$^{13}CO_2$ Breath tests and stable isotopes for investigating gastrointestinal functions

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(Acta gastroenterol. belg., **2000**, 62, **325-327**).

Key words : gastrointestinal function, breath test, digestion, absorption, fermentation, transit, stable isotopes.

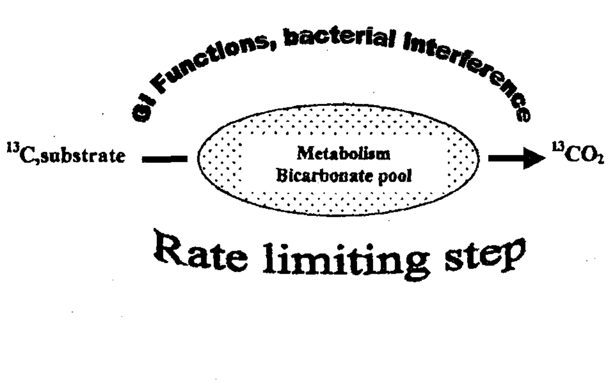
Introduction to breath tests

The $^{13}CO_2$ breath test (BT) is a non-invasive and reliable method to study the main gastrointestinal (GI) functions including the assimilation of food ingredients. Stable isotopes offer the possibility to monitor various metabolic events, including the fermentation processes in the colon.

The principle of $^{13}CO_2$ BT is the following :

Breath tests have the common characteristics that to an individual a substrate is administered, that bears the functional group in which a normally present ^{12}C atom has been replaced by the stable isotope ^{13}C. This functional group is cleaved enzymatically under specific circumstances, either during the transit through the gastro-intestinal tract, either during the absorption, or during further metabolism of the absorbed substrate. After cleavage the marked subgroups undergo a metabolic process that ends with expiration of the labelled CO_2. It is necessary that the speed determining (rate limiting) factor of the whole physiologic process is directly related to the genesis of $^{13}CO_2$. The $^{13}CO_2$ mixes with the body pool of CO_2 - HCO^-_3 and is breathed out. In this way the exhalation of $^{13}CO_2$ reflects the function to be investigated.

Schematically this process can be represented as follows :



The ^{13}C,substrate has to be chosen in such a way that the enzyme/function/bacteria is the rate-limiting step in $^{13}CO_2$ evolution to demonstrate by $^{13}CO_2$ measurement either enzyme activity, either a well-defined GI function, either bacterial metabolism.

When the excretion of the tracer in breath is expressed as % dose per hour and/or as cumulative % dose excreted over a defined time period, a dynamic analysis of the examined parameter of the GI tract is obtained in course of time.

$^{13}CO_2$ breath tests may be considered excellent investigation methods, as their scientific bases are sound and well-conceived, the results have been validated in an unequivocal way and their applications are accepted by an increasing number of scientists.

$^{13}CO_2$ breath tests can be combined with the ^{14}C,tracer or/and the H_2 measurement in breath. Additional information on the fermentation processes in the colon is obtained by labelling bioactive molecules with nitrogen-15 (^{15}N) and deuterium (^{2}H).

There is a call for non-invasive methods, which parallels the information of the classical methods, which are simple to perform and which can be executed at lower costs. $^{13}CO_2$ breath tests may meet these calls. They show great advantages over conventional methods as the gastrointestinal function can be displayed in course of time. Furthermore they are not invasive for the patient, and can even be performed at home. The medical doctors ask for tests that can be done in a repetitive way without major discomfort or radiation hasard for the patient and without long-lasting occupation of equipment and personel. In no way, however, it is claimed that $^{13}CO_2$ breath tests are exclusive tests. The breath tests have to be considered as important pieces of the clinical investigation puzzle. Therefore it is mandatory that interpretation of the test result should be done in close discussion with the medical doctor in the clinical unit.

Special attention should be paid to the execution of $^{13}CO_2$ breath tests. These tests seem very simple to perform, and some investigators even try to render the test

This lecture is part of an extensive presentation at the 46th Nestlé Nutrition Workshop "Gastrointestinal Functions", Montreal 26-30 Sept. 1999.

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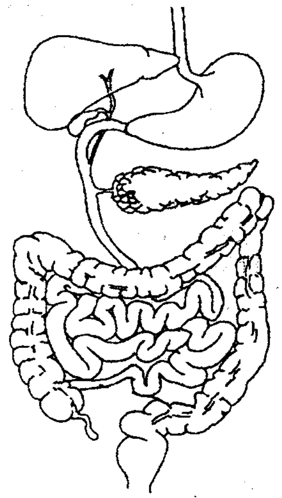
more simple by reducing the sample numbers or by changing other test conditions (meal, sampling time, calculations of results...). These modifications could lead to false interpretation of test results, and make them unsuitable to be used for interlaboratory comparison. The best way to safeguard uniformity in test design is to do breath tests in a *specialised clinical unit*, in analogy with other clinical units which dispose of their own examination facilities, e.g. radioscintigraphy, radiology...

Breath tests

At the laboratory "Digestion - Absorption", University Hospital Gasthuisberg, Leuven, the following BT's are used in clinical pratice.



Substrates used in $^{13}CO_2$ breath tests

Hepatic functions :

- demethylating and oxidative capacity : [^{13}C]aminopyrine (1)
- hepatic mass : [^{13}C]galactose (2)
- mitochondrial activity : [^{13}C]keto-isocaproic 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-, fermentative functions

- carbohydrates : [^{13}C]naturally enriched compounds, starch, lactose (10-12)
- lipids : [^{13}C]mixed triglyceride (13,14)
- proteins : [^{13}C],[^{15}N]egg-white proteins (15)
- fermentation process : lactose-[^{15}N]ureid (16) ; [^{15}N],[^{2}H]proteins (17)

Bacterial overgrowth - bile acid malabsorption

- the only [^{14}C]substrate in use, i.e. glycocholic acid + 3 days fecal collection + [^{3}H] PEG transit marker correction (18-20)

Note : mathematical expression of the functions

- an elegant method has been developed to express the meaning of GI events by mathematical formula (21,22)

Further developments are currently studied to express different gastrointestinal functions by mathematical analysis when several markers are used simultaneously. In the near future we will try to make it possible to measure by a single meal gastric emptying ([^{14}C]octanoic acid), protein digestion ([^{13}C]leucine-protein), orocecal transit time and small intestine half emptying time (hydrogen marker+mathematical convolution/deconvolution method) and fermentation pattern ([ring,^{2}H]-phenylalanine-protein, lactose-[^{15}N]ureid), plus fecal protein loss ([^{15}N]leucine-protein).

Apart from the ^{14}C,tracer (estimated dose 0.2mSv) these methods can be applied in children also, and they are very suitable to be used for nutritional and pharmacological research.

These elegant studies have been made possible only by close collaboration with the medical doctor in the hospital. Even when the tests are executed routinely for diagnostic purposes, it is a constant reflection on how helpfull these tests can be for the individual patient.

How attractive the test designs might be, standardisation of test execution and harmonisation of test protocols are needed for collaboration and for interlaboratory comparison of results. First attempt has been made by the european concerted action BIOMED PL93-1239, which has been co-ordinated by the laboratory. The results of these collaborative efforts have been entirely published (23). The on-going evolution in mathematical analysis of test results will contribute markedly to the better understanding of basic gastrointestinal processes in the individual. The mathematical expression of their results represents a new area for forthcoming application and further development. Mathematics are a welcome exposition of physiologists view of how the process of science can lead to reliable results, fantastic as those often seem to be.

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