Jan); 69(1): 79-82

7.   Davidson GP; Robb TA. Detection of primary and secondary sucrose malabsorption in children by means of the breath hydrogen technique. Med J Aust; 1983 (Jul 9);2(1): 29-32

8.   Davidson, G.P.; Robb, T.A.; Kirubakaran, C.P. Bacterial contamination of the small intestine as an important cause of chronic diarrhea and abdominal pain: diagnosis by breath hydrogen test. Pediatrics; 1984 (Aug); 74(2): 229-35

9.   Lembcke, B.; F:olsch, U.R.; Caspary, W.F.; Ebert, R.; Creutzfeldt, W. Influence of metronidazole on the breath hydrogen response and symptoms in acarbose-induced malabsorption of sucrose. Digestion; 1982; 25(3): 186-93

10.   Yolken, R.H.; Hart, W.; Oung, I.; Shiff, C.; Greenson, J.; Perman, J.A. Gastrointestinal dysfunction and disaccharide intolerance in children infected with human immunodeficiency virus. J Pediatr; 1991 (Mar); 118(3): 359-63

11.   Badiga, M.S.; Jain, N.K.; Casanova, C.; Pitchumoni, C.S. Diarrhea in diabetics: the role of sorbitol. J Am Coll Nutr; 1990 (Dec); 9(6): 578-82

12.   Jain, N.K.; Patel, V.P.; Pitchumoni, C.S. Sorbitol intolerance in adults. Prevalence and pathogenesis on two continents. J Clin Gastroenterol; 1987 (Jun); 9(3): 317-9

13.   Vernia, P.; Frandina, C.; Bilotta, T.; Ricciardi, M.R.; Villotti, G.; Fallucca, F. Sorbitol malabsorption and nonspecific abdominal symnptoms in type II diabetes. Metabolism. 1995(Jun); 44(6):796-9

14. Vaaler, S.; Bj:rneklett, A.; Jelling, I.; Skrede, G.; Hanssen, K.F.; Fausa, O, ; Aagenaes, O. Sorbitol as a sweetener in the diet of insulin-dependent diabetes. Acta Med Scand; 987; 221(2): 165-70

15. Beaugeri, L.; Flourie, B.; Lemann, M.; Achour, L.; Franchisseur, C.; Rambaud, J.C. Sorbitol absorption in the healthy human small intestine is increased by the concomitant ingestion of glucose or lipids. Eur J Gasgroenterol Hepatol. 1995 (Feb);7(2):125-8

16. Corazza, G.R.; Strocchi, A.; Rossim R.; Sirolam D.; Gasbarrini, G. Sorbitol malabsorption in normal volunteers and in patients with coeliac disease. Gut; 1988 (Jan); 29(1): 44-8

17. Coyne, M.J.; Rodriguez, H. Carbohydrate malabsorption in black and Hispanic dialysis patients. Am J Gastroenterol; 1986 (Aug); 81(8): 662-5

18. Beaugerie, L.; Flourie, B.; Marteau, P.; Pellier, P.; Franchisseur, C.; Rambaud, J.C. Digestion and absorption in the human intestine of three sugar alcohols. Gastroenterol 1990 (Sep);00(3):717-23

19. Hyams, J.S.; Etienne, N.L.; Leichtner, A.M.; Theuer, R.C. Carbohydrate malabsorption following fruit juice ingestion in young children. Pediatrics; 1988 (Jul); 82(1): 64-8

20. Kneepkens, C.M.; Jakobs, C.; Douwes, A.C. Apple juice, fructose, and chronic nonspecific diarrhoea. Eur J Pediatr; 1989 (Apr); 148(6): 571-3

21. Rumessen, J.J.; Gudmand-H:yer, E. Malabsorption of fructose-sorbitol mixtures. Interactions causing abdominal distress. Scand J Gastroenterol; 1987; 22(4): 431-6

22. Rumessen, J.J.; Gudmand-H:yer, E. Functional bowel disease: malabsorption and abdominal distress after ingestion of fructose, sorbitol, and fructose-sorbitol mixtures. Gastroenterology; 1988 (Sep); 95(3): 694-700

23. Nelis, G.F.; Vermeeren, M.A.; Jansen, W. Role of fructose-sorbitol malabsorption in the irritable bowel syndrome. Gastroenterology; 1990 (Oct); 99(4): 1016-20

24. Wursch, P; Koellreutter B; Schweizer TF. Hydrogen excretion after ingestion of five different sugar alcohols and lactulose. Eur J Clin Nutr; 1989 (Dec).; 43(12):819-25

25. Yuan. J.; Shen, X.Z.; Zhu, X.S. Effect of berberine on transit time of human small intestine. Chung Kuo Chung His I Chieh Ho Tsa Chih. 1994 (Dec); 14(12:718-20

26. Douwes, A.C.; van Caillie, M.; Fernandes, J.; Bijleveld, C.M.; Desjeux, J, F. Interval breath hydrogen test in glucose-galactose malabsorption. Eur J Pediatr; 1981 (Nov); 137(3): 273-6

27. Grasset, E.; Evans, L.A.; Dumontier, A.M.; Heyman, M.; Faverge, B.; Beau, J.P.; Desjeux, J.F. [Intestinal malabsorption of glucose and galactose. Study of a family]. Arch Fr Pediatr; 1982 (Dec); 39 Suppl 2: 729-33

28. Kneepkens, C.M.; Vonk, R.J.; Fernandes, J. Incomplete intestinal absorption of fructose. Arch Dis Child; 1984 (Aug); 59(8): 735-738

29. Kneepkens, C.M.; Jakobs, C.; Douwes, A.C. Apple juice, fructose, and chronic nonspecific diarrhoea. Eur J Pediatr; 1989 (Apr); 148(6): 571-3

30.   Rumessen, J.J.; Gudmand-H:yer, E. Absorption capacity of fructose in healthy adults. Comparison with sucrose and its constituent monosaccharides. Gut; 1986 (Oct); 27(10): 1161-8

31.   Truswell, A.S.; Seach, J.M.; Thorburn, A.W. Incomplete absorption of pure fructose in healthy subjects and the facilitating effect of glucose. Am J Clin Nutr; 1988 (Dec); 48(6): 1424-30

32.   Rumessen, J.J. Fructose and related food carbohydrates: Sources, intake, absorption and clinical implications. Scand J Gastroenterol. 1992 (Oct); 27(10)819-28

33.   Ravich, W.J.; Bayless, T.M.; Thomas, M. Fructose: incomplete intestinal absorption in humans. Gastroenterology; 1983 (Jan); 84(1): 26-9

34.   Ladas, S.D.; Haritos, D.N.; Raptis, S.A.   Honey may have a laxative effect on normal subjects because of incomplete fructose absorption. Am J Clin Nutr 1995 (Dec);62(6):1212-5

35.   Rumessen, J.J.; Gudmand-H:yer, E. Functional bowel disease: malabsorption and abdominal distress after ingestion of fructose, sorbitol, and fructose-sorbitol mixtures. Gastroenterology; 1988 (Sep); 95(3): 694-700

36.   Rumessen, J.J.; Gudmand-H:yer, E. Malabsorption of fructose-sorbitol mixtures. Interactions causing abdominal distress. Scand J Gastroenterol; 1987 (May); 22(4): 431-6

37.   Lembcke, B.; Bornholdt, C.; Kirchhoff, S.; Lankisch, P.G. Clinical evaluation of a 25 g D-xylose hydrogen (H2) breath test. Z Gastroenterol; 1990 (Oct); 28(10): 555-60

38.   Levine, J.J.; Seidman, E.; Walker, W.A. Screening tests for enteropathy in children. Am J Dis Child; 1987 (Apr); 141(4): 435-8

39.   Akesson, B.; Flor:en, C.H.; Olsson, S.A. Intestinal xylose degradation and bile acid deconjugation in patients with continent or conventional ileostomy. Acta Chir Scand; 1987 (Mar); 153(3): 221-3

40.   King, C.E.; Toskes, P.P. Comparison of the 1-gram [14C]xylose, 10-gram lactulose-H2, and 80-gram glucose-H2 breath tests in patients with small intestine bacterial overgrowth. Gastroenterology; 1986 (Dec); 91(6): 1447-51

41.   McKay, L.F.; Brydon, W.G.; Eastwood, M.A.; Smith, J.H. The influence of pentose on breath methane. Am J Clin Nutr; 1981 (Dec); 34(12): 2728-33

42.   Bjrnecklett, A.; Jenssen, E. Relationships between hydrogen (H_2) and methane (CH_4) production in man. Scand J Gastroenterol. 12:985, 1982

13. What about H. Pylori and Ulcers?

About 20 million Americans develop at least one ulcer during their lifetime. Each year ulcers affect about 4 million people, with about 1% having surgery because of persistent symptoms or problems from ulcers, and about 6, 000 people dying because of ulcer-related complications. Ulcers can develop at any age, but they are rare among teenagers and even more uncommon in children. Usually, duodenal ulcers occur for the first time between the ages of 30 and 50 years, but stomach ulcers are more likely to develop after the age of 60. *Stomach* ulcers develop more often in *women* than men, but *duodenal* ulcers occur more frequently in *men* than women, for reasons which are not clear.

Helicobacter pylori (H pylori) is a bacterium which causes chronic inflammation (gastritis) of the inner lining of the stomach. It was first described as *Campylobacter pylori* in 1982 by Australian investigators Barry Marshall and Robin Warren, who proposed it to be the underlying cause of gastric and peptic ulcers. The bacteria was later properly identified as *Helicobacter pylori (H pylori)* and (against strong opposition) they promoted the concept of an infective agent causing gastric ulcers. Marshall and Warren came to this conclusion because in their studies 80 percent of patients with stomach ulcers and virtually all patients with duodenal ulcers had the bacteria. The 20 percent of patients with stomach ulcers who did not have H pylori were those who had taken NSAIDs (non-steroidal anti-inflammatory drugs) such as aspirin and prostaglandin inhibitors, which are common causes of stomach ulcers. Their convictions were strong enough to drive them to infect themselves with H pylori, and they developed the gastritis they described in patients. Even this evidence did not resolve the question, since many physicians believed it was an iatrogenic disease (physician-inflicted during the process of obtaining the biopsy sample for examination). It took great effort to gain the support of other investigators who followed the lead of Marshall and Warren in carefully investigating this off-the-wall explanation of ulcers, and who demonstrated its widespread presence and high incidence of infection among patients with stomach or duodenal ulcers.

Evidence linking H pylori to ulcers mounted over the next 10 years as numerous studies from around the world confirmed the presence of

H pylori in most people with ulcers. Moreover, careful investigators proved that using antibiotics to eliminate H pylori healed ulcers and prevented their recurrence in a majority of cases. It was difficult for gastroenterologists to accept this new concept and discard the old methods of treating ulcers, which had been based for over a century on the concept that they were due to life-style, stress and diet.

H pylori infection is thought to be acquired by ingesting contaminated food and water, and through person-to-person contact, though its contagion has not been established. The infection is more common in crowded living conditions with poor sanitation. In countries with poor sanitation, perhaps 90% of the adult population will be infected. Thirty percent of the adult population in the United States are infected with H pylori, which increases to 50% by age 60 years. One out of six patients with H pylori infection will develop ulcers of the stomach or duodenum. Most individuals seem to be infected during childhood, and their infection probably lasts a lifetime unless effectively eradicated with antibiotic treatment, though there is some evidence that a natural resistance to the infection can be developed, since some people spontaneously lose their ulcers and no longer test positive for H pylori. Most people will never have symptoms or problems related to the infection and studies have not yet explained why only certain people develop H pylori-related symptoms or ulcers. Perhaps hereditary or environmental factors (such as stress) cause some individuals to develop problems. Alternatively, ulcers may result from infection with more virulent strains of bacteria.

H pylori survives in the stomach wall because the bacteria can penetrate the stomach's protective mucous lining and survive in a micro-climate with a higher pH than that of the stomach contents. That is because the bacterium produces the enzyme *urease*. Urease generates ammonia (NH_3) and CO_2 from urea (commonly found in meat). The CO2 diffuses away and the NH_3 neutralizes the stomach's acid around the capsule of the bacterium, enabling it to survive.

The presence of the bacteria causes the ulcers to develop, but the factors involved in ulcer development are not totally clear. Evidence suggests that they are not caused directly by the NH_3 which is generated,[1, 2] though there are data which relate the gastric injury to the ammonium production.[3, 4] Epidemiological studies have shown that a large proportion of healthy people have antibodies against H pylori,[5] suggest-

ing that some people have spontaneously eliminated the organism. Graham and co-workers[6] showed that the incidence of infection was higher than the incidence of ulcers, so many infected people do not develop ulcers. Debongnie, et al. ,[7] showed that the number (concentration) of bacteria is not a determining factor for the onset of ulceration in infected patients. An explanation for these and other similar observations has not yet been presented.

Conventional approaches to ulcer diagnosis generally require invasive procedures. An upper GI series involves taking an x-ray of the esophagus, stomach, and duodenum to locate an ulcer after the patient swallows a chalky barium-liquid to make the ulcer visible by x-ray. An alternative diagnostic test involves endoscopy, during which the patient is lightly sedated and a small flexible instrument with a camera on the end is inserted through the nose or mouth into the esophagus, stomach, and duodenum. If the doctor performs an endoscopy, multiple samples of stomach tissue can be obtained and one of several tests can be performed on the tissue.

Early tests depended on culturing the bacteria from gastric biopsy specimens or measuring the pH shift induced by NH_3 generated from a biopsy specimen exposed to urea.[8] Histology, which visualizes the bacteria under the microscope, and culture, which involves incubating the tissue and watching for growth of H pylori organisms, can also be applied.

Blood tests such as the enzyme-linked immunosorbent assay (ELISA) identify and measure H pylori antibodies.[9-10] The body produces antibodies against H pylori in an attempt to fight the bacteria. The advantages of blood tests are their low cost and availability to doctors. The disadvantage is the possibility of false-positive results in patients previously treated for ulcers since the levels of H pylori antibodies fall slowly after the eradication of H pylori.

Currently approved breath-tests measure isotopes of CO_2 in exhaled breath after it is released from specially-labeled urea.[1-16] Patients drink a solution containing urea which has been labeled with isotopic carbon. The H pylori bacteria break down the urea to generate CO_2 which is absorbed into the blood stream and exhaled in the breath. Breath samples are collected after either 10 or 20 minutes following ingestion of

the urea and analyzed to determine if some of the CO_2 came from the labeled urea.

Urea breath-tests are at least 90% accurate for detecting the presence of H pylori and are particularly suitable for follow-up treatment to see if bacteria have been eradicated.

Two isotopes of carbon have been used for the H pylori breath-test. Each has significant disadvantages: (a) $^{13}CO_2$ is a stable, heavy isotope of carbon and is safe to use, but it requires expensive analytical instrumentation (a mass spectrometer, at least at the present time); and (b) $^{14}CO_2$ can be detected and measured with less expensive equipment, but it is a radio-isotope and can not be used with women of child-bearing potential or with children, so much of the market is not available. Both instruments are considered to be beyond the scope of most gastroenterology laboratories, so the test is, by necessity, farmed out to other Service Laboratories.

Because the diagnosis of H pylori infection is currently based on measuring isotopes of CO_2 which require special instrumentation for their detection, the QuinTron MicroLyzers cannot be applied to this important diagnostic breath-test.

Whether the bacteria are active or latent is not determined from the breath-test. The National Digestive Diseases Information Clearinghouse does not recommend treating an H pylori infection which is inactive (not causing ulcer symptoms), since the unnecessary use of antibiotics is discouraged.

References

1.   Graham, D.Y.; Go, M.F.; Evans, D.J., Jr. Review article: urease, gastric ammonium/ammonia, and Helicobacter pylori—the past, the present, and recommendations for future research. Aliment Pharmacol Ther 1992 Dec;6(6):659-69

2.   el Nujumi, A.M.; Rowe, P.A.; Dahill, S.; Dorrian, C.A.; Neithercut, W.D.; McColl, K.E. Role of ammonia in the pathogenesis of the gastritis, hypergastrinaemia, and hyperpepsinogenaemia I caused by Helicobacter pylori infection. Gut 1992 Dec;33(12):1612-6

3.   Triebling, A.T.; Korsten, M.A.; Dlugosz , J.W.; Paronetto, F.; Lieber, C.S. Severity of Helicobacter-induced gastric injury correlates with gastric juice ammonia. Dig Dis Sci 1991 Aug;36(8):1089-96

4 .   Fusamoto, H.; Sato, N.; Kamada, T.   Chronic effect of intragastric ammonia on gastric mucosal structures in rats. Dig Dis Sci 1991 Jan;36(1):33-8

5.   Meyer, B.: Werth, B.; Beglinger, C.; Dill, S.; Drewe, J.; Vischer, W. A.; Eggers, R. H.; Bauer, F. E.; Stalder, 0. A. Helicobacter pylori infection in healthy people: a dynamic process? Gut. 1991 Apr; 32(4): 347-50

6.   Graham, D. Y.; Malaty, H. M.; Evans, D. G.; Evans, D. J. Jr; Klein, P. D.: Adam, E. Epidemiology of Helicobacter pylori in an asymptomatic population in the United States.Effect of age, race, and socioeconomic status. Gastroenterology. 1991 Jun; 100(6): 1495-501

7.   Debongnie, J. C.; Pauwels, S.; Raat, A.; de Meeus, Y.; Haot, J.; Mainguet, P. Quantification of Helicobacter pylori infection in gastritis and ulcer disease using a simple and rapid carbon-14-urea breath test. J NucI Med. 1991 Jun 32(6); 1192-9

8.   Loffeld, R.J.; Stobberingh, E.; Arends, J.W. A review of diagnostic techniques for Helicobacter pylori infection. Dig Dis 1993;11(3):173-80

9.   Marchildon, P.A.; Ciota, L.M.; Zamaniyan, F.Z.; Peacock, J.S.; Graham, D.Y. Evaluation of three commercial enzyme immunoassays compared with the 13C urea breath test for detection of Helicobacter pylori infection. J Clin Microbiol 1996 May;34(5):1147-52

10.   Chittajallu, R.S.; Neithercut, W.D.; Ardill, J.E.; McColl, K.E. Helicobacter pylori-related hypergastrinaemia is not due to elevated antral surface pH. Studies with antral alkalinisation. Scand J Gastroenterol 1992;27(3):218-22

11.   Graham, D. Y.; Klein, P. D.; Evans, D. J. Jr.; Evans, D. G.; Alpert, L. C.; Opekun, A. R.; Boutton, T. W. Campylobacter pylori detected noninvasively by the 13C-urea breath-test. Lancet. 1987 May 23:1(8543): 1174-7

12.   Marshall, B. J.; Surveyor, I. Carbon-14 urea breath test for the diagnosis of Campylobacter pylori associated gastritis. J Nucl Med. 1988 Jan; 29(1): 11-16

13.   Surveyor, I.; Goodwin, C. S.; Mullan, B. P.; Geelhoed, E.; Warren, J. R.; Murray, R. N.; Waters, T. E.; Sanderson, C. R. The 14C-urea breath-test for the detection of gastric Campylobacter pylori infection. Med J Aust. 1989 Oct 16:151(8): 435-9

14.   Marshall, B. J.; Plankey, M. W.; Hoffman, S. R.; Boyd, C. L.; Dye, K. R.; Frierson, H. F., Jr.; Guerrant, R. L.; McCallum, R. W. A 20-minute breath test for Helicobacter pylori. Am J Gastroenterol. 1991 Apr; 86(4): 438-45

15.   Coelho, L. G.: Chausson, Y.; Passos, M. C.; Sadala, R. U.; Costa, E. L.; Sabino, C. V.; Queiroz, D. M.; Mendes, E. N.: Rocha, G. A.; Oliveira, C. A.; et aI. [l4C-urea breath test to diagnose gastric Helicobacter pylori colonization]. Gastroenterol Clin Biol. 1990:14(11):801-5

16.   Good, D. J.; Dill, S.; Mossi, S.; Beglinger, C.; Stalder, G. A.; Meyer-Wyss, B. Sensitivity and specificity of a simplified, standardized 13C-urea breath test for the demonstration of Helicobacter pylori]. Schweiz Med Wochenschr. 1991 May 18; 121(20):764-6

14.   Breath-Tests in Babies

$\mathbf{M}$ost people throughout the world are able to drink milk in infancy and early childhood.   After age 3-5 years the majority of the world's population begins to lose that ability by reducing their production of the intestinal enzyme *lactase*.   Although milk intolerance is rare in infancy, other causes of H_2 and/or CH_4 production make the test useful in neonates and infants.

The ability to produce lactase enzyme is seen in almost all babies, but neonates frequently produce H_2 early in their postnatal life.   Barr and co-workers[1] studied sequential morning breath-H_2 levels in breast-fed and formula-fed infants during the first five months of life.   All babies (except for two whose stools did not produce H_2) produced H_2-levels greater than 10 ppm, which peaked at two months and fell off significantly after that.   Similar reports have been made by other authors.   Cheu and Brown,[2] MacLean and Fink,[3] Rogerro, et al.,[4] Lifschitz and co-workers[5] and Mobassaleh and co-investigators[6] reported that premature neonates as well as term neonates produce H_2 for a variable time after birth, but that they must ingest a given level of carbohydrate before H_2 is produced in measurable amounts.   Laforgia *et al.*[7] demonstrated that over 27% of newborn babies had positive lactose and lactulose breath-tests.

Most investigators agree that this evidence of low lactase levels in the newborn does not mean that the infant is a lactose malabsorber, nor that milk should be withheld from their diets.   In most cases (Lifschitz[5] reported exceptions) the level of H_2 production falls to less than 10 ppm within a few weeks or months.

There are many causes other than lactase deficiency for lactose malabsorption or breath-H_2 production in babies.   Cheu and co-workers in the U.S.[2, 8] and Garstin and Boston in England[9] have described the use of breath-H_2 measurements to detect the development of neonatal necrotizing enterocolitis (NEC) before any other symptoms have become evident.   Their reports were supported by Rauter, *et al.*[10]   This observation is important because early diagnosis is essential for effective treatment.   In their studies, the investigators sampled alveolar air daily from patients identified as likely candidates for the syndrome.   Elevated

breath H_2-levels were detected between eight and 28 hours before any clinical signs of NEC were observed.

Bodanszky, *et al.*,[11] reported an increasing incidence of positive H_2-tests as villous damage progressed in children with coeliac disease. In non-coeliac patients, Giardia lamblia infestation was the most frequent cause of a positive test.   Zaniboni, et al.,[12] reported that the xylose breath-H_2 test augmented the "blood xylose" test for an indirect test of mucosal damage in children. According to the investigators, the patient should be demonstrated to not have lactase deficiency for the breath-test to be useful. Nose and co-investigators[13] detected bacterial overgrowth through the lactose-loading breath-H_2 test and used it to diagnose blind or stagnant loop syndromes in infants and children. Shermeta[14] used the H_2 breath test to provide an early warning mechanism to indicate carbohydrate overload which might be produced in the process of adapting the bowel with short gut syndrome.

Children with active gastroenteritis and diarrhea have been reported to have significantly lower breath-H_2 production following a standard disaccharide dose than do non-diarrheic controls.[15] Furthermore, a recovery period much longer than 24 hours from the last diarrheic episode is necessary to obtain reliable data from breath-H_2 tests administered to infants.[16]

Miller, et al.,[17] and Yolken and co-workers[18] studied lactose (and d-xylose and sucrose) malabsorption in children infected with human immunodeficiency virus. An increase in positive H_2-tests was reported by both groups, though the children did not always show clinical symptoms of malabsorption such as diarrhea. Other investigators who work with adults have reported an increased incidence of bacterial overgrowth associated with HIV infection, which may have contributed to the positive breath-H_2 tests in the reports cited here.

Stephensen, Sack and Sack[19] have developed a non-invasive test of gastric acid secretion for use in infants and young children.   It is based on the reaction of ingested magnesium metal with hydrochloric acid in gastric juice to produce hydrogen gas, which is excreted in the breath and in belches.   The test compared well with the standard intubation test performed on different days. This test should be important

in HIV patients, who are frequently hypochlorhydric, thus increasing the likelihood of ingested bacteria reaching the intestinal tract.

Finally, a common cause of chronic diarrhea in children may be related to their consumption of fruit juices and sorbitol (frequently used as an artificial sweetener). Several groups of investigators have indicated that some fruit juices and sorbitol cause clinical symptoms and breath-H_2 production, particularly in young children. Hyams and co-workers[20] have performed breath-H_2 tests in healthy children and those with chronic non-specific diarrhea after the ingestion of apple, pear and grape juices and a 2% sorbitol solution. The degree of H_2 exhalation following ingestion of fruit juices was roughly correlated with the sorbitol concentration in each of the juices (highest in pear juice, less in apple juice and least in grape juice). No differences in H_2 production were seen between healthy children and those with chronic non-specific diarrhea. Kneepkens and co-workers[21, 22] demonstrated that fructose in apple juice was an important contributor to this malabsorption (with 80% coming from fructose and 20% contributed by sorbitol). They showed that the fructose effect is reduced by the addition of glucose or galactose to the juice, which presumably reflected activation of the hypothesized fructose carrier.

Ladas and co-workers[23] pointed out that honey contains fructose in excess of glucose and has been shown to produce expired H_2 and abdominal complaints. Ament[24] also drew attention to the presence of sorbitol and the imbalance between fructose and glucose (this time in apple and pear juices) as the cause of chronic, non-specific diarrhea in infants and children. Hyams and Leichtner[25] called attention to the somewhat unsuspected condition of chronic diarrhea caused by "non-excessive" apple juice consumption. It is probably appropriate to consider this possibility and withdraw apple juice or, better yet, test for its tendency to produce elevated breath-H_2 after consumption in otherwise healthy children who have chronic non-specific diarrhea. Such a procedure would be appropriate before embarking on an expensive, time-consuming and frequently unpleasant and unrewarding gastroenterological evaluation of such patients.

Collecting Alveolar Air Samples from Babies

Because of the low total volume and the high breathing frequency of babies, special attention must be paid to the problem of collecting a

suitable alveolar air sample for analysis in these patients.

When no correction factor for sample contamination is used, an effort must be made to collect a reliable alveolar air sample. Many physicians attempt to time the withdrawal of a sample into a syringe from a nasopharyngeal catheter or nasal prongs. It was demonstrated that samples collected by such techniques consist of a variable and unpredictable mixture of alveolar and non-alveolar (dead space) air. Most investigators have recognized the need for a correction factor for neonates based on other components in the sample which should be constant from sample to sample. These have primarily included O_2, CO and CO_2 (the discussion and references are presented in another chapter devoted to this topic).

Laboratories which do not have access to gas analyzers to be used to generate the correction factor have attempted to collect an end-expiratory sample in as reliable a manner as possible. In an attempt to improve the method of timing syringe withdrawal, QuinTron has developed two systems which can reduce the variability of sample contamination and increase the reliability of the pattern of testing for breathtests.

The patented QuinTron ScissorValve permits the operator to divert the last portion of sequential breaths or the last part of a crying-breath into a sample collection bag. Although it is usually not a pure alveolar air sample, particularly in neonates, it provides an improvement over the attempted timing pattern for syringe withdrawal.

For children who can follow instructions for blowing through a mouth-piece, QuinTron's KidSampler will improve the collection. It is an ultra-low volume "Tee-piece" combined with a small gas-collecting bag and a special (child-size) dead space "discard bag" which has a numbered scale on the side of the bag. The scale permits adjusting the volume of the bag to accommodate the reduced dead space of young children. It is based on an estimated dead space of about 2 ml/pound of body weight (1ml/pound for dead space plus an equal volume to flush out the laminar flow pattern in the system, including the patient's airway). The selected volume is set by repeatedly folding the bag over (or rolling it up) from the large end until it reaches the proper size, based on

the body weight of the patient, and then stapling or "paper-clipping" it to limit its volume.

Some laboratories use a collection system assembled with a suitable face mask, an ultra-low volume one-way breathing valve and a small impermeable gas-collecting bag (the system is available from QuinTron as the BabySampler) to collect a mixed expired air sample. The sample is assumed to be about one-half alveolar air, so the analysis can be simply doubled in all samples. This approach reduces the error when compared with samples timed with syringe-withdrawal.

Collecting an expired air sample and applying the correction factor for sample dilution will provide even more reliable values for both H_2 and CH_4. The Model SC MicroLyzer was developed for this application, and provides the simplest, most reliable method of correcting the sample for contamination.

References

1.	Barr, R.G.; Hanley, J.; Patterson, D.K.; Wooldridge, J. Breath hydrogen excretion in normal newborn infants in response to usual feeding patterns: evidence for "functional lactase insufficiency" beyond the first month of life. J Pediatr; 1984; 104(4): 527-33

2.	Cheu, H.W.; Brown, D.R. Breath hydrogen excretion in the premature neonate. Am J Dis Child; 1990 (Feb); 144(2): 197-202

3.	MacLean, W.C., Jr; Fink, B.B. Lactose malabsorption by premature infants: magnitude and clinical significance. J Pediatr; 1980 (Sep); 97(3): 383-8

4.	Roggero, P.; Mosca, F.; Motta, G.; Mangiaterra, V.; Perazzani, M.; Offredi, M.L.; Marini, A.; Careddu, P. Sugar absorption in healthy pre-term and full-term infants. J Pediatr Gastroenterol Nutr; 1986 (Mar-Apr); 5(2): 214-9

5.	Lifschitz CH; Smith EO; Garza CY. Delayed complete functional lactase sufficiency in breast-fed infants. J Pediatr Gastroenterol Nutr; 1983; 2(3): 478-82

6.	Mobassaleh, M.; Montgomery, R.K.; Biller, J.A.; Grand, R.J. Development of carbohydrate absorption in the fetus and neonate. Pediatrics; 1985 (Jan); 75 (1 Pt 2): 160-6

7.	Laforgia, N.; Benedetti, G.; Altavilla, T.; Baldassarre, M. E. Grassi, 0. A.; Bonsante, F.; Mautone, A. Significance of lactose breath test in the newborn. Minerva Pediatr 1995 (Oct);47(10):433-6

8.	Cheu, H.W.; Brown, D.R.; Rowe, M.I. Breath hydrogen excretion as a screening test for the early diagnosis of necrotizing enterocolitis [see comments]. Comment in: Am J Dis Child 1989 (Nov); 143(11):1262-3. Am J Dis Child; 1989 (Feb); 143: 156-9

9.	Garstin, W.I.; Boston, V.E. Sequential assay of expired breath hydrogen as a means of predicting necrotizing enterocolitis in susceptible infants. J Pediatr Surg: 1987 (Mar); 22(3): 208-10

10.   Rauter, L.; Startinig, B.; Mutz, I. Determination of hydrogen in expiratory air of premature infants in suspected necrotizing enterocolitis. Monatsschr Kinderheilkd 1992 (May);140(5):296-9

11.   Bod:anszky, H.; Hor:ath, K.; Bata, A.; Horn, G.; Simon, K. Hydrogen breath test in small malabsorption. Acta Paediatr Hung; 1987; 28(1): 45-9.

12.   Zaniboni MG; Lambertini A; Romeo N; Albini P; Pozzi M. Usefulness of the integration between the values of the 1-hour blood xylose test and the hydrogen breath-test after xylose oral load for the indirect evaluation of mucosal damage. Pediatr Med Chir; 1985 (Mar-Apr); 7(2): 243-7

13.   Nose, O.; Kai, H.l; Harada, T.; Ogawa, M.; Maki, I.; Tajiri, H.; Kanaya, S.; Kimura, S.; Shimizu, K.; Yabuuchi, H. Breath hydrogen test in infants and children with blind loop syndrome. J Pediatr Gastroenterol Nutr; 1984 (Jun); 3(3): 364-7

14.   Shermeta, D.W.; Ruaz, E.; Fink, B.B.; MacLean, W.C., Jr. Respiratory hydrogen secretion; a simple test of bowel adaptation in infants with short gut syndrome. J Pediatr Surg; 1981 (Jun); 16(3): 271-4

15.   Solomons, N.W.; Garcia, R.; Schneider, R.; Viteri, F.E.; von Kaenel, V.A. H2 breath tests during diarrhea. Acta Paediatr Scand; 1979 (Mar); 68(2): 171-2

16.   Joseph F Jr; Roseberg AJ. Low breath hydrogen production in post-diarrheic infants. Acta Paediatr Scand; 1991 (Aug-Sep); 80(8-9): 792-4

17.   Miller, T.L.; Orav, E.J.; Martin, S.R.; Cooper, E.R.; McIntosh, K.; Winter, H.S. Malnutrition and carbohydrate malabsorption in children with vertically transmitted human immunodeficiency virus 1 infection. Gastroenterology; 1991 (May); 100 (5) (Pt 1): 1296-302

18.   Yolken, R.H.; Hart, W.; Oung, I.; Schiff, C.; Greenson, J.; Perman, J.A. Gastrointestinal dysfunction and disaccharide intolerance in children infected with human immunodeficiency virus. J Pediatr; 1991 (Mar); 118(3): 359-63

19.   Stephensen, C.B.; Sack, R.B.; Sack, D.A. Comparison of noninvasive breath hydrogen test for gastric acid secretion to standard intubation test in infants and young children. Dig Dis Sci; 1987 (Sep); 32(9): 978-84

20.   Hyams, J.S.; Etienne, N.L.; Leichtner, A.M.; Theuer, R.C. Carbohydrate malabsorption following fruit juice ingestion in young children. Pediatrics; 1988 (Jul); 82(1): 64-8

21.   Kneepkens, C. M; Jacobs, C.; Douwes, A.C. Apple juice, fructose, and chronic nonspecific diarrhea. Eur J Pediatr; 1989 (Apr); 148(6): 571-3

22.   Kneepkens, C.M.; Vonk, R.J.; Fernandes, J. Incomplete intestinal absorption of fructose. Arch Dis Child; 1984 (Aug); 59(8): 735-8

23.   Ladas, S.D.; Haritos, D.N.; Raptis, S.A. Honey may have a laxative effect on normal subjects because of incomplete fructose absorption. Am J Clin Nutr 1995 (Dec);62(6):1212-5

24.   Ament, M.E. Malabsorption of apple juice and pear nectar in infants and children: clinical implications. J Am Coll Nutr 1996 Oct;15(5 Suppl):26S-29S

25.   Hyams, J.S.; Leichtner, A.M. Apple juice. An unappreciated cause of chronic diarrhea. Am J Dis Child; 1985 (May); 139(5): 503-5

15. Normalizing Breath-Gas Measurements

Contamination of the alveolar sample is seen most commonly when the patients are neonates or infants who can not cooperate with the sample collection. In some clinics an attempt is made to collect the end-expiratory portion of a breath by synchronizing the withdrawal of a syringe-sample through nasal prongs or a catheter placed in the oro-laryngeal space. Using this technique, breath samples in infants are significantly contaminated with room air or dead space air because of the high breathing frequency which makes timing difficult and because neonates have a low tidal volume/dead space ratio (with perhaps 50% of each breath comprising dead space). In addition, such attempts in neonates are usually accompanied by crying, which may cause hyperventilation and dilute the alveolar samples if crying continues for longer than a half-minute prior to the sample collection. With other crying patterns, high CO_2 levels can be seen due to breath-holding during the bout of crying. Other techniques use a one-way, non-rebreathing valve and a collection device. Broadbent et al.,[1] demonstrated that either of these collection methods produces samples of highly variable composition, with an unpredictable contribution of alveolar and non-alveolar air.

These errors can be corrected by using a sample-correction factor based on the principle of relating the trace gases to the value for CO_2 which would have been measured if alveolar composition was constant and predictable (as it should be).

The Principle Behind the CO_2 Correction Factor

The CO_2 correction factor is based on the concept that carbon dioxide is present in alveolar air at a virtually constant concentration, while it is present in only trace concentrations in room air. Therefore, if an alveolar air sample is accidentally mixed with room air, the concentration of carbon dioxide in the sample will be reduced, as will that of any trace gas also present. By knowing the degree to which the CO_2 is diluted, it is possible to apply a correction to the analysis of the trace-gas as well, and be able to estimate the "alveolar" concentration of the sample which was contaminated. The sample concentrations of H_2 and CH_4 are multiplied by the factor calculated from:

$$\frac{\text{Alveolar } CO_2 \text{ concentration}}{\text{Sample } CO_2 \text{ concentration}}$$

CO_2 is the physiological regulator of breathing, and the whole breathing system is dedicated to keeping the *alveolar CO_2-pressure (PA_{CO2}) constant at 40 mm Hg* (torr*)*. Therefore, CO_2 is the most reliable "normalizing" component in the sample because it ordinarily has the most constant alveolar composition of any gas in the sample.

The alveolar P_{CO2} remains constant at 40 torr (mm Hg) among normal individuals if ventilation is normal. The *percent carbon dioxide* in an alveolar sample at 40 mm Hg is affected by the altitude (barometric pressure) at which the sample is collected. Alveolar air with a P_{CO2} of 40 torr in Miami (at sea level) will have a concentration of about 5.5% CO_2 in dry air (40÷(760-37), while if it is measured in Denver, where barometric pressure is closer to 625 torr, the dry CO_2 in alveolar air will be near 6.8% (40 ÷ 625-37).

Significant differences in barometric pressure exist at different altitudes, as demonstrated with Denver and Miami. However, using a single correction factor will simplify the process without inducing a significant error because all samples will be normalized to the same (constant) CO_2 level. Except for research studies where the absolute alveolar pressure for the trace gases may be important, using a correction factor of 5% or 5.5% CO_2 will be adequate for normalizing breath trace-gases to alveolar concentration.

Other Approaches to Normalization

The absolute value of the correction factor is less important than the fact that all the samples are normalized to the same CO_2 concentration, such as 5.5%. However, if it is possible to do it more precisely, the absolute accuracy of the breath trace-gas concentrations will be improved.

Early investigators demonstrated the benefit of normalizing the H_2 values on the basis of observed oxygen levels,[1] assuming that O_2-concentration is constant in alveolar air, at least over a short period of time. Others used carbon monoxide levels,[2, 3] which is present in trace concentrations as a product of hemoglobin breakdown or as a residual from smoking, and is presumed to be constant during the period of the test. (This assumes that the subject is not exposed to smoke during the period of the test.)

Oxygen (O_2) and carbon monoxide (CO) have been used to correct for sample contamination for one of two reasons: (1) economical cost of O_2 analyzers, or (2) convenience, based on measuring H_2 and CO with the same instrument (e.g., a molecular sieve column on a gas chromatograph). Either of these methods are better than not making a correction at all, but the fact is that with reference to the trace-gases, O_2-concentration in alveolar air may be more variable than the concentration of carbon dioxide.

Most investigators now relate the composition of the sample to normal alveolar CO_2 composition.[4-8] This has been made more accessible by the development of the QuinTron Model SC MicroLyzer, which measures CO_2 at the same time the trace gases are measured. It calculates the correction factor and applies it to the H_2 and CH_4 levels in the sample, then it displays the corrected values for the trace-gases.

Perman, et al.,[9] called attention to the fact that if total ventilation changes markedly, a change in the relationship between the CO_2 response and the H_2 dilution and CH_4 dilution resulting from the ventilation change will be observed in the sample. This observation can be explained by the fact that body storage of CO_2 is much greater than it is for the other gases, so the relationship between CO_2 change and H_2 or CH_4 change will be nonlinear. This effect will be seen with all gases used to normalize the sample by this principle, but it will ordinarily have little effect on the clinical conduct of the breath-tests for disaccharide malabsorption or bacterial overgrowth, and a CO_2-based correction is, even then, better than alternative methods.

Strocchi, Ellis and Levitt[10] have suggested that the reproducibility of trace gas measurements needs more attention, and that normalization of H_2 and CH_4 with CO_2 reduced the variability more than any other technique, but attention to the expiratory technique could improve the accuracy of the tests. They showed that a 15-second breath-hold prior to exhaling the sample reduced the variability of replicate analyses. If the breath-hold period is reproducible, the maneuver should decrease the variability ordinarily seen, but it will not likely replace the CO_2 correction factor.

References

1.   Broadbent, R.; Robb, T.A.; Davidson, G.P. Reproducibility of expired breath hydrogen levels in the neonate: a comparison of two methods for sample collection. Clin Chim Acta; 1983 (Feb 7); 127(3): 337-42

2.   Hopper, A.O.; Smith, D.W.; Ostrander, C.R.; Cohen, R.S.; Stevenson, D.K. Use of end-tidal carbon monoxide to correct end-tidal hydrogen in neonates. J Pediatr Gastroenterol Nutr; 1983 (Nov); 2(4): 659-62

3.   Sannolo, N.; Vajro, P.; Dioguardi, G.; Mensitieri, R.; Longo, D. Gas chromatographic quantitation of breath hydrogen and carbon monoxide for clinical investigation in adults and in children. J Chromatogr; 1983 (Sep 9); 276(2): 257-65

4.   Hsien-Chi, N.; Schoeller, D.A.; Klein, P.D. Improved gas chromatographic quantitation of breath hydrogen by normalization to respiratory carbon dioxide. J Lab Clin Med; 1979; 94:755

5.   Breuer, N.; Ptok, A.; G:otze, H.; Goebell, H. [Methodological aspects of the use of the hydrogen (H2) breath test]. Z Gastroenterol; 1986 (Feb); 24(2): 80-4

6.   Cheu, H.W.; Brown, D.R.; Rowe, M.I. Breath hydrogen excretion as a screening test for the early diagnosis of necrotizing enterocolitis [see comments]. Am J Dis Child; 1989 (Feb); 143(2): 156-9

7.   Cheu, H.W.; Brown, D.R. Breath hydrogen excretion in the premature neonate. Am J Dis Child; 1990 (Feb); 144(2): 197-202

8.   Kien, C.L.; Liechty, E.A.; Myerberg, D.Z.; Mullett, M.D. Dietary carbohydrate assimilation in the premature infant: evidence for a nutritionally significant bacterial ecosystem in the colon. Am J Clin Nutr; 1987 (Sep); 46(3): 456-60

9.   Perman, J.A.; Modler, S.; Engel, R.R.; Heldt, G. Effect of ventilation on breath hydrogen measurements. J Lab Clin Med; 1985 (Apr); 105(4): 436-9

10.   Strocchi, A.; Ellis, C.; Levitt, M.D. Reproducibility of measurements of trace gas concentrations in expired air. Gastroenterology; 1991 (Jul); 101(1): 175-9

16. Breath-Sample Collection Techniques

The accuracy and reliability of breath-testing is dependent on the care applied to collecting the sample and the technique used in transfering it to the analyzer for analysis. If it has been incorrectly collected or if it was contaminated with room air prior to the analysis, the resultant error cannot be remedied by paying attention to how the sample is analyzed in any instrument except for the Model SC MicroLyzer which has the built-in capability of correcting for contamination.

The general lack of detail about sample collection procedures in the literature leads one to suspect that many gastroenterologists and their staff members do not understand or appreciate the importance of eliminating dead space air from samples to be analyzed as "alveolar air." A variable volume of the first portion of an exhaled tidal breath is simply a wash-out of the airways which was filled with the last portion of room air inhaled with the preceding breath. That volume can be approximated as one ml per pound of body weight, usually generalized as being about 150 ml for the average adult. With a normal tidal volume of about 500 ml/breath, the first 1/3 or so is dead space air. Because of the laminar pattern of airflow through the major airways, roughly twice that volume must be exhaled before all of the dead space air is washed out, to avoid contaminating alveolar air with a portion of slowly mixed dead space air. The problem is even greater with neonates, in whom dead space volume is represented by close to 50% of the tidal volume because of the disproportionately larger airway diameter in babies as compared with adults.

Most published articles describing the use of breath trace-gases simply indicate that "expired air" or "end-expired air" samples were analyzed, without a description of the methods and precautions used for the sample collection. Since that step is so critical to the success of the test, a description of the process should be included in the reported methodology, including the application of a correction factor for sample contamination if it is applied. Although they are not common, sufficient reports have been found which reported on the collection procedure so that we can present a view of how samples are generally collected and handled.

Most patients who provide breath samples for analysis exhale a volume greater than the usual 500 ml tidal volume during the sample-collection procedure. By collecting the last portion of this larger volume, a more reliable alveolar sample can be assured. However, it requires attention to discarding the first portion of the expirate and increasing the expired volume so an adequate sample can be collected for analysis. Casual sampling of exhaled air without concern for this technique can result in having unacceptable reproducibility in sample analysis.

When an analyzer is available which requires only very small samples, it is possible to collect an alveolar air sample in a syringe and introduce it directly into the analyzer. End-expiratory samples can be collected by holding a syringe in the air-stream and withdrawing a sample near the end of the expiratory effort, which can then be introduced to the instrument or to an evacuated tube. Some practice is required to develop a reliable technique which does not include dead space air in the sample. Kneepkens and co-workers[1] used a plastic syringe with a side-hole near the top. The patient blew into the open end of the syringe, and the exhaled air was vented through the side-hole. At the end of expiration, the plunger was pushed forward to cover the side-hole and a stopcock was closed on the open end to capture the sample in the syringe. They then transferred the sample to a vacuum tube for transport to the analyzer. The weakness of those methods is that there is no verification of the expired volume before the sample is collected.

The use of a modified Haldane-Priestley device to collect an alveolar air sample is widespread. This consists of a "discard" bag of known volume (approximately 400 ml for an adult) with a side-arm on the mouthpiece which permits the attachment of a syringe or collection bag. The patient blows into the device, and when the discard bag is filled, the end-expired air is diverted into the side-arm where it is captured during a continuing expiratory effort. This simple device works well for all patients who are able to follow instructions, provided the operator understands the importance of having the first portion of the patient's air pass into the discard bag before the sample is collected.

Murray and co-workers[2] studied the use of plastic syringes for holding breath samples. Hydrogen retention was measured at room temperature, refrigerator temperature and freezer temperature (-20ºC) for up to 72 hours. The use of a conventional twist-in-lock closure was compared to the Critocap® syringe closure. They found that Critocaps were superior to conventional twist-in-lock closures, and long-term reliability was maximal when samples were placed in environments less than 5 ºC. Ellis, Kneip and Levitt[3] demonstrated that a substantial loss of trace-sample-component is seen when either glass or plastic syringes are stored for long periods of time, but that using either a mineral oil or water seal at the end of the syringe barrel markedly reduces the leakage of H_2 and other gases to negligible levels. Gardiner, et al.,[4] compared four different collection techniques in babies (postnasal catheter, nasal prongs, Rahn-Otis end-tidal sampler, and a modification of the Wiggins' blowout - a party toy) and found that the variability between the techniques was less than the inherent variability of repeated breath hydrogen assays using the same technique. Accordingly, the choice could be made on the basis of personal (or patient) preference.

A careful study by Strocchi, Ellis and Levitt[5] showed that attention to the expiratory technique could improve the accuracy of the tests. They found the least variability when the samples were collected into collection bags through a modified Haldane-Priestley tube after a 15-second breath-hold, followed by normalizing the results on the basis of adjusting to an equivalency of 5% CO_2.

The greatest challenge facing the technician or operator who is doing the breath-test is in getting reliable alveolar air samples from neonates and from babies who are too young to follow instructions for blowing into a collection bag. Breath collection from the newborn baby and infant is even more difficult because of the requirement of a system with a small dead space, owing to the small tidal volume in the newborn baby.[6] Methods that have been used include pediatric face masks[7-9] or nasal prongs and oropharyngeal or nasopharyngeal catheters.[10-13] These latter techniques require a high degree of technical expertise and the results depend heavily on the skills of the operator. Drawing a sample into the syringe in rhythm with the infant's breathing pattern cannot be standardized, especially if the breathing pattern is rapid and irregular, as in small infants. The procedure of using nasal prongs has been widely used, with the accumulation of a small aliquot of alveolar air collected in a syringe at the end of each exhalation of the baby. Others use the

patented QuinTron ScissorValve™ or collect mixed expired air in a collection bag with a one-way breathing valve. Most investigators recognize that even with the best technique they can develop, some contamination of the sample is likely. If they were all contaminated to the same degree, this would not be a problem - but they are not. Consequently, they depend on "normalizing" the breath sample, whether it was collected as expired or end-expired air in adults or by nasal prongs or direct collection of expired air in infants. The correction is based on mathematically adjusting the concentration of CO_2[10, 14-16] or O_2[17-19] in the sample to that which would normally be expected if the sample was pure alveolar air. Some investigators have assumed that measuring changes in mixed expired air samples without normalization will faithfully reflect changes in trace-gas composition. That assumption is probably safer than saying that all samples collected with nasal prongs are representative of alveolar air without measuring it; though, at best, the trace-gases in expired air samples are diluted with air of dead space composition by about 30% in adults and nearly 50% in neonates, so the values are lower than they would be with alveolar or corrected alveolar samples.

Tadesse and co-workers[20] developed a modified open-flow method, in which the child's head is put into a perspex box and a sample of expired air is sucked from the box by means of an air pump. They compared their method with a modified Haldane-Priestly device (described below) and a nasopharyngeal catheter, and concluded that their method was more satisfactory and as reliable as the other techniques for collecting mixed expired air samples.

Some investigators have devised elegant systems which are designed to collect alveolar samples reliably. Yeung and co-workers[21] described an automatic electronically operated sampler which uses a hot-wire sensor to detect expiration and automatically collects a portion of sequential end-expirates in a syringe. Alveolar air occupied 87% of the sample volume, with highly reproducible results.

QuinTron Devices for Sample Handling

Based on our history of an earlier experience with pulmonary physiology and instrumentation (which, by the way, generated the MicroLyzer series of breath analyzers), the QuinTron GaSampler and its associated

devices used as accessories for the analyzers were natural develop-ments for the support of breath-testing. They will be described here, with reference to the QuinTron order number, to allow the reader to get more information if desired. The physiological basis for the proper use of the devices will be emphasized where needed.

Of historical importance is the Haldane-Priestley Tube, which has been used to permit the collection of alveolar (end-expiratory) air for over 50 years. It is diagramed below, to show its simple use.

Haldane-Priestley Tube
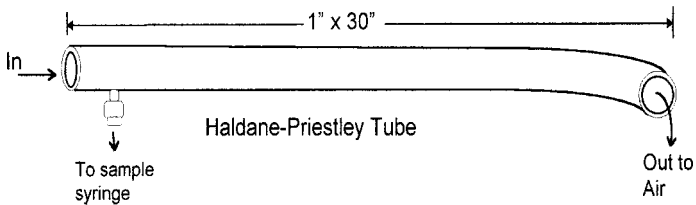
The "Tube" is used to discard the dead space air and flush out the volume of expired air which contains the transition from dead space to alveolar air. The patient blows into the tube and exhales completely. At the end of the breath, he/she occludes the end of the tube by keeping it in the mouth and holding the breath. At that time, the technician quickly pulls a sample into the syringe, and it is subsequently analyzed. The sample consists of the last portion of expired air, which came from the alveoli and is dead-space free. This is a simple procedure, and has been demonstrated to be a satisfactory way to collect alveolar air samples for many years.

QuinTron GaSampler™

The QuinTron GaSampler is a modified Haldane-Priestley collec-tion device. However, instead of using the long, unwieldy tube, it was replaced with a mouthpiece and a simple plastic bag having about 400 ml capacity. A side-port is attached to the mouthpiece, on which was fitted a gas-impermeable bag of variable volume. The bag is fitted with two ports, a large one with a one-way valve which is attached to the side-arm of the mouthpiece, and a smaller port with a female Luer Lock fitting to accept a standard syringe, which is used to transfer the sample to the MicroLyzer or to a sample holding bag. Collection bags are available in standard 750 ml volume, and in smaller volumes of about

250 ml (generally used for infants and children). A special "ultra-mini-collection bag" of about 150 ml volume can be used for neonates when combined with other collection systems. The collection bags are available in either re-usable or single-use bags.

The GaSampler consists of an alveolar air collection bag, a discard bag and a Tee-piece with a mouthpiece attached. The system was designed for the task of sampling and storing alveolar air samples before analysis.

The GaSampler can be used by untrained technicians (or even by a patient without supervision, after having its operation explained) to collect a sample of alveolar air for subsequent analysis.

The three major components of the system are pictured above. The discard bag (#QT00843-P) is assembled in-line with the mouthpiece on the other end of the Tee-Mouthpiece Assembly (#QT00854-P), with a one-way flap-valve inserted into the right-angle port, the "Tee-piece." (If this valve seat is removed, be sure that flow goes in the right direction (out) when it is reassembled.) The 750 ml collection bag (#QT00841-P for a reusable bag or #QT00830-P for a 750 ml single-use bag) is attached to the Tee-piece. The large port on the collection bag also contains a flap-valve (within the bag) which allows air to go only in one direction, to fill the bag. That port cannot be used to empty the bag or for removing a sample. A different, small plastic sampling port is located at the other end of the collection bag (on the same surface as the large port). It has an air-tight plug in the Luer-taper fitting which

accepts a standard stopcock or a syringe, and is used for sampling <u>from</u> the bag, as discussed later.

Collecting a Sample

It is recommended that a stopcock be put into the Luer port of the Collection Bag prior to collecting the sample in order to minimize losing or altering the composition of the sample when it is transferred to the syringe or sample holding bag in preparation for analysis.

After an approximately normal inspiration (avoid filling the lungs maximally), the patient (or subject) places the mouthpiece in his/her mouth, forming a tight seal around it with the lips. For children or patients who can not understand the purpose of the procedure it may be necessary to use a nose-clip; others may just squeeze their nose to assure that all the expired air goes through the mouth and into the GaSampler. A normal expiration is then made through the mouth (do not blow hard) to empty the lungs of as much air as required to provide the alveolar sample. The first portion of the expired air will be used to fill the discard bag, after which the valves on the Tee-piece and in the collection bag will open (when the pressure goes up in the discard bag) and the remaining expired air will be diverted into the collection bag. Stop when both bags are full. When an adequate sample is collected, instruct the patient to stop expiring and remove the mouthpiece.

After the patient removes the GaSampler from his/her mouth, the collection bag (with the sample inside) should be removed from the Tee-piece and the red-cap put in its place. The sample can be analyzed immediately or stored for later analysis. Using the cap plug assures that sample volume will not be lost due to a leak through the flap-valve. It also prevents the contamination of the sample due to gas diffusion through the valve-leaflet in the large port if it is stored for a long time period prior to analysis. If the GaSampler is used to collect the next sample from the same patient, transfer the sample to a syringe or to a sample holding bag and empty the collection bag by flattening it against the table-top. Use the same discard bag on the Tee-piece for subsequent samples from the same patient. The discard bag and the Tee-Mouthpiece should be replaced with a new one for the next patient.

Sample Holding Bag

The Sample Holding Bag (#QT00842-P) is a small bag of about 250 ml capacity, which is used to hold samples until the analyses can be completed. It is similar to the Mini-Collection Bag, except that it is equipped with only a single small port and has no large port. It is filled directly from the Collection Bag through a male-male connector which is supplied with the order for Sample Holding Bags. As indicated above, it is recommended that a stopcock be put into the Luer port of both bags prior to transferring the sample, to minimize losing or altering sample composition when it is transferred to the syringe.

The sample holding bag volume is sufficient to allow several sample analyses with the MicroLyzer, and they are inexpensive enough for the GaSampler system to be used for the collection of additional samples so they can all be analyzed at the same setting.

KidSampler™ (GaSampler™ For Small Children)

The KidSampler for small children was designed for children who can follow verbal instructions. It is based on the same principal as that for the larger discard bag and mouthpiece used with older children and adults. The special, scaled-volume Discard Bag (# QT00843-PSV) is folded and clipped or stapled at the level indicated by their body weight (which relates the dead space to proportionately smaller volumes). It is attached to one end of the reduced-volume Tee-Piece (#QT00859-P) which reduces the dead-space air added to the collected sample but permits it to fit standard toddler masks, mouthpieces and collection bags. The Infant Face Mask (QT00882-L), Toddler Mask (QT00883-L) or Child's Mask (QT00884-L) should be attached to the other end of the Tee-Piece.

The 250ml Mini-Collection Bag (QT00834-P for single-use or QT00844-P for multi-use) is installed on the side-arm of the Tee-Piece.

It is smaller than the standard bag in order to be less cumbersome and to accommodate smaller samples more easily. It has a large port with a one-way valve (sealed with a red cap during storage) which connects to the Tee-Piece, and a small port with a Luer fitting to accept a standard syringe for withdrawing the sample from the bag. It is recommended that a stopcock be put into the Luer port prior to collecting the sample, to minimize losing the sample when it is transferred to the syringe.

It is feasible to use the KidSampler for a rebreathing system and allow the baby's increased tidal breaths to "overflow" into the Mini-Collection Bag if it is applied with the Discard Bag collapsed and when the lungs are filled. Such rebreathing (or even crying into the face mask) will avoid contaminating the sample with room air, since after a few breaths, the air in the Discard Bag will be equilibrated with alveolar air, so that any air breathed out will have a concentration near that of alveolar air. Such rebreathing should not exceed about 10-15 seconds to prevent the build-up of gases (CO_2 as well as H_2 and CH_4) in the alveolar air due to blood recirculation. If the sample collection is not successful with the first effort, it is feasible to remove the mask and let the baby recover for a minute (which will be sufficient unless the baby is crying vigorously and, thereby, hyperventilating). The valve in the Mini-Collection Bag will permit the mask to be removed and reapplied to collect an adequate sample size. When the system is reapplied, be sure the Discard Bag is collapsed and the lungs are filled so the sample-collection begins with a full exhalation. A stopcock should be put into the Luer sampling port prior to collecting the sample, to minimize losing or altering sample composition when it is transferred to the syringe from the Mini-Collection Bag.

BabySampler™

When it is not likely that a valid alveolar air sample will be collected with the KidSampler, it is better to collect a mixed expired air sample, consisting of dead space air mixed with alveolar air, in a collection bag. The one-way system called the BabySampler is recommended for neonates or small babies whose tidal volumes are smaller, and who have a high breathing frequency and a low tidal volume/dead space ratio, so that dead space washout is likely to encroach on most of the expired air. This can be accommodated with the QuinTron BabySampler. The special ultra-low volume Tee-Piece is furnished with a one-way valve (permitting inspiration through that port) inserted into the side-arm. The face mask should be put on one end and the Ultra-Mini-Collection Bag (QT00835-P), about 100 ml volume, with its own one-way valve is attached to the opposite end of the special Tee-Piece. An adapter (QT00855-L) is required when the neonatal face mask (QT00881-L) is used, but toddler and child-size masks will fit directly on the Tee-Piece.

This system allows the inhalation of room air by the baby, with the expired air going to the Ultra-Mini-Collection Bag. The tidal volume should be sufficiently small so that an average of 3-5 breaths can be collected, to give a reliable representation of mixed expired air. Such samples are usually valid for comparing trace-gas concentrations from one sample to another, since the proportion of dead space air contributing to the sample will be similar among the samples unless there is a

marked change in the breathing pattern. Try to start the collection at the beginning of inspiration to permit the collection of a full expiration, and stop at the end of a complete exhalation. It should be recognized that if the sample is diluted by up to half with dead space air, the response will, likewise, be reduced by half and the concentration should be appropriately adjusted in all samples. Even with that recognition, it is, of course, recommended that a correction factor be applied to normalize the samples to a constant CO_2 concentration.

AlveoSampler™

The QuinTron AlveoSampler (QT00827-P) is a disposable device based on the now familiar Haldane-Priestley tube. It permits single-patient use of a system to collect an alveolar sample in a standard syringe for immediate analysis. Ordinarily a 20 ml syringe is used, since a sample size of 20 ml is adequate for the analyzer. This device will remove the danger of inter-patient cross-infection and will save time and money related to the cost of cleaning and sterilizing reusable sample-collection bags and components.

The AlveoSampler is assembled by putting the stopcock on the syringe. Have the stopcock open and push the plunger of the syringe all the way in. Attach the stopcock and syringe to the AlveoSampler by inserting the male end of the stopcock firmly into the side-hole in the middle of the mouthpiece.

The patient takes a normal breath and, at the end of inspiration, puts the AlveoSampler mouthpiece into his/her mouth and exhales normally through the mouth and into the bag (not too rapidly, not too slowly). The discard bag has a small vent hole, so as the patient exhales, the polyethylene bag will be filled with air and will be vented through the hole so exhalation can continue. When the polyethylene bag is filled, all the dead space air will be flushed out of the airways, and the remaining air comes from the alveoli.

While the patient continues to blow through the AlveoSampler, and keeps the bag inflated, withdraw 20 ml of alveolar air into the syringe before the patient stops blowing. Be sure the patient keeps his/her mouth tightly closed around the mouthpiece until the sample is collected. Turn the stopcock off when the syringe is filled with the sample. The patient can now stop exhaling and you can remove the Syringe Assembly from the mouthpiece. The sample can be analyzed shortly after collection, or it can be stored in the syringe (not longer than four hours) for later analysis.

ScissorValve™

QuinTron has developed a patented (US Pat# 5,026,027) single-port ScissorValve (QT00890-P) which has unique applications in gas collection procedures. (It is called the ScissorValve because of the scissors-motion which moves a collection bag into position over the breathing port). It is used to collect the alveolar portion of an expired breath. To prevent the possibility of cross-infection among patients, the ScissorValve should be used only with single-use bags. The ScissorValve is particularly useful in pediatrics breath-testing. The ScissorValve accepts all sizes of single-use face masks, and has minimal dead space, so the sample will reliably represent alveolar air.

A simple collecting procedure is used to collect an alveolar air sample from adults and children who can follow instructions, and even from infants who are crying (taking big breaths). With a suitable face mask and collection bag attached, have the patient breathe normally through the valve. At a point near the end of an expiratory maneuver (or near the end of a prolonged crying expiration), simply squeeze the scissors handle. This will rotate the collection bag to the mask-opening and allow the rest of the expiration to go into the bag. When an appropriate sample is collected, relax on the scissors and the spring-action of the valve will return the bag to its original position, isolating the sample. Remove the bag from the port and put the red cap in place in order to prevent sample loss. For infants and small children, the single-use Mini-Collection Bag (250 ml capacity) should be used; for larger children and adults, the single-use GaSampler Collection Bags (750 ml capacity) may be more suitable. Reusable collection bags are not recommended for use with the ScissorValve because the fingers going into the bag-port open the one-way valve, and inspiring from the bag is possible after some air is put into it. This makes inter-patient cross-infection possible and should be avoided.

EasySampler®

The patented (US Pat# 5,467,776) EasySampler was designed to provide a method for filling vacuum tubes with a sample suitable for analysis with the Model SC MicroLyzer. It is necessary to use that analyzer because there are several sources of sample dilution which require correction when the sample is analyzed. There is a residual

volume of air in the tube, though it is evacuated as far as practical in its preparation. There may be slight contamination with dead space or room air during sample collection.

Since the volume in the tube is limited to about 12 ml, the sample loop is not well flushed, so some residual carrier gas (room air) may dilute the sample in the sample loop. The EasySampler is preferred by clinics and laboratories which analyze samples collected elsewhere and

mailed in for analysis. This is because of the convenience of the tube for handling, the stability of the sample in a glass tube which allows long-term storage, and the simple, straight-forward technique of using the EasySampler. Sample dilution is not a problem because the SC MicroLyzer is designed to correct for dilution of the trace gases by reference to the decreased concentration of CO_2. Ordinarily, a correction factor of 1.2 to 1.5 is required to compensate for all the factors contributing to the dilution, but the correction factor applied to each sample permits a reliable picture of the pattern of trace-gas response to the challenge-dose of sugar ingested for whatever test is performed.

It is necessary to use unsterile vacuum tubes without anticoagulant for the sample collection because standard sterile tubes contain high concentrations of H_2,[22] which will contaminate the sample.

SamplXtractor ™ for Model SC MicroLyzer

QuinTron has developed a system to simplify the extraction of the sample from a vacuum tube filled with the EasySampler, and reliably inject it into the Model SC MicroLyzer. Prior to the use of the extractor, it was necessary to withdraw the sample with a syringe, which would develop negative pressure created by the process of emptying the tube. This was difficult for some technicians, and led to more variability in the correction factor than some laboratories felt comfortable with. The SamplXtractor (#QT01162-EX) is pictured below.

The vacuum tube containing the sample is snapped into the tube shuttle inside the needle tower, with the cap of the tube up. The shuttle handle is moved upward to carry the tube up, around the double-needle. The handle of the 3-way stopcock at the top of the needle tower is then moved to the horizontal position, which opens the water-line to the needle. With the 3-way stopcock open, a syringe pump is used to displace the sample in the tube with water from a reservoir and drive it through a sample drying tube, on its way to the MicroLyzer. The drying tube removes water vapor which would contaminate the columns if it inadvertently got into the system. The sample loop of the MicroLyzer is filled with the air from the tube, after which it is analyzed in the standard way and compared with reference gas composition. The correction factor is applied by flipping a switch on the MicroLyzer so the top two

meters indicate the corrected values for <u>alveolar</u> concentrations of H_2 and CH_4 in the sample. The value for the correction factor is displayed in the CO_2 meter when the correction factor is applied.

After the sample is extracted and before the tube is removed, turn the 3-way stopcock handle up, which closes the line from the needle so water in the system does not drain back into the reservoir, thereby leaving the line filled with air. If air gets into the line, flush the needle with water, then turn the stopcock handle up and flush the line returning to the reservoir until the line is free of air bubbles. The water-filled tube is removed after extracting the sample by dropping the tube shuttle and pressing the tube release button at the back of the needle tower behind the shuttle.

References

1.　Kneepkens, C. M.; Vonk, R. J.; Bijlevel; Fernandes, J. The daytime breath hydrogen profile: technical aspects and normal pattern. Clin Chim Acta. 1985 Apr 30; 147(3): 205-13

2.　Murray, R. D.; Kerzner, B.; MacLean, W. C. Jr; McClung, H. J.; Gilbert, M. Efficient storage system for breath hydrogen. J Pediatr Gastroenterol Nutr. 1985 Oct; 4(5): 711-3

3.　Ellis, C. J.; Kneip, J. M.; Levitt, M. D. Storage of breath samples for H2 analyses. Gastroenterology. 1988 Mar; 94(3): 822-4

4.　Gardiner, A. J.; Tarlow, M. J.; Sutherland, I. T.; Sammons, H. G. Collection of breath for hydrogen estimation. Arch Dis Child. 1981 Feb; 56(2): 125-7

5.　Strocchi, A.; Ellis, C.; Levitt, M. D. Reproducibility of measurements of trace gas concentrations in expired air. Gastroenterology. 1991 Jul; 101(1): 175-9

6.　Smith, C.A.; Nelson, N.M. The physiology of the newborn infant. 2nd ed. Springfield, Illinois. Charles C. Thomas, 1976

7.　Rogerro, P.; Mosca, F.; Motta, G., et al. Sugar absorption in healthy preterm and full-term infants. J. Pediatric Gastroenterol Nutr. 1986; 5:214-9

8.　Lifschitz, C.H.; O'Brian Smith, E., Gavza, C. Delayed complete functional lactase sufficiency in breast-fed infants. J Pediatr Gastroenterol Nutr. 1983; 2:478-82

9.　Van der Klei-van Moorsel, J.M.; Douwes, A.C.; van Oeveren, J.P. New principle for estimation of H_2 in expired air. Eur J Pediatr. 1984; 141:221-4

10.　Cheu, H. W.; Brown, D. R. Breath hydrogen excretion in the premature neonate. Am J Dis Child. 1990 Feb; 144(2): 197-202

11.　MacLean, W.C.; Beverly, B.F. Lactose malabsorption by premature infant: magnitude and clinical significance. J Pediatr. 1980; 97:383-8

12. Stevenson, D.K.; Cohen, R.S.; Ostrander, C.R.; et al. A sensitive analytical apparatus for measuring H_2 production rates. II. Application to studies in human infants. J Pediatr Gastroenterol Nutr. 1982; 1:233-7

13. Stevenson, D.K.; Shabin, S.M.; Ostrander, C.R.; et al. Breath H_2 in preterm infants: correlation with changes in bacterial colonization of the gastrointestinal tract. J Pediatr 1982; 101:607-12

14. Breuer, N.; Ptok, A.; Gotze, H.; Goebell, H. Methodological aspects of the use of the hydrogen (H2) breath test. Z Gastroenterol. 1986 Feb; 24(2): 80-4

15. Hopper, A. O.; Smith, D. W.; Ostrander, C. R.; Cohen, R. S.; Stevenson, D. K. Use of end-tidal carbon monoxide to correct end-tidal hydrogen in neonates. J Pediatr Gastroenterol Nutr. 1983 Nov; 2(4): 659-62

16. Kien, C. L.; Liechty, E. A.; Myerberg, D. Z.; Mullett, M. D. Effects in premature infants of normalizing breath H2 concentrations with CO2: increased H2 concentration and reduced interaliquot variation. J Pediatr Gastroenterol Nutr. 1987 Mar; 6(2): 286-9

17. Broadbent, R.; Robb, T. A.; Davidson, G. P. Reproducibility of expired breath hydrogen levels in the neonate: a comparison of two methods for sample collection. Clin Chim Acta. 1983 Feb 7; 127(3): 337-42

18. Pereira, S. P.; Khin-Maung-U; Duncombe, V. M.; Bolin, T. D.; Linklater, J. M. Comparison of an in vitro faecal hydrogen test with the lactulose breath test: assessment of in vivo hydrogen-producing capability in Burmese village

19. Sannolo, N.; Vajro, P.; Dioguardi, G.; Mensitieri, R.; Longo, D. Gas chromatographic quantitation of breath hydrogen and carbon monoxide for clinical investigation in adults and in children. J Chromatogr. 1983 Sep 9; 276(2): 257-65

20. Tadesse, K.; Leung, D. T.; Lau, S. P. A new method of expired gas collection for the measurement of breath hydrogen (H2) in infants and small children. Acta Paediatr Scand. 1988 Jan; 77(1): 55-9

21. Yeung, C. Y.; Ma, Y. P.; Wong, F. H.; Kwan, H. C.; Fung, K. W.; Tam, A. Y. Automatic end-expiratory air sampling device for breath hydrogen test in infants. Lancet. 1991 Jan 12; 337(8733):90-3

22. Jensen, W.E.; O'Donnell, R.T.; Rosenberg, I.H.; Karlin, d.A.; Jones, R.D. Gaseous contaminants in sterilized evacuated blood collection tubes. Clin Chem. 1982; 28:1406

17. Selecting the Correct MicroLyzer™

The correct choice of MicroLyzer for a laboratory should be made on the basis of the needs of the individual laboratory. Considerations should include the kind of practice engaged in by the physicians and the expected number of patients which will need breath-tests. The CLIA regulations have exempted breath-testing from their certification for now (since 1996), so the test can be done in a laboratory or office not certified by the HHS.

Selecting an Instrument for H_2 Analysis Only

If the customer selects an instrument only for the measurement of H_2 (usually based on an expected low workload or for a specific research application), he can choose between the Model CM2 and the Model 12i MicroLyzers. These less expensive MicroLyzers will detect H_2 as well as the other QuinTron analyzers, but they will allow a small percentage of lactose malabsorbers to avoid detection due to conversion of H_2 to CH_4. By the way, other analyzers which are limited to only H_2 analysis also have this limitation.

The main difference between the Model CM2 and the Model 12i MicroLyzers is in how H_2 is separated from interfering components which might be in the sample, and in some of the features present in the Model 12i MicroLyzer which are not included in the Model CM2.

The Model CM2 MicroLyzer (QT00120-M) was designed as a clinical instrument and is recommended only if cost is the determining factor. It can be as accurate and reliable as the other instruments if attention is paid to its analytical technique, and if it is operated properly.

The Model CM2 Clinical MicroLyzer uses a special SivRite-10™ cartridge for separating H_2 from any other "reducing gas" which might be present in alveolar air samples, like carbon monoxide, methane, alcohols, ketones, etc. which might interfere with the analysis.

The cartridge contains a material called "Molecular Sieve" which makes it a "gas chromatographic" column, and allows different gases to percolate through at different rates, depending on their physical characteristics. The size of the cartridge has been adjusted so that all the H_2 in a 20 ml sample will be flushed through before any other reducing gas gets to the end of the cartridge. Thus, if a 20 ml syringe is filled with sample, then flushed through the cartridge, the H_2 will be transferred to the sample loop of the MicroLyzer. The sample loop is small enough so it is adequately flushed with the 20 ml sample. When the volume of gas in the sample loop is introduced into the analyzer, it will be analyzed and the H_2 concentration will be displayed on the meter.

It is important to use a 20 ml sample size to assure that the H_2-containing portion will reach the sample loop, and to allow methane and other contaminant-reducing gases to be trapped in the cartridge. Unless they are removed, they will be moved forward with successive samples, and can appear at the end of the cartridge with later sample flushes. It is therefore necessary to "backflush" the cartridge with room air in between samples. The analytical method requires a backflush of 40 ml to assure that the contaminants are removed from the cartridge so they will not interfere with subsequent samples. The sensor is specifically sensitive to H_2, so other reducing gases which get through the cartridge do not show a great effect on the sensor output.

Indicating Drierite® granules are added to the SivRite-10 cartridge to alert the user when the cartridge is exhausted. The granules turn pink (from blue) when moisture from the expired air sample builds up in the cartridge after the molecular sieve is no longer active (due largely to the accumulation of moisture). When ¾ of the material has changed color, the cartridge should be discarded and replaced with a new one.

It should be emphasized that for maximum accuracy with the CM2 MicroLyzer, it is necessary to pay close attention to the flushing volume and to back-flush the cartridge after each sample analysis with the Model CM2 MicroLyzer. This removes other reducing gases which would migrate slowly through the cartridge and interfere with subsequent sample analyses.

The Model 12i MicroLyzer (QT00112-M) has an internal gas chromatographic column through which the sample is flushed after it is introduced into the instrument. Molecular sieve material in the column permanently retards most components which might interfere with the measurement, so H_2 appears by itself at the sensor and is accurately quantitated. On the basis of the mechanism of separating H_2 from interfering gases with an internal, constantly flushing separating column, and based on some of the features not present on the Model CM2, the Model 12i is the most accurate, reliable "H_2-only" analyzer on the market. This is because:

a. It is a conventional gas chromatograph. There is a continuous flow of dry room air carrier gas through the separating column, so the column is kept flushed with no chance of contamination from previous

samples. It uses a continuous, fresh air sample as a baseline for the measurement and measures any increase in H_2 from that in the air.

b. A sample loop is used to introduce the same size sample each time an analysis is done. The analyzer requires only a 20 ml sample of air for the analysis, so it is applicable to babies and children as well as adults. An important advantage is that the proper flushing volume is not critical, as long as the minimum volume necessary to adequately flush the sample loop is used.

c. The Model 12i MicroLyzer has output terminals for a strip-chart recorder. This permits the recording of a permanent record, which can be a part of the patient's file. This hard-copy record is preferred by many physicians and by most insurance carriers.

d. The sensor is a solid-state device with no limit to its effective life-span. Some Model 12 MicroLyzers (the predecessor of the Model 12i) have been used for more than 20 years without replacement of the sensor! This is not a guarantee, but there is very low maintenance and few sensor replacements in the MicroLyzers.

Why CH_4 Analysis Should be Included in the Test

The main reason for adding the measurement of methane in tests for lactose malabsorption is to clear up questions about false-negative H_2 tests, but it also may extend our understanding about the dynamics of trace-gases in the colon.

As described in earlier sections, some malabsorbers who have negative H_2 breath-tests may generate CH_4 instead. These patients will be recognized if CH_4 is measured as part of the routine test. If the malabsorber generates neither H_2 nor CH_4 following ingestion of a nonabsorbed sugar, the patient must be a "non-producer," either as a result of having a sterile gut or of having rapid-transit diarrhea and/or a hostile pH (acidity too severe for the existence of hydrogen-producing bacteria).

There is overwhelming evidence in the literature (documented elsewhere) that most patients or subjects who fail to produce significant increases in H_2 after the administration of lactulose excrete increased

levels of CH_4. In one study a linear relationship was found between the amount of a disaccharide mixture ingested and H_2 produced over a 10-hour period. If CH_4 was formed, the sum of <u>both</u> gases followed a linear dose-effect relationship, indicating an interaction between the two components. Others have demonstrated an effect of CH_4-production on fasting H_2 baseline values, breath-H_2 area under the curve following lactulose and orocecal transit time, suggesting that knowledge of CH_4 status is necessary for the proper interpretation of the H_2 breath-test.

Instruments for H_2 and CH_4 Measurements

Two models are available for the measurement of H_2 and CH_4. The Model DP MicroLyzer (QT00126-M) is used for the measurement of H_2 and CH_4. The Model SC MicroLyzer (QT00130-M) measures H_2 and CH_4, and uses CO_2 to correct for any dilution of the alveolar sample by dead space air or sampling error.

With either instrument, special consideration should be paid to the location of the analyzer with respect to the patient. If the test is performed with the patient in the same room as the analyzer, it will detect CH_4 only if it exceeds the room-air level (since the instrument is zeroed in room air, even if it contains CH_4). If the analyzer is located in a

separate room it is possible that the CH_4-level may differ from that in the patient's area, particularly if the rooms are heated with a gas forced-air furnace. In that case, it is advisable to collect a sample of air in the patient's room (by using a large syringe) at zero time and analyze it along with the sample. This will detect conditions where the patient's sample shows a trace of CH_4 but it is from the room and not generated by the patient. Of course, such levels of CH_4 will not affect the interpretation of breath-tests since they are based on the change in H_2 and CH_4 in the samples analyzed.

The Model SC MicroLyzer has a unique feature which makes it the analyzer of choice for breath trace-gas studies. It has the ability to detect and correct for contamination of the sample with room air or dead space air during the collection procedure. Such contamination can result from improper sample collection, in which some of the respiratory dead space air is captured with the sample, or the technician inadvertently contaminates the sample with room air during handling. The Model SC MicroLyzer measures CO_2, compares it with what the alveolar CO_2 should be, and corrects each sample H_2 and CH_4 values for the contamination.

A recent modification to the operation of the Model SC MicroLyzer permits a practical method of introducing an alveolar air sample collected with a vacutainer tube (via the patented QuinTron EasySampler system). Unfortunately, the system can be used only with the Model SC, which permits the measurement of CO_2 in the sample which is analyzed. Even if the sample is diluted slightly during its collection in the evacuated tube and diluted further during the filling of the sample loop in the instrument (due to a limited volume for flushing the sample loop in the instrument), the ratios of H_2 and CH_4 to CO_2 will remain constant with air (non-alveolar) dilution, and the correction factor can be applied to provide a measure of alveolar trace-gases in a 10-12 ml sample, which was stored in a glass, gas-impermeable storage tube.

A device to assist in the transfer of the tube-sample to the SC MicroLyzer has recently been developed. The SampleXtractor is described and illustrated in a previous chapter. It uses the water-replacement principle to drive the air sample into the sample loop of the analyzer so that the transfer is made easily and reproducibly, with the CO_2 correction factor applied to correct for any possible sample dilution during the analytical procedure.

Using a Potentiometric Recorder with the MicroLyzer

The advisability of including a recorder for registering the MicroLyzer output should not be overlooked. Many insurance companies prefer hard-copy records, and most research projects demand the generation of permanent data records. The presence of a hard-copy record will minimize the likelihood of losing data, and will permit the discovery of transposition errors in copying results to data sheets, whether for clinical or research use. These considerations are important for either the Model 12i for H_2 or for the Models SC and DP used for H_2 and CH_4. A single channel output is required for H_2 and CH_4 recorded with the Model DP, since they are generated sequentially from the same sensor.
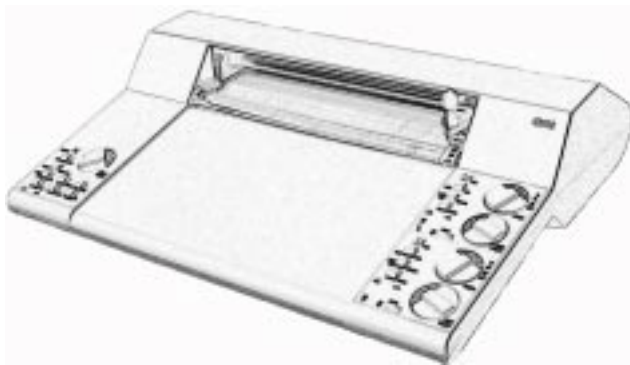
There is a separate recorder terminal for the CO_2 output of the Model SC MicroLyzer, and it can be recorded simultaneously with H_2 and CH_4 on a two-channel recorder if desired. However, the purpose of a permanent analytical record can be served by recording the H_2 and CH_4 tracings on a single chart and writing the correction factor (from the CO_2 module) and the corrected H_2 and CH_4 values on the chart, if desired. This will save the price of a two-channel recorder.

FOR MORE INFORMATION
PLEASE CONTACT US!

QuinTron

QuinTron Instrument Company

Milwaukee, WI 53215 USA

Phone: 1-414-645-4442

Fax: 1-414-645-3484

Toll Free (USA & Canada)

1-800-542-4448

www.EuinHron-I G5 .com