Noninvasive Techniques for the Evaluation of Gastrointestinal Function

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The study of gastrointestinal function during infancy has been impeded by the invasiveness, impracticality, and imprecision of the conventional techniques of internal medicine transposed to newborn and young infants. Quantitative data on the intraluminal phase of digestion are usually obtained in adults from analyses of duodenal fluid, whereas mucosal functions are investigated with biopsies and intestinal perfusion techniques. However, the performance of biopsy and intubation procedures is more difficult in infants, and cannot be practiced in asymptomatic infants. Noninvasive techniques for the evaluation of gastrointestinal function are, therefore, appealing to clinicians and investigators dealing with infants.

This review will discuss well-established measurements using fluids obtained noninvasively: serum, urine, feces, and breath. Appropriate applications and limitations of the various methodologies will be considered, with particular emphasis on recently introduced techniques of breath analysis in the pediatric population. For purposes of discussion, techniques are organized according to the phase of digestion or absorption assessed by a particular method (Table 1).

INTRALUMINAL PHASE

Assessment of Pancreatic Function

Fat Digestion

Lipolysis is the process by which dietary fat or triglyceride is hydrolyzed by pancreatic lipase. Lipolysis results in the production of insoluble end-products in the form of long-chain fatty acids and β-monoglycerides. The quantitative fecal fat determination remains the most reliable measure of lipolysis. The infant should be maintained on a normal diet containing a minimum of 35% of total calories as fat for 2 days prior to the beginning of stool collections. Stools are collected for 72 hr in infants with diarrhea. Charcoal or carmine
TABLE 1. Tests of digestion and absorption

<table>
<thead>
<tr>
<th>Function</th>
<th>Test</th>
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<td><strong>Intraluminal phase</strong></td>
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<td><strong>Pancreatic function</strong></td>
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<td>Triglyceride → fatty acids, monoglycerides</td>
<td>Quantitative fecal fat</td>
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<td>Carbohydrate → oligosaccharides, disaccharides</td>
<td>Serum carotene</td>
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<td>Protein → peptides, amino acids</td>
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<td>Micellar solubilization</td>
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<td>Quantitative fecal fat</td>
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<td>¹³C-Lipid breath tests (multiple substrates)</td>
<td>¹³C-Glycocholate breath test</td>
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<td><strong>Mucosal phase</strong></td>
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<td><strong>Specific functions</strong></td>
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<tr>
<td>Fat absorption</td>
<td>Quantitative fecal fat</td>
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<tr>
<td>Disaccharides → monosaccharides</td>
<td>¹³C-Lipid breath tests</td>
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<td>Peptides → amino acids</td>
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<td>Fecal reducing substances</td>
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red markers administered at the beginning and end of a 72-hr balance period should be used in those without diarrhea, and all stools passed from the appearance of the first marker until the appearance of the second marker should be collected. A strict record of intake is to be maintained from which the dietary fat ingested during the 72-hr balance period can be calculated. Results are then expressed as a coefficient of absorption by the equation:

\[
\frac{\text{Dietary fat} - \text{fecal fat}}{\text{Dietary fat}} \times 100 = \text{coefficient of absorption}
\]

Normal values for infants are related to diet and age (11,26).

Measurement of serum carotene, a precursor of vitamin A, is useful as a screening test for efficiency of lipolysis in the older child and adult, but does not establish the extent of fat malabsorption (24). In early infancy, abnormally low serum carotene values may reflect limited dietary intake of carotene-containing foods, including carrots, squash, sweet potatoes, and green vegetables. Carotene measurements cannot estimate the adequacy of lipolysis in the infant whose diet consists entirely of breast milk or formulas.

Oral administration of carbon-labeled (¹⁴C or ¹³C) dietary fats, followed by collection and measurement of labeled carbon dioxide excreted in breath, permits assessment of fat absorption without 72-hr stool collection. Appropriate enrichment of labeled CO₂ in breath following administration of labeled
lipid indicates normal digestion, absorption, and oxidation of fat. Since $^{14}$C represents a small but finite radiation burden to the child, Watkins et al. have validated the use of $^{13}$C-labeled lipids in breath tests (30). $^{13}$C is a nonradioactive naturally occurring stable isotope representing approximately 1.1% of all carbon atoms, and is differentiated from $^{12}$C by its mass using a mass spectrometer. Administration of the labeled long-chain triglyceride $^{13}$C-triolein has recently been shown to establish the presence of steatorrhea in children ages 3 months to 17 years with lipolytic defects (28) (Fig. 1). The $^{13}$C-triolein breath test does not, however, differentiate lipolytic from other intraluminal phase (micellar solubilization) or mucosal abnormalities. Subsequent administration of $^{13}$C-palmitic acid and measurement of expired $^{13}$CO$_2$ in breath appears to discriminate lipolytic function from micellar phase and mucosal function, since palmitic acid is a free fatty acid which does not require lipolysis for digestion but depends on micellar solubilization and normal mucosal function for absorption (Fig. 1).

It is important to note that the usefulness of $^{13}$C-lipid breath tests has not yet been established in small, immature infants or in populations with extensive malnutrition. Both groups may have altered rates of CO$_2$ production at rest, potentially affecting the results of $^{13}$C breath tests. In addition, substrates are generally expensive and facilities for the analysis of $^{13}$CO$_2$ are not widespread. With greater use, the price per gram of substrate should decrease and com-
mercial services for isotopic measurements on breath should expand. It is, therefore, anticipated that these procedures will become both cost effective and accessible for general use.

Carbohydrate Digestion

Unlike fat, hydrolysis of starch and glucose polymers by measurement of starch content in stools does not accurately assess adequacy of intraluminal carbohydrate digestion. Starch balance cannot be determined in a fashion analogous to fat balance because bacterial fermentation in the colon significantly modifies starch escaping small bowel absorption. Performance of starch balance requires measurement of lactic acid, glucose, dextrins, and starch in 3-day collections of feces while simultaneously quantifying the starch content in the diet (10). The practicality of this method is limited.

The same fermentation processes which modify starch escaping small bowel absorption result in gaseous products which can be measured noninvasively, providing an index of starch digestion and absorption. Endogenous H$_2$ production is exclusively due to fermentation of luminal substrate by intestinal bacteria. H$_2$ elaborated by bacterial processes is not metabolized in man, and is excreted in the lungs in proportion to its production in the intestine (16). Breath H$_2$ measurement has been established as a sensitive indicator of carbohydrate malabsorption, and incomplete digestion or absorption of starches and flours may be detected using this methodology (2). In a preliminary report, Mackie et al. (17) have utilized H$_2$ measurements to demonstrate that 15% to 50% of a starch load is malabsorbed in individuals with pancreatic insufficiency secondary to chronic pancreatitis. Breath H$_2$ tests for detection of disaccharide and monosaccharide malabsorption will be discussed below.

Protein Digestion

Accurate noninvasive measures of protein digestion are currently not available. Fecal nitrogen determinations are affected by the large contribution of endogenous sources of nitrogen to the intraluminal nitrogen content, and by bacterial catabolism of nitrogen in the colon (12,13). Controversy exists regarding whether quantitative fecal nitrogen determinations are linearly related to the amount of dietary nitrogen ingested and malabsorbed (8,23). However, 72-hr collections of feces followed by nitrogen determination are sufficient to distinguish children with pancreatic insufficiency from normal controls and patients with celiac disease. Recent studies using experimental animals (7) and in vitro fecal incubation studies (20) suggest that breath H$_2$ measurements following ingestion of proteins, or carbohydrate-containing proteins (glyco-proteins), may be useful in quantifying protein absorption. These techniques have not been validated at present.
Assessment of Micellar Solubilization

The products of lipolysis, fatty acids, and monoglycerides, must gain aqueous solubility for absorption across the intestinal mucosa. Bile acids cluster in complexes called “micelles” which are capable of bringing fatty acids and monoglycerides into solution. Duodenal concentrations of bile acids inadequate for the formation of micelles may occur in infants with cholestatic liver disease, small bowel bacterial overgrowth syndromes associated with deconjugation of bile acids, and in ileal disease or resection which interrupts the enterohepatic circulation.

Assessment of micellar solubilization may be performed by quantitative fecal fat determination. Whereas neither this test nor the triolein breath test will alone discriminate between abnormalities of lipolysis and micelle formation, the $^{13}$C-palmitic acid breath test should, because palmitic acid requires micellar solubilization only. A labeled carbon breath test using trioctanoin, a medium-chain triglyceride, would also be expected to achieve this discrimination, since absorption of trioctanoin does not require micellar solubilization, but digestion of this substrate is enhanced by lipolysis (Fig. 1). Ileal dysfunction or bacterial overgrowth causing defective micellar solubilization may also be detected by a breath test using $^{13}$C-glycocholic acid. In this test, glycocholic acid is labeled on the carboxyl carbon atom of the glycine moiety. The glycine is liberated when the amide bond linking the glycine to the bile acid is hydrolyzed by intestinal bacteria present in the colon or, in the case of small bowel bacterial overgrowth, in the upper small intestine. Subsequently, the liberated glycine is metabolized by the bacteria or within the body, resulting in elaboration of labeled CO$_2$ in expired air. In individuals with ileal resection or disease, large amounts of bile acid are exposed to colonic bacteria and deconjugated, resulting in a measurable increase in the excretion of $^{13}$CO$_2$. An identical process occurs in the upper small bowel in the case of small bowel bacterial overgrowth. $^{13}$C-Glycocholate breath tests have recently been validated for the detection of both ileal dysfunction and bacterial overgrowth in infants (29).

MUCOSAL PHASE

Nonspecific Mucosal Function

The functional integrity of the jejunum may be assessed by measurement of D-xylose absorption or excretion. D-Xylose is a 5-carbon monosaccharide absorbed chiefly by passive transport in the proximal small intestine. Serum samples for xylose determination are obtained at 30-min intervals for 2 hr following administration of a xylose test dose of 0.5 g/kg (maximum 5 g). The values obtained are compared with a blank or fasting xylose concentration. Some investigators have advocated the use of a single determination at 1 hr
following administration of xylose, but the accuracy of this approach is controversial (6,9). In children old enough to void on command, all urine is collected for 5 hr following the administration of xylose and the quantity of xylose in the urine is determined. Normal values based on age are available (25).

Xylose absorption is not a measure of carbohydrate absorption, since uptake of xylose is independent of intestinal mucosal disaccharidases, pancreatic exocrine secretions, and bile salts. Intraluminal bacterial overgrowth may modify the results of the test. Xylose absorption is affected by delayed gastric emptying, and excretion may be impaired in patients with abnormal intravascular volumes or renal disease.

Specific Mucosal Functions

Uptake of fatty acids and monoglycerides may be assessed by quantitative fecal fat determination. A xylose excretion test may then be used to distinguish fat malabsorption due to mucosal disease from that due to intraluminal phase defects. Alternatively, serial $^{13}$C-labeled breath tests will discriminate abnormalities in absorption of fat from abnormalities in lipolysis (Fig. 1).

Hydrolysis of Disaccharides and Uptake of Monosaccharides

Tests for Carbohydrates in Feces

Sugars escaping small bowel absorption enter the colon where a portion is fermented by bacteria to short-chain fatty acids. This process may be detected by measuring the fecal pH. A value of 5.5 or less is indicative of carbohydrate malabsorption (1). Unfermented sugar passes into the feces, and may be detected by the Clinitest method for reducing sugars (14). Measurements of fecal pH and reducing substances are useful screening tests for sugar malabsorption, but are not quantitative.

Disaccharide and Monosaccharide Absorption Tests

Oral tolerance tests in which a dietary carbohydrate such as lactose, sucrose, or glucose is administered to test a specific disaccharidase or transport function, require repeated determinations of serum glucose, and can be distorted by variations in gastric emptying and intermediary glucose metabolism. These uncontrolled variables lead to difficulties in interpretation of oral tolerance tests (15,18). A fundamental limitation of oral sugar tolerance tests is that, in contrast with fat absorption tests, unabsorbed sugar cannot be measured by these means. Oral tolerance tests for the identification of lactase and sucrase deficiency should therefore be interpreted with caution.
**Lactose and Sucrose Breath H\textsubscript{2} Tests**

Simplified H\textsubscript{2} collection, storage, and measurement techniques have been developed for detection of childhood lactose and sucrose malabsorption (4,19). Samples of expired air are collected by nasal prong (19) from fasting patients before and at 30-min intervals for 3 hr after administration of 20% lactose or sucrose solution (2 g/kg; maximum 50 g). A rise in expired air H\textsubscript{2} concentration indicates malabsorption of the substrate (Fig. 2). The amount of nutrient carbohydrate escaping absorption can be estimated by measuring H\textsubscript{2} produced after sequential administration of equivalent amounts of nonabsorbable (lactulose) and potentially absorbable carbohydrate (5). This procedure is based on the demonstration that H\textsubscript{2} increases linearly with increasing amounts of lactulose, and that equivalent amounts of H\textsubscript{2} are produced from different carbohydrate substrates by fecal flora from individual patients (5,21).

Criteria for interpretation of breath H\textsubscript{2} tests have largely been established on the basis of studies in older children and adults with primary disaccharidase deficiencies. Comparisons of mucosal histology and enzyme activity with breath H\textsubscript{2} excretion indicate that increases in expired air H\textsubscript{2} concentrations in children with isolated lactase deficiency are of greater magnitude and occur more rapidly after an oral lactose load when compared with children with lactase insufficiency secondary to mucosal injury (4). These differences in magnitude and time course of the H\textsubscript{2} response among primarily and secondarily deficient individuals may simply be attributable to the amount of carbohydrate escaping small bowel absorption. In early infancy, however, additional factors may affect the relationship between absorptive capacity for a given sugar, and H\textsubscript{2} production and excretion. The colonic contents of normal neonates and of infants with carbohydrate malabsorption are generally acidic (3). Using an in vitro fecal incubation system, studies in our laboratory have established that H\textsubscript{2} production within human colonic ecosystems is inhibited at acid pH (21). Acidification of colonic contents by repeated administration of nonabsorbable carbohydrate confirmed the effect of acid pH on H\textsubscript{2} production in vivo (Fig. 3). Thus, the presence of acid luminal contents at the time a test carbohydrate is administered may affect resultant H\textsubscript{2} production.

Similarly, the H\textsubscript{2} signal in certain infants may be blunted by low mesenteric blood flow. H\textsubscript{2} excretion is dependent on transfer of H\textsubscript{2} into the portal circulation. Premature newborns may have decreased mesenteric perfusion secondary to left-to-right cardiac shunts, hypoplastic left heart, polycythemia, and placement of umbilical catheters. The effect of intestinal ischemia on H\textsubscript{2} excretion was examined in adult and newborn dogs by insufflating ileal segments with H\textsubscript{2}, modifying mesenteric perfusion by hemorrhage or occlusion, and monitoring of H\textsubscript{2} excretion in breath (22). Breath H\textsubscript{2} measurements were shown to decrease in ischemia and rise upon relief of ischemia (Fig. 4), suggesting that altered intestinal perfusion may affect the H\textsubscript{2} signal.
These variables should be considered when interpreting breath H\(_2\) measurements in infants, since they potentially affect the relationship between the absorptive capacity for a given carbohydrate and H\(_2\) production/excretion. Recognition of these variables may ultimately add to the utility of breath H\(_2\) measurements for evaluation of gastrointestinal function.

**Peptide and Amino Acid Absorption**

Noninvasive tests for specific assessment of peptide hydrolysis, and peptide and amino acid absorption are currently unavailable. Conversely, transmucosal protein loss from the circulation can be detected by measurement of random fecal alpha-1-antitrypsin concentration in stool (27).

**CONCLUSIONS**

In summary, gastrointestinal function in infancy may be assessed by a variety of noninvasive techniques. Many of the methods are adequate only for...
screening purposes, whereas others, such as fecal fat determinations, yield quantitative information. Breath tests are particularly attractive because they are accurate and painless, and permit the study of digestive and metabolic processes otherwise not readily accessible to investigation. Important questions regarding sensitivity and specificity of breath tests in infants remain to be resolved, but the promise of these simple procedures is considerable.

ACKNOWLEDGMENTS

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REFERENCES


DISCUSSION

_Dr. Aurrichio_: Do you have any experience on the breath test in patients with cystic fibrosis? What kind of relationship is there between the increase in the breath content of hydrogen and the bacterial overgrowth?

_Dr. Perman_: Approximately 50% of our patients with cystic fibrosis have elevated basal hydrogen excretion. There are a number of interpretations of the elevated basal hydrogen excretion in cystic fibrosis, all of which need to be explored. One is the fact that perhaps glycoproteins, which are substrates for hydrogen production, are increased in the lumen of patients with cystic fibrosis. There are other possible interpretations. It is conceivable that a portion of the elevated basal hydrogen excretion even in fasting patients with cystic fibrosis may be due to malabsorption of carbohydrates. All our patients who had cystic fibrosis and elevated basal hydrogen excretions had been on antibiotics, and it is conceivable that what we are seeing is a change in the flora due to the antibiotics.

With regard to bacterial overgrowth, we don’t have a close correlation between the number of organisms, for example, in the upper small bowel and basal hydrogen excretion, but in each situation where we have aspirated duodenal contents and documented overgrowth, an elevated basal hydrogen was found.