Testing Intestinal Function

Possibilities Offered by $^{13}\text{C} \text{O}_2$ Breath Tests and Stable Isotopes

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The $^{13}\text{C} \text{O}_2$ breath test is a reliable, noninvasive method of studying the principal gastrointestinal functions, including the assimilation of food ingredients. Stable isotopes offer the possibility of monitoring various metabolic events as well.

**THE PRINCIPLE OF THE $^{13}\text{C} \text{O}_2$ BREATH TEST**

Breath tests have in common the fact the subject is given a substrate in which $^{12}\text{C}$ atoms normally present in the functional group are replaced by the stable isotope $^{13}\text{C}$. This functional group is cleaved enzymatically under specific circumstances, during either transit through the gastrointestinal tract, absorption, or further metabolism of the absorbed substrate. After cleavage, the labeled subgroups undergo a metabolic process that ends with the expiration of labeled $\text{CO}_2$. It is necessary that the speed-determining (rate-limiting) factor of the whole physiologic process is directly related to the genesis of $^{13}\text{C} \text{O}_2$. The $^{13}\text{C} \text{O}_2$ mixes with the body pool of $\text{CO}_2/\text{HCO}_3^-$ and is expired. In this way, the exhalation of $^{13}\text{C} \text{O}_2$ reflects the function to be investigated. The process is shown schematically in Fig. 1.

For $^{13}\text{C} \text{O}_2$ measurement to be used to demonstrate a well-defined gastrointestinal function, an enzyme activity, or bacterial metabolism, the $^{13}\text{C}$ substrate has to be chosen in such a way that the enzyme or function is the rate-limiting step in $^{13}\text{C} \text{O}_2$ production. When the excretion of the tracer in the breath is expressed as percent dose per hour or as cumulative percent dose excreted over a defined time period, a dynamic analysis of the variable examined is obtained over time.

The $^{13}\text{C} \text{O}_2$ breath tests are excellent investigative methods, as their scientific basis is sound and well conceived, the results have been validated in an unequivocal way, and their application is accepted by a growing number of scientists.
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$^{13}$CO$_2$ BREATH TESTS AS CLINICAL DIAGNOSTIC TOOLS

Originally, breath tests were designed as diagnostic tools for use in gastrointestinal and hepatology clinics. Fig. 2 shows the breath tests used in clinical practice in the Digestion-Absorption Laboratory of the University Hospital Gasthuisberg, Leuven.

Substrates Used in $^{13}$CO$_2$ Breath Tests

Hepatic function:
- Demethylating and oxidative capacity: $[^{13}$C$]$aminopyrine (1)
- Hepatic mass: $[^{13}$C$]$galactose (2)
- Mitochondrial activity: $[^{13}$C$]$ketoisocaproic acid (3)

Transit measurement:
- Gastric emptying: $[^{13}$C$]$octanoic acid and $[^{13}$C$]$glycine (4,5)
- Orocecal transit: lactose-$[^{13}$C$]$ureide (6,7)
- Small intestinal transit: by mathematical deduction (8)

*Helicobacter pylori* in stomach:
- $[^{13}$C$]$urea (9)

Digestive, absorptive, and fermentative functions:
- Carbohydrates: $[^{13}$C$]$naturally enriched compounds, starch, lactose (10–12)
- Lipids: $[^{13}$C$]$mixed triglyceride (13,14)
• Proteins: $[^{13}\text{C}]$, $[^{15}\text{N}]$ egg white proteins (15)
• Fermentation process: lactose-$[^{15}\text{N}]$ ureide (16); $[^{15}\text{N}],[^{2}\text{H}]$ proteins (17)

Bacterial overgrowth or bile acid malabsorption:
• The only $[^{14}\text{C}]$ substrate in use (i.e., glycocholic acid + 3 days fecal collection + $[^{3}\text{H}]$ polyethyleneglycol transit marker correction) (18–20)

**Mathematical Expression of the Functions**

An elegant method has been developed to express the meaning of gastrointestinal events by a mathematical formula (21,22). This is discussed later in the chapter.

**Good Reasons for Performing $^{13}\text{CO}_2$ Breath Tests**

A wide range of gastrointestinal techniques are used to investigate gastrointestinal function in clinical practice. These techniques classically are composed of intubation and perfusion to study pancreatic exocrine excretion (23), biopsy to determine brush border enzyme activity (24), and aspiration of intestinal juice to culture for bacterial overgrowth (25). These techniques are complemented by radiography for morphologic examination of the small intestine and colon (26), by scintigraphy to demonstrate gastrointestinal segmental transit (27), and by endoscopy with biopsy (28). Less invasive techniques are being developed, such as endoechography (29) and nuclear magnetic resonance imaging (30). Investigation methods based on the analysis of feces and urine for intestinal absorptive capacity are still in daily diagnostic use in hospitals.

Although these techniques are considered reference methods in clinical investigation, they may have some serious shortcomings. For example, intubation, perfusion, aspiration, and biopsy are static methods (i.e., they provide values obtained under nonphysiologic conditions). Methods based on imaging can display dynamic information, and further developments in these techniques may improve clinical investigation in gastroenterology. There remains, however, a need for noninvasive methods providing the same information as the classic methods, but which are simpler and cheaper to perform. $^{13}\text{CO}_2$ breath tests may meet these requirements.

If asked, patients request tests that can be executed in a simple way and whenever possible at home (i.e., with minimal absence from family life or work). Doctors ask for tests that can be done repetitively without major discomfort or radiation hazard for the patient, and without time-consuming involvement of equipment and personnel. Public health asks for tests with minimal hospital costs and which do not cause a problem of waste disposal. These advantages are all offered by $^{13}\text{CO}_2$ breath tests.

The isotope ratio mass spectrometry technique in current use allows breath $^{13}\text{CO}_2$ to be measured in centralized units. Breath samples are sent to the unit and results are available the next day after overnight measurement. It is not claimed that $^{13}\text{CO}_2$ breath tests can yet replace the classic tests; at present, they must be considered
strictly as important adjuncts to the overall plan of clinical investigation. Thus, it is mandatory that the interpretation of the results should only be made after detailed discussion with the clinician requesting the test.

Special attention must be paid to the execution of the $^{13}\text{CO}_2$ breath tests. These tests appear very simple to perform, and some investigators have tried to make them even simpler by reducing the sample numbers or by changing the other test conditions (e.g., meals, sampling time, calculation of results). These modifications can lead to a false interpretation of the test results and make them unsuitable for interlaboratory comparison. The standardized conditions under which these tests are performed, the way the samples are analyzed, and the fact that the calculations can be expressed in relation to an international standard represent a unique advantage over other methods of clinical investigation. An attempt has been made to ensure standardization by the European concerted action BIOMED PL93-1239 project (31). The best way to safeguard uniformity in test design is to do breath tests in specialized clinical units, along the lines of those performing other investigations involving highly technical analytic procedures such as radioscintigraphy. A centralized unit has the additional advantage that breath tests can be performed on several patients at the same time. Samples can be sorted by clinical presentation and the analysis performed overnight.

ADVANTAGES OF $^{13}\text{CO}_2$ BREATH TESTS

Three clinical or experimental situations occur in which $^{13}\text{CO}_2$ breath tests have a real advantage over classic tests.

1. Combinations of tests. With the combined use of $^{13}\text{C}$ and $^{14}\text{C}$ labeling, it is possible to measure two gastrointestinal functions simultaneously. This makes it possible to demonstrate the influence of one function on the other, for example the rate of lipid digestion following the rate of gastric emptying (32). The use of carbon-13 as the sole label to measure gastric emptying as well as orocecal transit time in a single test is under current investigation (see later).

2. Patients can serve as their own controls. For example, to demonstrate a dose-response relation of a prokinetic drug on gastric emptying or orocecal transit (33), or to investigate the digestibility of pretreated food (34). Under these conditions, interindividual variations such as differences in CO$_2$ production, gastric emptying, and so on, which can influence the test results, are excluded.

3. When several tests are used to monitor different functions in the same patient. In cases such as cystic fibrosis, breath tests could be used to assess the absorption of carbohydrates, lipids, and proteins and to monitor liver function and gastrointestinal transit. In this way, it may also be possible to optimize pulmonary function. The breath tests can be complemented by other functional tests based on the use of stable isotopes, including the measurement of energy expenditure. Once suitable instruction has been given, all these tests can be done at home without discomfort to the patient.
$^{13}\text{CO}_2$ BREATH TESTS IN NUTRITIONAL AND PHARMACEUTICAL RESEARCH

Lipids

The test molecule of choice is the 1,3 distearyl-2,$[^{13}\text{C}]$octanoyl glycerol, also called "mixed triglyceride" ($[^{13}\text{C}]$MTG) (13,14). The marker is incorporated in the lipid phase of the meal, which is taken in the morning. The test lasts 6 hours and every 30 minutes a breath sample is obtained. After 4 hours, a light meal is allowed. This does not affect the test results.

Figure 3 shows the mean $^{13}\text{CO}_2$ excretion curves in normal individuals and in patients with pancreatic insufficiency receiving different pharmacologic treatments. The curves are the averaged results from five subjects (unpublished observations). Curve 1 shows $^{13}\text{CO}_2$ excretion in normal individuals after a $[^{13}\text{C}]$-labeled breakfast (chocolate paste incorporating $[^{13}\text{C}]$MTG). Curve 4 shows $^{13}\text{CO}_2$ excretion when patients with pancreatic insufficiency had the same meal. When enzyme replacement therapy is given with the meal, improvement is seen in lipid absorption (curve 3). Maximal recovery of the tracer is obtained when gastric acid secretion is simultaneously inhibited by an Na$^+$/H$^+$-adenosine triphosphatase (ATPase) blocker (curve 2), as it is well known that pancreatic lipase is only active at nearly neutral pH. If the environment in the proximal small bowel is acid, enzyme supplementation is relatively ineffective at improving lipid digestion unless the acidity is suppressed.

This test can also be used to study the effect of lipids in relation to:

- Solubilization of lipid in a meal, caloric load, and so on
- Small intestinal conditions that influence lipid assimilation (e.g., transit)
- Inhibition of fat uptake (e.g., the efficiency of lipid assimilation in obesity)

In young children, the $[^{13}\text{C}]$MTG breath test is very useful for monitoring lipid absorption in relation to the composition of formula food and gastric motility. This test is of particular interest in patients with cystic fibrosis. As it can be performed at home, it avoids frequent visits to hospital. It is an elegant method of monitoring the effect of food composition or doses of enzyme supplements on lipid absorption without having to perform stool collections (35–37).

![FIG. 3. $[^{13}\text{C}]$ mixed triglyceride breath test to demonstrate duodenal lipase activity.](image-url)
Carbohydrates

To measure the degree and rate of carbohydrate absorption, advantage is taken of the fact that carbohydrates originating from maize or cane are $^{13}$C-enriched naturally by 0.02% (10). This apparently low percentage of enrichment is nevertheless sufficient to allow measurement of $^{13}$C enrichment in the breath with high accuracy.

The carbohydrate most abundantly present in human food is starch. One of the factors that plays a major role in starch assimilation is the physicochemical nature of the test molecule. Its chemical nature is determined by its amylose or amylopectin structure; its physical nature is influenced by pretreatment, specially by heat pretreatment. Heat treatment (gelatinization) has positive effects on the assimilation of starch. Gelatinized starch is more rapidly digested and absorbed than crystalline starch. Branched starch (amylopectin) is much better assimilated than starch with linear configuration (amylose) (38).

In patients with pancreatic insufficiency, starch is less well absorbed than in normal individuals (12). To eliminate the effect of impaired glucose metabolism, correction for glucose oxidation is necessary, as endocrine pancreatic function may also be disturbed. Thus, it is necessary to compare measurements of $^{13}$CO$_2$ excretion in normal controls and in patients after ingestion of a test meal containing $^{13}$C-glucose. Even when these corrections are applied, measurement of starch digestion and absorption remains a test of relatively low sensitivity in demonstrating exocrine pancreatic insufficiency.

The $^{13}$CO$_2$ breath tests are well suited for monitoring inhibition of starch hydrolysis by acarbose, an inhibitor of brush border alpha-glucosidase (39). The resulting $^{13}$CO$_2$ excretion curve resembles the malabsorption that occurs in pancreatic failure. Similar changes in $^{13}$CO$_2$ excretion curves have been obtained in cases where lipid or protein absorption have been inhibited.

Carbohydrates and Fermentation in the Colon: H$_2$ Excretion in Breath

Malabsorption of carbohydrates in the small intestine results in an increased influx of undigested material in the colon. Bacteria in the colonic lumen metabolize the carbohydrate moiety to short chain fatty acids and gases (40). The most prominent gas is hydrogen, which partly leaves the body in the breath. This phenomenon has been used to demonstrate bacterial overgrowth in the small intestine (41), to measure orocecal transit time (42), and most often to measure carbohydrate malabsorption (43). The combination of $^{13}$CO$_2$ and H$_2$ breath tests has the additional advantage that they demonstrate the fate of carbohydrates in foods, such as lactose (11), fructose (44), or carbohydrate-related food additives (45). In daily practice for the diagnosis of lactose absorption or lactose intolerance, both $^{13}$CO$_2$ and H$_2$ are measured. The same test procedure is applied to demonstrate sucrose malabsorption.

The metabolic fate of food additives (e.g., sorbitol or xylitol) or of prebiotics (e.g., inulin, polyfructoses) can also be monitored in this way.
Figure 4 shows how, by measuring $^{13}\text{CO}_2$ after the intake of labeled inulin, information can be obtained on energy salvage from nonabsorbable carbohydrates (46). The similar $\text{H}_2$ (right panel) and $^{13}\text{CO}_2$ excretion suggests that the $^{13}\text{CO}_2$ output is most probably derived from the oxidative metabolism of short chain fatty acids originating from anaerobic bacterial metabolism in the colon.

It has been argued that malabsorption of carbohydrates might be quantified by measuring breath $\text{H}_2$ output after a standardized intake of inulin or lactulose. This method has been abandoned, however, as quantitative measurement of the cumulative excretion of hydrogen over a long period of time is imprecise.

Proteins

Proteins are very important food constituents. Previously, intensive studies have been undertaken to explore the dynamics of various amino acids in the body using stable isotopes. However, few reports have been made on the absorption of proteins or on their metabolic fate in the colon in cases of protein malabsorption. The reason for this is mainly that no reliable substrate was available for monitoring the gastrointestinal events that take place when proteins are taken orally. Our group has described a technique by which it has become possible to incorporate $[^2\text{H}]$, $[^{13}\text{C}]$, or $[^{15}\text{N}]$ amino acids, or combinations of these, into eggs in a reproducible way (47). In an extensive study (48), Evenepoel investigated different aspects of the fate of ingested proteins in the gastrointestinal tract. In an initial series of experiments, the gastric phase was studied in relationship to protein digestion. Fig. 5 shows how gastric emptying precedes protein digestion in normal individuals. Protein assimilation follows the delivery of the gastric chyme to the duodenum. Simultaneous measurement of both intestinal functions became possible by using a combination of $[^{14}\text{C}]$ and $[^{13}\text{C}]$ tracers (48).

In a second experiment, we investigated the influence of the gastric phase and the role of acidity on protein digestion. Predigestion of proteins begins in the stomach under the influence of gastric acid, pepsinogen being converted to pepsin. In clinical practice, it is common for acid secretion in the stomach to be suppressed by $\text{H}_2$ blockers. How does this influence protein digestion? Our experiments show that
proton pump inhibition does not cause any change in gastric emptying. However, the rate of protein absorption is delayed by approximately 30% when gastric pre-digestion does not take place normally (49).

Food pretreatment also has a marked influence on the rate of protein absorption. Raw egg white leaves the stomach very rapidly, but the digestive phase of raw egg is seriously disturbed: after 6 hours of observation, only one third had been assimilated in comparison with cooked egg. In summary, after ingestion of raw egg, gastric emptying takes half the time it does with cooked egg, but assimilation is three times slower (34).

The application of this technique in ileostomy patients also allows the differentiation between endogenous and exogenous ileal effluents (34).

The most important aspect of our studies on the fate of proteins in the gastrointestinal tract has been the validation of a $^{13}$CO$_2$ breath test to demonstrate the absorption of proteins. Average values for $^{13}$CO$_2$ excretion in the breath of normal individuals have been obtained. The amount of the tracer recovered in the breath is markedly diminished in patients with exocrine pancreatic insufficiency. Serum concentrations of $[^{13}\text{C}]$leucine have also been determined. As expected, in the normal individual, the increase of $[^{13}\text{C}]$leucine in the serum following a protein meal precedes the excretion of $^{13}$CO$_2$ in the breath. In patients with pancreatic insufficiency, serum values are significantly lower than in normal controls. Differences between $^{13}$CO$_2$ excretion in the breath of normal individuals and in patients have been validated by intestinal perfusion studies and the quantitative recovery of trypsin activity after hormonal stimulation of the pancreas (15). The breath test is promising in monitoring the assimilation of protein from food and in demonstrating the effect of enzyme replacement therapy in cases of exocrine pancreatic insufficiency.

Until now, proteins have been labeled with $[^{13}\text{C}]$leucine only. Additional labeling with $[^{15}\text{N}]$leucine and [ring-$^{2}\text{H}_4$]tyrosine has been shown to be important in detecting protein malabsorption by measuring $[^{15}\text{N}]$ losses in the stool (17). This is the only way to differentiate between nitrogen losses originating from exogenous and endogenous sources, not taking into account the recirculating nitrogen pool. The recovery
of the [\(^2\)H\(_4\)] marker as ring-labeled phenolic compounds in the urine is a reliable way of measuring the degree of fermentation of proteinaceous compounds in the colon (17). Knowledge of this variable might be of value in controlling the formation of toxic compounds in the colon following dietary intake of proteins (50).

To summarize, the technique of multiple labeling of egg white proteins has made it possible to measure the digestion and absorption of proteins in the small intestine, their degree of fermentation in the large bowel, and their loss in the stools.

**GASTROINTESTINAL TRANSIT: GASTRIC EMPTYING AND OROCecal TRANSIT**

The rate at which food passes through the gastrointestinal tract is important in determining the nutritional value of food and the extent to which food components are subject to bacterial fermentation in the colon. Whatever the type of food, transit is always subject to complicated feedback mechanisms, either by control of target receptor cells or by direct neural or hormonal regulators. The process starts before food has been taken, as smell, taste, color, consistency, environment, and feelings of satiety have important effects on gut activity and function—the cephalic phase of food assimilation. The influence of these factors is reflected in gastric emptying and orocecal transit time.

**Gastric Emptying**

Gastric emptying determines the rate at which food is delivered to the duodenum. It is a codeterminant of food acceptability and food assimilation. Breath tests for gastric emptying can be represented in their most simple form by the data shown in Fig. 6. This figure shows gastric emptying in an individual after the intake of a liquid meal (curve 1), a solid meal with same energy content as the liquid meal (curve 2), and a solid meal with twice the energy density (curve 3). This method has multiple applications for food and pharmaceutical research (33,51–53). In com-
combination with other breath tests, it is an elegant way of showing how the digestion of lipid, carbohydrate, and protein is influenced by gastric emptying when food conditions are altered (by varying their composition, viscosity, consistency, energy density, and so on).

Orocecal Transit

Orocecal transit time (OCTT) is the time needed for food to pass from the mouth to the cecum. It defines the moment when assimilation of food ingredients by the host ceases and the breakdown process by bacteria in the colon begins. Orocecal transit time is determined by the use of a molecule, lactose ureide, in which the urea moiety is labeled with $^{13}$C. When also marked with a [$^{15}$N] tracer, the molecule is very well suited to monitoring the fermentation of N compounds in the colon. The rationale for the use of this molecule is that it is not absorbed in the small intestine, but is well fermented by bacteria in the colon. The method has been evaluated using radioisotopic modality (6). This $^{13}$CO$_2$ breath test offers many advantages in exploring pharmacologic modulation of the principal gastrointestinal functions of the proximal intestine (54).

Figure 7 shows how gastric and small intestinal transit can be measured in a single experiment (i.e., gastric emptying and orocecal transit time) by measuring the output of $^{13}$CO$_2$ in the breath (55). The development of appropriate mathematical methods (see later) made it possible to apply this technique of combined breath tests over a wide range of experimental conditions.

In the curve in Fig 7, only the $[^{13}C]$ label is shown and no data are given on the fate of the $[^{15}N]$ tracer from lactose-$[^{15}N]$ureide. This molecule is very suitable for monitoring the fate of bacterial N metabolites in the colon. Fig. 8 shows clearly the effect of providing a nonabsorbable carbohydrate (lactulose) with a meal: short chain fatty acids are generated from the carbohydrates by bacterial fermentation, which

![FIG. 7. Measurement of gastric emptying and orocecal transit time (OCTT) in a single experiment.](image)
in combination with $^{15}$N compounds (ammonia, urea, and so on) are utilized by colonic bacteria for cell division. This results in less $^{15}$NH$_3$ being delivered to the liver and, consequently, less $^{15}$N-labeled compounds are found in urine (16). This biological system is the key mechanism in avoiding overload of NH$_3$ in cases of hepatic encephalopathy. Lactose-$^{15}$N-ureide can be considered a "biological endoscope" for the study of N compounds in the human large intestine.

$^{13}$CO$_2$ BREATH TESTS IN PEDIATRICS

A need exists for noninvasive tests to investigate gastrointestinal and nutritional function in pediatric patients. Clinical problems particular to childhood require prompt and accurate methods of investigation, which are reliable and ethically acceptable. They should serve both for diagnostic purposes and also for assessing the effects of treatment. The use of $^{13}$C offers the possibility of applying $^{13}$CO$_2$ breath tests in infants and children, because their noninvasive nature makes them ideally suited for these patients. Another advantage of these noninvasive tests is that they can be performed in the ambulatory patient or at the bedside. It is not necessary for infants to be transported or to stay in hospital.

Methods of performing breath tests in children are currently being investigated in the Department of Pediatrics, Gastroenterology and Nutrition at the Catholic University of Leuven under the supervision of Professor Dr. G. Veereman-Wauters in collaboration with Dr. M. Van Den Driessche. In the pediatric population, the test meal is of particular importance. Children and infants need meals that taste good, look nice, and can be adapted to all age groups. Standardization of the methodology and the test meal is a key factor for success. Most of the breath tests described above have been adapted for use in children, even in preterm infants, and others are subject to ongoing research (5,35,37,55–60).
MATHEMATICAL ANALYSIS OF RESULTS, OBTAINED BY $^{13}\text{CO}_2$ BREATH TESTS

Mathematics are the most common way to express results obtained by instrumental analysis. In science mathematics fulfill the same role as words in philology, as expressed by the French philosopher Boileau (1638–1711):

"Ce qui se conçoit bien, s'enonce clairement et les mots pour le dire arrivent aisément."

Relating this statement to breath tests, one could say:

"A well conceived breath test provides those results which can easily be represented by a mathematical formula."

The $^{13}\text{CO}_2$ breath tests lend themselves particularly well to mathematical expression as they show a dynamic event over the course of time. A $^{13}\text{C}$-labeled substrate undergoes a series of steps before the labeled carbon isotope is excreted in breath as $^{13}\text{CO}_2$. In breath tests, the function or enzyme activity under investigation is the rate-limiting step, and any variable derived from the breath test curve will reflect the condition of this rate-limiting step. In breath tests for measuring gastrointestinal transit, it is important to obtain precise data describing the process under study alone, unaffected by the influence of any other steps in the complete process undergone by the marker molecule. In general, the problem is to isolate or separate the step of interest from all the other events. This has been done successfully in the case of gastric emptying (21). The unraveling of the rate-limiting step by mathematical analysis is still ongoing. A process of consecutive steps in which an entity undergoes an event resulting from a previous event in cascade may be described mathematically as a convolution. In a convolution, any part of the end result at a well-defined time needs that same amount of time to run through the two consecutive steps that make up the process. The total process is the sum of all the parts needing this total time, divided in any possible way over the separate steps. The action of isolating one of two events from the total is the reciprocal of convolution, called the "deconvolution." Deconvolution can be performed numerically. Separate events can be expressed as mathematical functions, so that the total process can also be expressed mathematically (61,62). This opens the way for the demonstration of physiologic or biochemical processes by unequivocal mathematical equations (8,21,54,63).

USE OF BREATH TESTS IN THE SEARCH FOR FUNCTIONAL FOODS

Many ways are found in which the methods described above could be used in food technology. These include monitoring food modifications to improve (or to inhibit) digestibility and absorption of food components; changing food composition to enhance energy uptake, protein quality, glycemic index, lipid profile, and so on; and testing foods that have been modified to improve their acceptability (the cephalic phase) by altering their consistency, smell, viscosity, and so forth.

New food technology can be directed toward the development of functional foods in relation to substrate utilization (64). One of the possibilities for making food
functional is to add or increase the concentration of a component with beneficial biological effects. These effects can be directed toward a function (e.g., immunologic or antioxidant) or toward a specific organ. The gastrointestinal tract is the main target organ for the development of functional foods (65). In gastroenterology, special attention is paid to the colon as the target organ for functional foods: the food then becomes a colonic food (i.e., a food ingredient not absorbed in the small intestine that reaches the colon and has health-enhancing properties). The human colon is an ecosystem, maintaining an equilibrium between harmful and beneficial bacteria, and between carbohydrates and proteins as substrates for bacterial fermentation (40). These balances are interlinked: excessive fermentation of proteins can yield toxic compounds (even co-carcinogens), whereas fermentation of carbohydrates by beneficial bacteria generates metabolites that are beneficial for the host. The former group includes ammonia, amines, mercaptanes, and phenolic compounds, whereas beneficial metabolites mainly consist of the short chain fatty acids, among which butyric acid constitutes an important metabolic fuel for colonic cells.

Colonic food that selectively promotes the growth of beneficial bacterial strains is called a prebiotic food. Polysaccharoses, specially polyfructoses (inulin), are considered to be prebiotics. Prebiotics are likely to have distinct advantages: stimulation of lactobacilli and bifidobacteria, production of short chain fatty acids, and removal of toxic compounds. Colonic food can also consist of viable bacteria, mainly lactobacillus strains; this is called probiotic food (66).

To investigate the potential of functional food, it is necessary to exert control over the main gastrointestinal functions. However, the gastrointestinal tract is a closed system with many different functions and it is inaccessible to direct investigation without disturbing normal physiologic events. $^{13}$CO$_2$ breath tests and the use of stable isotopes could be of great help in solving these problems. They allow measurement of the digestive and absorptive capacity of the small intestine, to demonstrate gastric emptying and small intestinal transit and to monitor metabolites formed by bacterial fermentation in the colon. These in vivo investigations can be completed by careful analysis of the feces for losses of food components, variations in bacterial population, and incorporation of tracers by bacteria. The proposed methods are non-invasive for the individual and risk free. They can be repeated as many times as necessary and, by centralizing the analytic unit, interlaboratory research is easily set up. Clinical investigation protocols can be established for the study of functional food in chronically ill patients with diseases confined to the gastrointestinal tract, especially the colon, such as constipation, ulcerative colitis, colon cancer, Crohn’s ileitis, and gastrointestinal fistulas. Patients with diseases that do not depend primarily on colonic events may also benefit from a healthy gastrointestinal environment, particularly patients with uremia.

GENERAL CONCLUSIONS

Our digestion-absorption laboratory in Leuven uses $^{13}$CO$_2$ breath tests to monitor a wide range of gastrointestinal and hepatic functions. In collaboration with clinicians, these tests are proven to be useful in medical practice. They are also well suited
to applications in nutritional and pharmaceutical research. Combined with the administration of molecules labeled with stable isotopes, $^{13}$CO$_2$ breath tests are a promising development in studies of general metabolism.

The mathematical expression of the results of breath tests is a new area for development and application. Mathematics is a welcome way for physiologists to demonstrate how scientific procedures can give reliable results.

REFERENCES


DISCUSSION

Dr. Zoppi: You said that it is impossible to distinguish between endogenous and exogenous nitrogen losses. Do you mean that typical metabolic balance studies with stool samples over 3 days are no longer useful?
Dr. Ghoos: If protein is not labeled, such studies are not useful.

Dr. Van-Daei: In your methodology for gastric emptying and orocecal transit time, you need to be sure that the marker is completely equilibrated with the food added to it. Are you 100% sure that octanoic acid is completely equilibrated with the formula, and that on acidification, a phase separation does not occur between the curd and the watery whey phase? If that happened, it would be possible for the lipid fraction to enter the small intestinal system in association with one of these two phases but not the other.

Dr. Ghoos: I am not completely sure, but we studied this using radioscintigraphy. We analyzed each step that occurs between eating the food and expiration of CO₂ in the breath, and we ended up with an almost 100% coincidence between the breath test and radioscintigraphy results. This work was published in the *American Journal of Physiology* (1).

Dr. Mansbach: You said that the treatment of the fat before ingestion has an effect on gastric lipase or on the gastric phase of digestion. Could you elaborate on that?

Dr. Ghoos: I can only deal with this in an indirect way. If triglycerides are mixed in saturated butter fat (i.e., as a solid meal when taken), then practically no labeled CO₂ is in breath until 2 hours after intake of the meal. But, if the same label is incorporated in chocopaste, which is a viscous semiliquid that can be smeared on bread, then labeled CO₂ appears in as short a time as 1 hour.

Dr. Roy: Could you discuss the technique for assessing bacterial overgrowth? Do you rely on early rise versus late rise in the CO₂ in breath, or do you have any markers associated with your stable isotope?

Dr. Ghoos: I have been looking for a breath test for bacterial overgrowth for more than 20 years, but in fact there is none. Using a breath test, discrimination cannot be done between bacterial overgrowth and accelerated small intestinal transit. If you really want to look for bacterial overgrowth, then a tube must be inserted. That is the only way to be sure.

Dr. Lentze: In the early studies on the lactose-ureide test for determining orocecal transit time it was found that when volunteers were given unlabeled lactose-ureide in high dose on day 1 and then given the labeled compound on day 2, the apparent transit time was faster than when the subjects were not preloaded. Has that puzzle been solved?

Dr. Ghoos: Normal flora does not have the capacity to split urea immediately from the lactose molecule. This means that to do the test for orocecal transit time, bacterial enzymatic activity must be activated the day before, so we now give one dose of 500 mg of the unlabeled lactose-ureide the evening before the test (2).

Dr. Koletzko: Would you just comment on the reproducibility or the intraindividual variation of this new test of transit time and protein digestion? Even used as a liver function test, we found reproducibility rather disappointing both in healthy children and in children with liver disease.

Dr. Ghoos: For gastric emptying, the coefficient of variation is 19%, and we are happy with that as it is less than for radioscintigraphy. For orocecal transit time, it is of the same order. Unfortunately, not many studies of orocecal transit time have been done by radioscintigraphy: it is difficult to determine the exact time that the chyme enters the colon using that technique.

Dr. Alpers: CO₂ in the breath is many steps removed from protein digestion and will probably be very dependent on the particular amino acid labeled. You intimated that you have, or are going to have, data in the blood immediately afterward. Do you have those data, is the amino acid still intact, and does that correlate differently or better with the CO₂ excretion? (3).

Dr. Ghoos: With respect to [¹³C]leucine, in normal individuals, a steep rise in [¹³C] occurs
in the serum and about 14 minutes after that the \( \text{CO}_2 \) excretion peaks. In patients with malabsorption, that steep rise of the \( ^{13}\text{C} \) does not occur, but it increases more slowly to a plateau value, and the \( \text{CO}_2 \) peak is less than the plateau value. In patients with pancreatic deficiency, a relatively larger rise is seen in \( ^{13}\text{C} \) leucine in the blood than would be expected from the expiration of \( ^{13}\text{C} \text{CO}_2 \) in breath. That is the first enigma. The second enigma in malabsorption is that after 6 hours, when the chyme would normally already be in the colon, the plateau of \( ^{13}\text{C} \) in the blood is still present, although \( \text{CO}_2 \) excretion is practically normal; in fact, the plateau of \( ^{13}\text{C} \) leucine is higher than found in normal individuals (4). We do not at present understand the reason for these two enigmas. I realize a lot of work remains to be done on the metabolism of these labeled amino acids. Until now, we have been discussing only \( ^{13}\text{C} \) leucine, but what happens to nitrogen? A very fine review by Reeds in the *Annual Review of Nutrition* relates to the recycling of nitrogen in the gut (5). Anybody interested in nitrogen metabolism in the gut should read it.

*Dr. Alpers:* But your preliminary results are consistent with the idea that if a large amount of amino acid is taken in that is not needed for protein synthesis, then a lot of it will be transaminated, and so the difference between what is found in the blood and what comes out as \( \text{CO}_2 \) may be very dependent on the nutritional status of the patient.

*Dr. Ghoos:* You are right. When investigating the catabolic state of an individual, we cannot use \( \text{CO}_2 \) and blood analyses alone; we need to combine those tests with other biological markers.

*Dr. Seidman:* Your data suggested that raw egg proteins are less well digested than cooked egg. What is the mechanism? Have you applied this to other proteins? For example, the proteins in milk are routinely consumed raw.

*Dr. Ghoos:* Until now, we have not studied milk proteins. As to the mechanism of reduced digestion, consider what happens when you try to pick up a cooked egg and a raw egg in your hand. I think the enzymes in the duodenum have the same problem as you have with your hands.

*Dr. Seidman:* I think that is too simplistic! Could there be some other explanation than digestion? Is it possible that the subjects who took the raw protein did not tolerate it, and vomited the protein?

*Dr. Ghoos:* I do not think so. But we are dealing with human beings. And as I mentioned at the start, executing breath tests is not always straightforward. Certainly in cases of malabsorption or intolerance of carbohydrates, abdominal complaints could be provoked, so you need to remain nearby the patient.

**REFERENCES**


