

¹³C–Breath Tests in Medical Research and Clinical Diagnosis

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1. Introduction

1.1 The Nature of ^{13}C -Breath Tests

Initially focused on investigating infants and pregnant women with their particularly high risks of being exposed to energy rich radiation, breath tests using the stable isotope ^{13}C instead of its radioactive counterpart ^{14}C nowadays have replaced ^{14}C -breath tests almost completely. It is common to the majority of ^{13}C -breath tests that a certain amount of the ^{13}C -labelled substrate is (usually orally) applied to the test person on an empty stomach in the early morning. (If a naturally labelled substrate is to be applied the individual should be adjusted to a constant ^{13}C content as much as possible differing from the ^{13}C content of the substrate by administering an appropriate diet. (If the substrate is synthesised from C_4 -plants with their Hatch-Slack cycle of photosynthesis the diet should preferentially consist of food from C_3 -plants with their Calvin cycle and their lower ^{13}C content when compared with C_4 -plants, and vice versa.)

The substrate contains one or more functional groups labelled with ^{13}C , the carbon of which is cleaved in the test person's organism in the course of enzymatic reactions like oxidation, decarboxylation or hydrolysis and directly or via intermediate metabolites exhaled in the form of [^{13}C]carbon dioxide. If the metabolic reaction to be investigated is the rate determining step for the elimination of carbon dioxide with breath the rate of this step and hence the rate of [^{13}C]carbon dioxide elimination reflects the actual metabolic situation with respect to that particular metabolic reaction.

The ^{13}C -contents in breath are usually measured mass spectrometrically or infrared spectrometrically. Infrared spectrometric measurement is conventionally done in the form of non-dispersive infrared spectrometry which is distinguished by simplicity, high serviceability and accuracy. In this variant of infrared spectrometry the infrared radiation is not dispersed into its spectral components by a prism, a grating or an interferometer. Selectivity for $^{13}\text{CO}_2$ or $^{12}\text{CO}_2$, respectively, is rather achieved by means of detectors filled with the isotopically pure $^{13}\text{CO}_2$ or $^{12}\text{CO}_2$. As far as further details of sampling, storing, transport and measuring the breath samples are concerned, we refer to K. Wetzel and H. Fischer: Recent Results of the Development and Application of ^{13}C -Breath Tests, Fischer ANALYSEN Instrumente GmbH, Leipzig 1999.

For some ^{13}C -breath tests it is sufficient to measure the ^{13}C -content of exhaled carbon dioxide at the time $t = 0$ and at only one time t after having taken in the substrate and to draw the diagnostic conclusion from the increase of the ^{13}C -content $a(t)$ during this period of time t . Many other ^{13}C -breath tests are distinguished by measuring the ^{13}C -content $a(t)$ for several or even many hours every ten to thirty minutes and plotting them into an $a(t)$ versus t diagram. Then also quantitative data can be evaluated on parameters like rate constants of digestion, absorption or cleavage of the applied substrate, half life times of gastric emptying or duration of the lag-phase.

It has been proved in hundreds of publications and conference presentations on the application of ^{13}C -breath tests in medical research and clinical diagnosis that such tests yield deep insights into metabolic processes and diagnostic results of high sensitivity and specificity to gastrointestinal, liver, pancreatic and other diseases. The latest summarising presentation of this field was given by the authors of this booklet in their monograph "Recent Results of the Development and Application of ^{13}C -Breath Tests" published by Fischer ANALYSEN Instrumente GmbH in December 1999.

1.2 Terminology of Tracer Investigations with Compounds Labelled with Stable Isotopes

Understanding and comparability of results of clinical and other investigations with stable isotopes is complicated, because there is no uniform, commonly accepted measure of the content of a stable isotope like ^{13}C in some substance or chemical compound. On the contrary at least five measures are used simultaneously. In the following the term ' ^{13}C -content' will be applied as generic term for the different names for the share of the stable isotope ^{13}C in some substance or in some site of a molecule. The (relative) isotopic abundance a is defined as the quotient of the amount of the isotope under consideration (in mol) divided by the total amount of the element (in mol), in case of carbon the sum of the amounts of ^{12}C and ^{13}C . (The amount of the radioactive isotope ^{14}C , which also occurs in nature, can be neglected in comparison to ^{12}C and ^{13}C .) A hundred times the abundance a is named isotopic abundance in atom%. The enrichment of an isotope, usually an isotope less abundant in nature, in comparison to its natural abundance is commonly given in the form of its excess abundance (in atom% excess) which is the difference abundance a minus natural abundance a_0 .

Chemical compounds with more than one carbon atom in their molecules are called uniformly labelled, if all their carbon atoms have the same or approximately the same isotopic composition deviating from natural isotopic composition. This is indicated by placing a capital "U" connected with a hyphen before the name of the respective chemical compound (or the site in a certain molecule).

If there is only a small deviation of the relative abundance of the isotope under consideration from its natural abundance, like in case of tracer experiments connected with high isotope dilution and in case

of isotope geochemistry, instead of the isotope abundance or excess abundance frequently the so-called δ -value of the isotope is given, i. e. the relative deviation of the abundance a_{sample} from the abundance a_{standard} of some standard in permil:

$$\delta = \left[\frac{a_{\text{sample}} - a_{\text{standard}}}{a_{\text{standard}}} \right] \times 1000\text{‰}$$

For carbon the so-called PDB standard is valid, the calcium carbonate of the fossil Belemnitella of the Pee Dee Formation in South Carolina with $a_0 = 1.1112372$ atom% corresponding to $\delta^{13}\text{C} = \pm 0.00000$. From that follows:

$$a = a_0 (1 + \delta^{13}\text{C}/1000) = 1.11123 (1 + \delta^{13}\text{C}/1000)$$

for the conversion of δ -values $\delta^{13}\text{C}$ into relative abundances a in atom%.

Tab.1 presents some natural substances and products prepared from natural foodstuffs together with their $\delta^{13}\text{C}$ -values.

Substance	$\delta^{13}\text{C}$-value [‰]
<u>Limestone:</u>	+4.9 — -4.9
<u>PDB Standard:</u>	± 0.00000
<u>Atmospheric carbon dioxide:</u>	-6.1 — -9.1
<u>Plants:</u>	
C ₃ -plants	-20.4 — -25.9
C ₄ -plants	-9.2 — -14.6
<u>Human breath:</u>	
American	-16.4 — -21.0
Japanese	-19.9 — -22.5
European	-19.8 — -24.3
<u>Carbohydrates:</u>	
Beet sucrose	-22.3
Cane sucrose	-12.9
Maize starch	-11.8
Potato starch	-26.4
<u>Proteins:</u>	
Casein	-24.5
Soy	-23.4
<u>Lipids:</u>	
Maize oil	-14.8
Soy oil	-23.4
<u>Natural oil and gas:</u>	-21 — -69

Tab. 1. Natural substances and products prepared from natural substances together with their $\delta^{13}\text{C}$ -values. (All $\delta^{13}\text{C}$ -values refer to PDB standard, the calcium carbonate of the fossil Belemnitella of the Cretaceous Pee Dee Formation in South Carolina, USA.)

Frequently isotope abundances are given as excess abundances a_{excess} , i. e. as the difference between the measured relative abundance a in breath and the abundance a_0 in the PDB standard:

$$a_{\text{excess}} = a - a_0 = (a - 1.11123) \text{ in atom\%}$$

In ^{13}C -breath tests excess abundance means the difference between the measured relative abundance a in breath and the abundance $a_{t=0}$ in breath immediately before tracer intake:

$$a_{\text{excess}} = a - a_{t=0}$$

In an analogous way δ -values are often given in the form of their deviations DOB (DOB = delta over baseline) from the $\delta^{13}\text{C}$ values δ_{baseline} measured before the administration of the labelled substrate:

$$\text{DOB} = \delta - \delta_{\text{baseline}}$$

Especially if the chemical element, like in case of carbon, consists of two stable isotopes, the enrichment is often given as isotope ratio, i.e. as quotient of the amounts of these isotopes (measured in mol), with the less abundant isotope either in the numerator or in the denominator.

1.3 Evaluation of ^{13}C -Breath Tests

There is a variety of ways for presenting and evaluating ^{13}C -breath test results. The most common ones are presented in the following.

- 1) The percentage ^{13}C -dose recovery per hour (PDR) is defined as the expired ^{13}C -dose per hour in % of the administered ^{13}C -dose:

$$\%^{13}\text{C dose/h} = (\text{C - excess in breath} / \text{C - excess administered}) \times 100 \text{ in \%}$$

- 2) The cumulative percent ^{13}C -dose recovery (CPDR) is defined as the total ^{13}C -dose eliminated with breath during a certain time after tracer administration in % of the tracer intake. For determining both CPDR and PDR CO_2 -production must be calculated by means of the equations:

$$\text{CO}_2 - \text{production} = 300 \text{ mmol} / (\text{BSA} \cdot \text{h}),$$

where

$$\text{BSA} = 0.024265 \cdot W^{0.5378} \cdot H^{0.3964}.$$

In the latter formula W means the body weight in kg and H the body height in cm.

- 3) In case of investigating gastric emptying using ^{13}C -breath tests two approaches are available (Y. Ghoo (1996): ^{13}C -breath tests at the laboratory "Digestion – Absorption" of the University Hospital Gasthuisberg, Leuven, Belgium).

The first model is derived from the χ^2 -distribution in statistics:

$$\%^{13}\text{C dose/h} = a \cdot t^b \cdot e^{-ct},$$

where t means the time and a, b and c are parameters to be determined by non-linear regression analysis. The expression $\ln a$ is called gastric emptying coefficient:

$$\text{GEC} = \ln a$$

GEC is reliable for describing the rate of gastric emptying.

The second approach takes the cumulative percent ^{13}C -dose recovery (CPDR), i. e. the total ^{13}C -dose eliminated with breath during a certain time after tracer administration in % of the tracer intake, as a starting point:

$$\text{cumulative percent } ^{13}\text{C-dose recovery CPDR} = m \cdot (1 - e^{-kt})^\beta$$

with the time t and the total cumulative percentage m of the recovered dose. The coefficients m, k and β are determined by non-linear regression analysis.

Since the data of the CPDR-curve are obtained from PDR-values by numerical integration, deriving the above given CPDR-equation in time yields an equation for PDR:

$$\text{PDR} = m \cdot k \cdot \beta^{-kt} \cdot (1 - e^{-kt})^{\beta-1}$$

Thus a non-linear regression analysis can be performed on the originally measured data to obtain values for m, k and β for each ^{13}C -breath test.

Taking CPDR equal to m/2 in the above equation yields the half-life of gastric emptying (HLF):

$$\text{HLF} = (-1/k) \cdot \ln(1 - 2^{-1/\beta})$$

It is the subject of the present booklet to describe the ^{13}C -breath tests known at present with respect to their medical and clinical relevance, to the metabolism of their substrates and to the procedure of carrying them out. As for the techniques of breath sampling and measuring their isotopic composition, which are common to all ^{13}C -breath tests, we refer to the above mentioned monograph.

The ^{13}C -breath tests are primarily arranged according to the anatomical areas in which they are applied for investigating metabolic processes and diagnosing corresponding diseases, namely:

- ^{13}C -breath tests for investigating processes and diagnosing diseases in the gastric and duodenal area
- ^{13}C -breath tests for investigating exocrine pancreatic function and diagnosing pancreatic diseases
- ^{13}C -breath tests for investigating liver function and diagnosing liver diseases
- ^{13}C -breath tests for investigating processes and diagnosing intestinal diseases in jejunum, ileum, caecum and colon.

Within these four sections the tests are preferentially arranged alphabetically according to the initial letter of the substrate used. If the test can be applied in more than one anatomical area, it is described in the first relevant section of the above list. (In the particular lists of references the quotations are arranged according to their year of publication.) Otherwise we refer to the table of contents.

The description of each test is divided into five parts, the first part characterizing the relevance of the respective test to medical research and diagnosis, the second part the metabolism of the substrate, the third one the procedure of carrying the test out. The diagnostic validity is described in the fourth part, while the fifth part contains references of the most important publications describing the indications and the procedures of carrying out the respective tests.

At the end of the first part of the description of each test the reader finds an estimation regarding the suitability of the particular test for clinical diagnosis. The basis of this estimation is Tab. 2. If the total value of the test according to this table is at least twelve, the suitability is described as excellent, if it is ten or eleven, we denominate it as good. Tests with total values in the range of seven to nine are regarded as satisfactory. The suitability for clinical diagnosis of tests with a total value of less than seven is usually described as controversial.

In the third part („Procedure“) of the description of the individual tests (chapters 2, 3, 4 and 5) we present a proposal for carrying out suitable tests under clinical routine conditions.

In case of ¹³C-breath tests with the substrates acetate, aminopyrine, galactose, glucose, glycocholic acid, lactose, leucine, methacetin, mixed triglyceride, octanoic acid, trioleine, urea and xylose the software of our ¹³C-analysers FANci2 or HeliFANplus, respectively, leads the user also with respect to the activities described in the third part of the description. Corresponding explanations are given in the last passage of this part.

2. ¹³C–Breath Tests for Investigating Processes and Diagnosing Diseases in the Gastric and Duodenal Area

[1-¹³C]ACETATE BREATH TEST

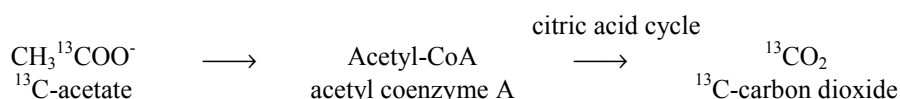
Indication / Relevance to Medical Research and Diagnosis:

[¹³C]acetate breath test is recommended for the evaluation of gastric emptying of liquid meals, particularly in diabetic patients with symptoms of gastroparesis. In case of semiliquid test meals the [¹³C]acetate breath test serves to determine the gastric emptying rate of the liquid constituents in that test meal. The test is also useful in paediatrics. Aldehyde dehydrogenase deficiency does not considerably affect ¹³C-excretion with breath.

Suitability for clinical diagnosis: excellent (for evaluating gastric emptying of liquid meals)

Metabolism of Substrate:

Citric acid cycle transforms [1-¹³C]acetate via acetyl-CoA into carbon dioxide and water:



Procedure:

After a nocturnal fasting period 100 or 75 mg of sodium[1-¹³C]acetate (90% ¹³C) are given together with a 370 kcal semiliquid test meal consisting of 100 ml of coffee, in which the tracer is dissolved, 20 ml of milk, 100 ml of orange juice, 45 g of mixed-grain bread, 20 g of butter and two scrambled eggs (370 kcal). This test meal should be taken within 15 minutes. Breath samples are taken at the beginning of the test and then in 10 minute intervals over two hours.

For measuring gastric emptying time under clinical routine conditions we propose to take the results of Pfaffenbach B, Schaffstein J, Adamek RJ et al. (1996) and Biskup H, Heine E and Wutzke KD (1999) as a starting point and to proceed as follows: After a nocturnal fasting period 100 or 75 mg of sodium[1-¹³C]acetate (90% ¹³C) are given together with a 370 kcal semi liquid test meal consisting of 100 ml of coffee, in which the tracer is dissolved, 20 ml of milk, 100 ml of orange juice, 45 g of mixed-grain bread, 20 g of butter and two scrambled eggs (370 kcal). The test meal should be taken within 15 minutes. On a trial basis breath samples could be collected immediately before and then 20, 40, 60, 80, 100, 120 and 180 minutes after tracer intake. In normals DOB values can be expected to pass a maximum 60 to 120 minutes after tracer intake.

The software of FANci2 or HeliFANplus, respectively, leads the user according to the following guidelines: (After a nocturnal fasting period) a dose of 2.0 mg per kg body weight sodium [1-¹³C]acetate (99.0 atom% ¹³C) (together with a 370 kcal semiliquid test meal consisting of 100 ml of coffee, in which the tracer is dissolved, 20 ml of milk, 100 ml of orange juice, 45 g of mixed-grain bread, 20 g of butter and two scrambled eggs) is given. Including the basal sample taken immediately before tracer intake 17 breath samples should be collected at 15 minute intervals. FANci2 or HeliFANplus, respectively, then displays the gastric emptying coefficient (GEC)* of the investigated individual.

* Following the χ^2 -distribution in statistics gastric emptying coefficient can be defined as:

$$\text{GEC} = \ln a,$$

where a is a parameter to be determined by non-linear regression analysis of the function:

$$\%^{13}\text{C dose/h} = a \cdot t^b \cdot e^{-ct}$$

with the time t and the parameters b and c as well as a to be determined by nonlinear regression analysis. The percentage dose per hour (¹³C% dose/h) is defined as the expired ¹³C-dose per hour in % of the administered ¹³C-dose:

$$\%^{13}\text{C dose/h} = (\text{¹³C - excess in breath} / \text{¹³C - excess administered}) \times 100 \text{ in \%}$$

Diagnostic Validity:

For gastric emptying rate of liquids [¹³C]acetate breath test clearly correlates with ^{99m}Tc-scintigraphy (r = 0.80; p < 0.001). Four of five patients with delayed gastric emptying by scintigraphy also showed delay in the breath test.

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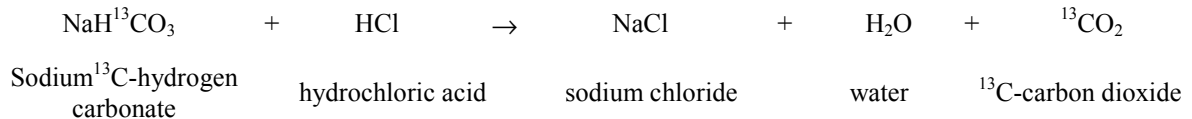
[¹³C]BICARBONATE ([¹³C]HYDROGEN CARBONATE) BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

The ¹³C-bicarbonate breath test is a good means of measuring gastric emptying, preferentially of liquid and semi-liquid meals, of measuring energy expenditure while walking or cycling and of diagnosing atrophic gastritis. Furthermore the test can be used for studying the effects of infused amino acids or lipids on glucose metabolism and for investigating metabolic processes under the conditions of total parenteral nutrition. In view of the low physical strain connected with its implementation the ¹³C-bicarbonate breath test is also employed for determining basal metabolic rate, especially in seriously ill individuals.

Suitability for clinical diagnosis: satisfactory.

Metabolism of Substrate:



Procedure:

Fasting subjects receive 250 mg NaH¹³CO₃ incorporated in the liquid or solid meal to be assessed with respect to its gastric residence time. Immediately after consuming a solid meal subjects drink 300 ml of distilled water. Breath samples are taken at 10-min intervals for 120 min. For investigating metabolic processes under the conditions of total parenteral nutrition see Bresson JL, Mariotti A, Narcy P et al. (1990).

Diagnostic Validity:

The test clearly differentiates between gastric emptying of liquid and solid meals. In combination with the administration of glucose and amino acids or lipids, respectively, the test can be used for studying the effects of infused amino acids or lipids on glucose metabolism. Recovery of ¹³C in breath from infused NaH¹³CO₃ increases during euglycaemic hyperinsulinaemia.

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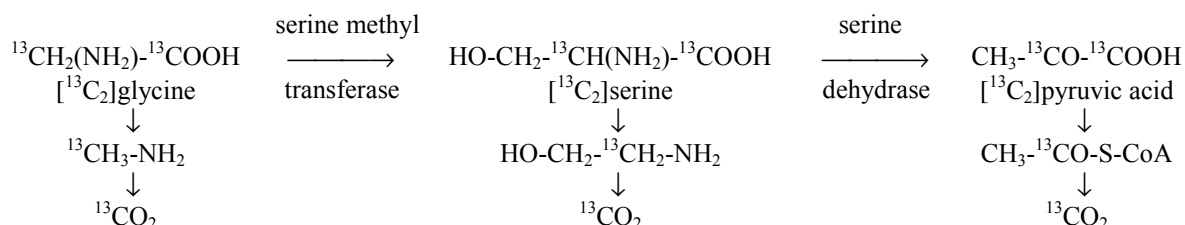
[¹³C₂]GLYCINE BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

The ¹³C-glycine breath test is useful for studying amino acid and protein metabolism and for measuring gastric emptying, particularly for discriminating between different inborn disorders of amino acid metabolism. Especially in combination with the ¹⁴C-octanoic acid breath test the ¹³C-glycine breath test is used for studying the influence of octreotide, a long-acting synthetic octapeptide analogue of somatostatin, on the gastric emptying of solids and liquids.

Suitability for clinical diagnosis: satisfactory.

Metabolism of Substrate:



Procedure:

After an overnight fast the test meal is ingested within a period of 10 minutes. It consists of 60 g of white bread and an egg, the yolk of which is baked separately from the egg white. The subjects then drink 100 mg of [1-¹³C]glycine (99% ¹³C) dissolved in 150 ml of water. Breath samples are collected immediately before tracer intake and then every 15 minutes for four hours.

Diagnostic Validity:

The combined ¹⁴C-octanoic acid/¹³C-glycine breath test is suited to demonstrate that subcutaneous injection of a single physiological dose of octreotide induces a marked delay in gastric emptying of both solids and liquids in young healthy volunteers, especially since the absorption and metabolism of both substrates remains unaltered after administration of octeotride.

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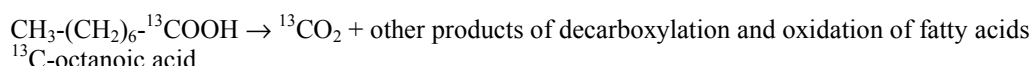
[1-¹³C]OCTANOIC ACID ([1-¹³C]OCTANOATE) BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

Like [1-¹³C]palmitic acid breath test [1-¹³C]octanoate breath test can be used for estimating fatty acid oxidation, particularly medium chain fatty acid oxidation, for evaluating mitochondrial function during stress situations, e. g. alcohol-induced oxidative stress, for studying gastric emptying, especially for gastric emptying of solids, and for investigating the influence of special pharmaceuticals like cisapride, octreotide, capsaicin or N-dimethyl-N-isopropyl-8,9-anhydroerythromycin A 6,9-hemiacetal on gastric emptying. Particularly diabetes mellitus and gastrotonia patients are investigated with this test. The [1-¹³C]octanoate breath test can also be applied in pediatry, particularly for investigating patients treated with valproic acid and for diagnosing preterm infants. (Valproic acid [dipropyl acetic acid] is an anticonvulsant drug with severe side effects.) In addition the influence of physical exercise on fat metabolism can be studied by this test.

Suitability for clinical diagnosis: good

Metabolism of Substrate:



Procedure:

Individuals orally receive 3.5 mg/kg body mass of [1-¹³C]octanoate or a test meal consisting of 100 mg [1-¹³C]octanoate in one scrambled egg, 50 g of mixed-grain bread, 20 g of butter and 200 ml of orange juice (total caloric value of 280 kcal). Breath samples are taken immediately before the meal and then every 10 or 15 min for 4 or 6 hours, respectively. Other authors collected breath samples every 15 minutes during the first hour and then every 30 minutes during the following two hours. For investigating side effects of valproic acid in epileptic children 1.0 mg/kg body mass of the tracer (90 atom% excess) are administered after a 14 to 15 hours fast. Breath samples are obtained at 15 minute intervals during the first hour and then at 30-minute intervals for the following two hours, the investigated individuals resting quietly during the test.

Recently Choi M-G, Camillery M, Burton DD et al. (1998) repeatedly troubled for simplifying and enhancing reproducibility of the test: Persons to be investigated received an omelette consisting of two egg whites and one yolk dosed with 100 mg [1-¹³C]octanoate. The omelette was placed on a slice of whole-wheat bread and given with a glass of skimmed milk for a total caloric value of 240 kcal and a nutrient composition of 35 % protein, 25 % fat, 40 % carbohydrate and 2.5 g of fibre. The reproducibility of determining gastric emptying time revealed to be as high as scintigraphy with ^{99m}Tc-pertechnetate, if twelve breath samples were collected during a period of six hours. Other attempts to further simplifying the test in order to introduce it into clinical routine application yielded controversial results so far.

The software of FANci2 or HeliFANplus, respectively, leads the user according to the following guidelines: (After a nocturnal fasting period) a dose of 2.0 mg per kg body weight [1-¹³C]octanoic acid (99.0 atom% ¹³C) is taken in (together with a test meal consisting of one scrambled egg, 50 g of mixed grain bread, 20 g of butter and 200 ml of orange juice (total caloric value of 280 kcal)). Including the basal sample taken immediately before tracer intake 17 breath samples should be collected at 15 minute intervals. FANci2 or HeliFANplus, respectively, then displays the gastric emptying coefficient (GEC)* and the half-life of gastric emptying (HLF)** of the investigated individual. If GEC ≥ 2.8 after 4 h, then normal gastric emptying kinetics can be assumed. GEC < 2.8 points to delayed gastric emptying.

* Following the χ^2 -distribution in statistics gastric emptying coefficient can be defined as:

$$\text{GEC} = \ln a ,$$

where a is a parameter to be determined by non-linear regression analysis of the function:

$$\%^{13}\text{C dose/h} = a \cdot t^b \cdot e^{-ct}$$

with the time t and the parameters b and c as well as a to be determined by nonlinear regression analysis. The percentage dose per hour (¹³C% dose/h) is defined as the expired ¹³C-dose per hour in % of the administered ¹³C-dose:

$$\%^{13}\text{C dose/h} = (\text{¹³C - excess in breath} / \text{¹³C - excess administered}) \times 100 \text{ in \%}$$

GEC is reliable for describing the rate of gastric emptying.

**The half-life of gastric emptying (HLF) is defined as the time when $c = c_0/2$.

Diagnostic Validity:

Low interindividual and moderate intraindividual reproducibility. Comparison of Isotope Ratio Mass Spectrometry (IRMS) and Non-Dispersive Infrared Spectrometry (NDIRS) applied to the test yielded good correlation of results both for gastric emptying time ($r^2 = 0.918$) and duration of lag phase ($r^2 = 0.924$). Results of gastric emptying of solids are as reliable as ultrasonography or radioactive labelling of certain components in feces.

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Indication / Relevance to Medical Research and Diagnosis:

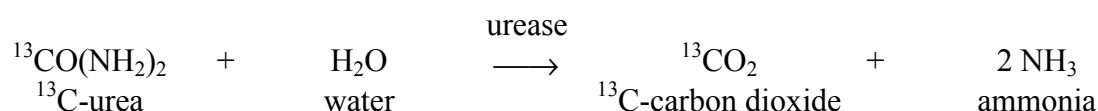
The [¹³C]urea breath test is indicated when symptoms of gastrointestinal disorders and gastroduodenal diseases like gastritis, gastric and duodenal ulcers, gastric adenocarcinoma or gastric lymphoma are observed. The test is considered to be the golden standard for diagnosing *Helicobacter pylori* infection.

There are controversial indications that this infection might also induce nonulcer dyspepsia, coronary heart disease, pernicious anaemia and migraine. The test is also suitable for the investigating or screening of asymptomatic persons, esp. individuals with elevated blood pressure. (It has been proven that *Helicobacter pylori* infection can induce elevated blood pressure.) The test is also suitable for (semi)-quantitative diagnosis.

Suitability for clinical diagnosis: excellent.

Metabolism of Substrate:

Helicobacter pylori produces urease, an enzyme which cleaves urea to form carbon dioxide and ammonia according to the equation:



Fast increase of ¹³C in exhaled breath indicates *Helicobacter pylori* infection.

Procedure:

Immediately after sampling the patient's breath after an overnight fast the patient receives orally 75 mg ¹³C-urea (99% ¹³C) dissolved in about 250 ml orange juice. 20 or 30 minutes after tracer intake a second breath sample is taken. (If the tracer amount is enhanced to 100 mg ¹³C-urea, the time interval between the first and the second breath sampling can be shortened to ten minutes without loss of diagnostic accuracy.) The difference between the δ-values of the two samples, the so-called delta over baseline (DOB) value, is the diagnostic criterion, the cut-off value being 3 to 5 ‰. For children with their higher endogenous carbon dioxide production a cut-off value of 3.5‰ has to be assumed.

Use of aqueous ¹³C-urea solutions, enlarging tracer dose to 100 mg ¹³C-urea, application of gelatine-capsuled substrate or rinsing oral cavity after tracer intake in order to prevent pre-gastric urea hydrolysis, shortening or prolonging the period of time between first and second breath sampling or substitution of orange juice by 0.1 N citric acid solution do not appreciably affect diagnostic results, nor does the administration of the tracer together with special commercial test meals instead of orange juice or citric acid solution for retarding gastric emptying. The posture of the patient during the test (supine, sitting and changed position by rolling) seems to influence the 5 and 10 minute δ¹³C-values rather than the 30 minute values.

Application of film-coated tablets enables shortening the duration of the test from 30 down to 10 minutes. Mouth rinsing after tracer intake is not required in this case.

For diagnosing *Helicobacter pylori* infection under clinical routine conditions we propose to take the results of Oksanen A, Bergström M, Sjöstedt et al. (1997) as a starting point and to proceed as follows: After an overnight fast adults receive an oral dose of 100 mg [¹³C]-urea (99 atom% ¹³C) dissolved in tap water, followed by further 20 ml of water. The individuals are instructed to remain in a sitting position and to avoid physical activity during the test. Breath samples are collected immediately before and 30 minutes after tracer intake. The cut-off value for distinguishing between healthy individuals and those with an acute *Helicobacter pylori* infection can then be supposed to be 3.5 ‰ delta over baseline. Sensitivity and specificity are 92 or 95 %, respectively, in that case.

The software of FANci2 or HeliFANplus, respectively, leads the user according to the following guidelines: (After a nocturnal fasting period) a dose of 75 mg ¹³C-urea (99.0 atom% ¹³C) (dissolved in 250 ml of orange juice) is taken in. Breath is sampled immediately before tracer intake and 30 minutes thereafter. FANci2 or HeliFANplus, respectively, then displays the DOB-value which is ≥ 4.0 for *Helicobacter pylori*-positive and < 4.0 for *Helicobacter pylori*-negative individuals.

Diagnostic Validity:

Both sensitivity and specificity are in the range of 96 to 98 or 99%, respectively. The effect of anthropometric parameters (age, sex, weight, height) on endogenous CO₂ production can be eliminated by normalising measuring results. ¹³C-measurements immediately before and 20 minutes after tracer administration may yield the most sensitive and specific diagnostic results. Especially in cirrhotics ¹³C-urea breath test is superior to serology in the diagnosis of *Helicobacter pylori* infection. For monitoring therapy the test should be carried out not before 5 weeks after its completion. In cirrhotic patients sensitivity and specificity were found to be 87 and 86 %, respectively.

Unlike serology which is also non-invasive, [¹³C]urea breath test is specific to actual *Helicobacter pylori* infection, while serology indicates both acute and past *Helicobacter pylori* infection.

Proton pump inhibitors used for eradication therapy may reduce urease activity not related to reduced bacterial load, thus giving rise to false negative [¹³C]urea breath test results. On the other hand high intragastric pH-values may also cause this effect in case of H₂-receptor antagonists used for eradication therapy. Therefore eradication therapy should be avoided or cut short, respectively, at least four weeks before the test. If this should not be desirable, H₂-receptor antagonists dissolved in citric acid should be used for eradication therapy instead of proton pump inhibitors. If proton pump inhibitors should have the preference for therapy at all, the antibiotics omeprazole or pantoprazole should be preferred to lansoprazole and esomeprazole.

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3. ¹³C-Breath Tests for Investigating Exocrine Pancreatic Function and Diagnosing Pancreatic Diseases

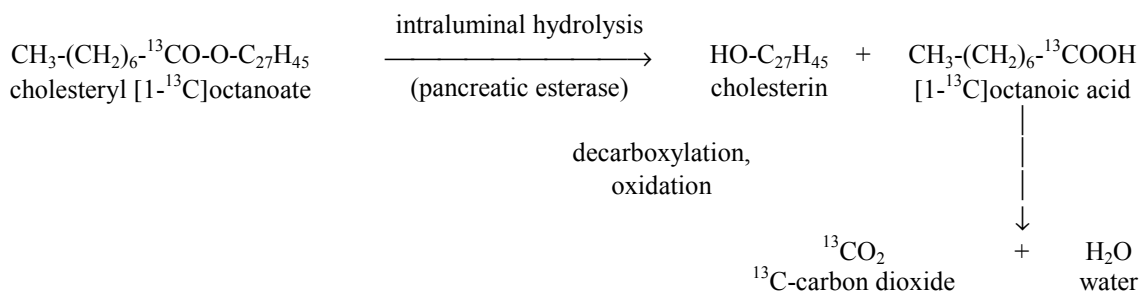
[¹³C]CHOLESTERYL OCTANOATE BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

The [¹³C]cholesteryl octanoate breath test is useful for the diagnosis of pancreatic diseases like fat malabsorption, exocrine pancreatic insufficiency, chronic pancreatic disease and biliopancreatic diversion.

Suitability for clinical diagnosis: controversial.

Metabolism of Substrate:



Procedure:

After a 12 h overnight fast 500 mg labelled cholesteryl-[1-¹³C]octanoate and 800 mg unlabelled cholesteryl octanoate are given as an emulsion in an isotonic liquid meal prepared in the following manner: Both the labelled and the unlabelled substrate are dissolved in 20 ml of olive oil heated to 90°C. For emulsifying the substrate 5 ml of glycerol and 5 g of lecithin are dissolved in 100 ml of water, to which 200 ml of normal saline solution and 60 ml of vegetable broth are added. The oil is gently poured into the aqueous phase and emulsified by stirring with a high speed mixer for 10 minutes. Prior to mixing 5 g of D-xylose is added in order to assess gastric emptying of the meal. Breath samples are collected immediately before tracer intake and every 15 minutes thereafter for six hours. The subjects are asked to remain seated and should not eat and smoke for the duration of the test.

Diagnostic Validity:

For the diagnosis of pancreatic disease using the three hour cumulative ¹³CO₂ recovery test the sensitivity is 68% and the specificity 75% which is similar to the results of faecal chymotrypsin and fluorescein dilaureate test.

References:

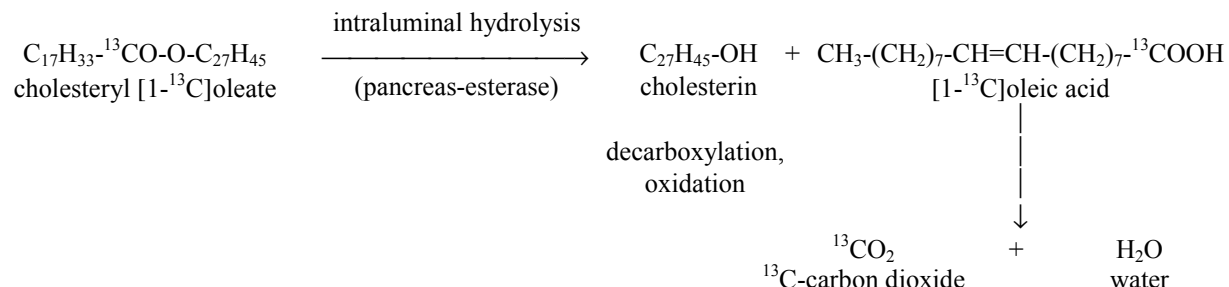
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[¹³C]CHOLESTERYL OLEATE

Indication / Relevance to Medical Research and Diagnosis:

The cholesteryl-[1-¹³C]oleate breath test can probably be applied for the diagnosis of exocrine pancreatic insufficiencies like fat malabsorption and chronic pancreatitis
Suitability for clinical diagnosis: Not yet clear.

Metabolism of Substrate:



Procedure:

After a 14 h overnight fast patients receive 14 ml of remnant-like emulsion by injection into an anticubital vein within a two min period. During the following ten hours the patients stay in a semi-recumbent position and allowed water only. Breath is sampled every ten min for the first 60 min, every 30 min for the next six hours and hourly for another three hours. After another 60 min the individuals are given a small snack and allowed to go home.

The emulsion to be injected is prepared thus: 135 mg triolein, 75 mg phosphatidylcholin, 70 mg cholesteryl-[¹³C]oleate and 24 mg cholesterol, all >99% pure, are emulsified by sonication for 1h in 8.5 ml of 2.2 vol-% glycerol in water. After sonification the mixture is centrifuged at 2500 g for ten min and then filtered through a 0.22 µm filter into sterile vessels. The emulsion preparations have to kept in sterile and pyrogen-free conditions, stored at -20°C and thawed 30 min prior to administration.

Diagnostic Validity:

The test is used for investigating the metabolism of lipoproteins with unsaturated fatty acids and will probably be also applied for diagnosing pancreatic disease.

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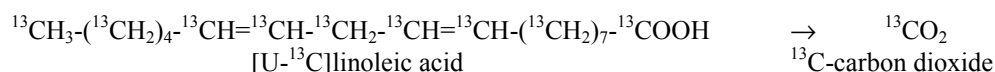
[U-¹³C]LINOLEIC ACID BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

The [U-¹³C]linoleic acid breath test can be used for studying the metabolism of polyunsaturated fatty acids, especially in newborn infants and lactating women, and for investigating the essential fatty acid status of cystic fibrosis patients.

Suitability for clinical diagnosis: Not yet clear, because too small a number of patients were investigated so far.

Metabolism of Substrate:



Procedure:

Newborn infants are breast-fed and receive 1 mg of uniformly labelled [¹³C]linoleic acid per kg body weight. Lactating women ingest the same dose. Breath samples are collected immediately before and then at 30-min intervals for six hours.

Diagnostic Validity:

The [U-¹³C]linoleic breath test seems to be an effective tool of investigating the metabolism of polyunsaturated fatty acids, especially in breast-fed infants. DOB-values in breath pass a maximum about 4 hours after tracer ingestion.

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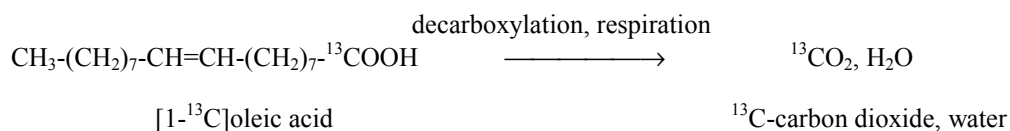
[1-¹³C]OLEIC ACID BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

[1-¹³C]oleic acid breath test can be applied for studying gastrointestinal fatty acid metabolism. In combination with [¹³C]palmitic acid and [¹³C]stearic acid breath test it is used for investigating the influence of chain length and saturation of fatty acids on their metabolic behaviour.

Suitability for clinical diagnosis: controversial.

Metabolism of Substrate:



Procedure:

After an overnight fast subjects consume [1-carboxyl-¹³C]oleic acid at a dose of 10 mg/kg body mass (99 atom% excess). The tracer is heated to 85°C in a mixture of double cream and olive oil and then emulsified with a mixture of casein, glucose and sucrose dissolved in water kept above 85°C. The emulsion is flavoured with chocolate milk-shake powder containing permitted emulsifiers to improve palatability and stability. This emulsion is consumed with 120 g white bread, 20 g strawberry jam and 10 g Flora margarine, together with the emulsion constituting a test meal with 3007 kJ, 30.0 g lipids (43 % saturated, 38 % monounsaturated and 19 % polyunsaturated fatty acids), 97.4 g carbohydrates and 19.9 g protein. Breath samples are taken immediately before tracer administration and then at hourly intervals for ten hours and again at 15 and 24 hours after tracer intake.

Diagnostic Validity:

In combination with ¹³C-palmitic acid and ¹³C-stearic acid breath test the ¹³C-oleic acid breath test might be valuable for studying the effects of fatty acid chain length and saturation on the gastrointestinal handling and metabolic disposal of fatty acids.

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[1-¹³C]PALMITIC ACID BREATH TEST

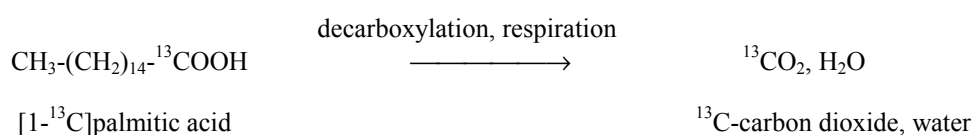
Indication / Relevance to Medical Research and Diagnosis:

Like [¹³C]octanoic acid breath test [¹³C]palmitic acid breath test is used for studying fatty acid oxidation, particularly in paediatrics and in patients treated with valproic acid. (Valproic acid [dipropyl acetic acid] is an anticonvulsant drug with severe side effects.) In combination with the [¹³C]trioctanoin and the [¹³C]triolein breath test it may enable additional insight into the mechanism of fat metabolism disorders (pancreatic insufficiency, mucosal disease, bile salt deficiency etc.). Moreover the [¹³C]palmitic acid breath test can be used for evaluating therapeutic efficiency of carnitine in patients with mild multiple acetyl-CoA dehydrogenase deficiency.

In combination with [¹³C]stearic acid and [¹³C]oleic acid breath test the [¹³C]palmitic acid breath test is used for investigating the influence of chain length and saturation of fatty acids on their metabolic behaviour.

Suitability for clinical diagnosis: satisfactory.

Metabolism of Substrate:



Procedure:

17 mg/kg body mass of 1-carboxyl-[¹³C]palmitic acid (80 - 99 % ¹³C) are given after a nocturnal or 4 h (for under 1 year old individuals) fasting period. Breath samples are collected immediately before and then at 30 minute intervals over six hours. Substrate is administered either orally or by short term infusion together with Lipomul the fatty-acid content of which – according to the manufacturer (The Upjohn Co., Kalamazoo, Mich.) – is 8 % C₁₆ (palmitic), 1 % C_{16:1}, 2 % C₁₈ (stearic), 30 % C_{18:1} (oleic), 56 % C_{18:2} (linoleic) and 2 % polyunsaturated fatty acids; 20 g fat /30 ml.

Other authors administer 10.0 mg/kg body mass (90 atom% excess) of the tracer for investigating side effects of valproic acid in epileptic children, who had been fasting for 14 to 15 hours.

Breath samples are obtained 15 minutes before and at 60 minute intervals over at least five or six hours after tracer intake, the investigated individuals resting quietly during the test.

Diagnostic Validity:

The [¹³C]palmitic acid breath test might be of some value in cases with primary or secondary carnitine deficiencies.

Combination of [¹³C]palmitic acid breath test with [¹³C]triolein or [¹³C]trioctanoin breath test seems to enable additional insight into the mechanism of fat metabolism disorders (pancreatic insufficiency, mucosal disease, bile salt deficiency etc.).

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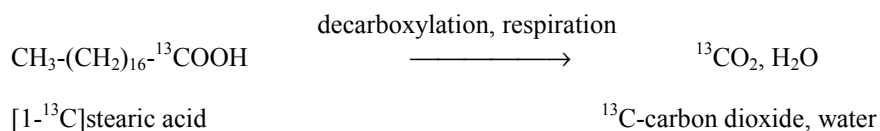
[¹³C]STEARIC ACID BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

[¹³C]stearic acid breath test can be applied for studying gastrointestinal fatty acid metabolism. In combination with [¹³C]palmitic acid and [¹³C]oleic acid breath test it is used for investigating the influence of chain length and saturation of fatty acids on their metabolic behaviour.

Suitability for clinical diagnosis: controversial.

Metabolism of Substrate:



Procedure:

After an overnight fast subjects consume [1-¹³C]stearic acid at a dose of 10 mg/kg body mass (99 atom% excess). The tracer is heated to 85°C in a mixture of double cream and olive oil and then emulsified with a mixture of casein, glucose and sucrose dissolved in water kept above 85°C. The emulsion is flavoured with chocolate milk-shake powder containing permitted emulsifiers to improve palatability and stability. This emulsion is consumed with 120 g white bread, 20 g strawberry jam and 10 g Flora margarine, together with the emulsion constituting a test meal with 3007 kJ, 30.0 g lipids (43 % saturated, 38 % monounsaturated and 19 % polyunsaturated fatty acids), 97.4 g carbohydrates and 19.9 g protein. Breath samples are taken immediately before tracer administration and then at hourly intervals for ten hours and again at 15 and 24 hours after tracer intake.

Diagnostic Validity:

In combination with ¹³C-palmitic acid and ¹³C-oleic acid breath test the ¹³C-stearic acid breath test might be valuable for studying the effects of fatty acid chain length and saturation on the gastrointestinal handling and metabolic disposal of fatty acids.

References:

- Jones AE; Stolinski M, Smith RD, Murphy JL and Wootton SA (1999): Effect of Fatty Acid Chain Length and Saturation on the Gastrointestinal Handling and Metabolic Disposal of Dietary Fatty Acids in Women. *Brit Journ Nutr* 81, 37 – 43
- Jones PJH, Penchards PB and Clandinin MT (1985): Absorption of ¹³C-labelled Stearic, Oleic, and Linoleic Acids in Humans: Application to Breath Tests. *The Journal of Laboratory and Clinical Medicine* 105, 647 – 652
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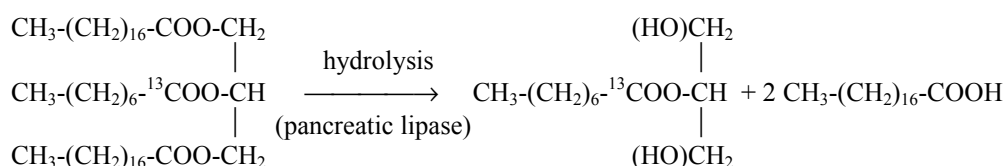
1,3-DISTEARYL-2-[1-¹³C]OCTANOYLGLYCEROL ([¹³C]MIXED TRIGLYCERIDE) BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

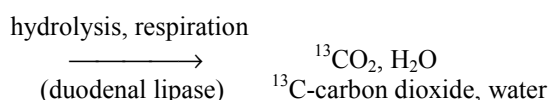
1,3-distearyl-2-[1-¹³C]octanoylglycerol, containing a ¹³C-medium chain fatty acid in the 2-position and long chain fatty acids in the 1- and 3- positions, as a substrate of a ¹³C-breath test may be used to investigate celiac disease, the development of fat digestion in infancy, lipid digestion in cystic fibrosis patients and to detect exocrine pancreatic insufficiency. The test is useful to follow the evolution of pancreatic disease and to monitor the effect of pancreatic enzyme replacement therapy. It is neither a diagnostic test of pancreatic disease nor a test of steatorrhea but assesses duodenal lipase activity. Intestinal lipolysis is not reduced in cystic fibrosis liver disease when measured with the ¹³C-mixed triglyceride breath test. This affirms the test's application as a measure of fat digestion not affected by inadequate intraluminal bile salts or liver disease.

Suitability for clinical diagnosis: satisfactory.

Metabolism of Substrate:



1,3-distearyl-2-[1-¹³C]octanoylglycerol 2-[1-¹³C]octanoylglycerol stearic acid



The rate limiting step is the hydrolysis of 1,3-distearyl-2-octanoylglycerol to form 2-octanoylglycerol catalysed by pancreatic lipase.

Procedure:

The test is carried out in the morning after an overnight fast, the test meal consisting of 0.25 g of butter per kg body mass to which 16 mg of the ¹³C-mixed triglyceride per gram of butter has been added, and 100 g of toast. Breath samples are taken before the meal and at 30 minute intervals for a period of six hours after tracer intake.

The software of FANci2 or HeliFANplus, respectively, leads the user according to the following guidelines: (After an overnight fast adults are given an oral dose of 4 mg per kg body weight of 1,3-distearyl-2-[1-¹³C]octanoylglycerol (mixed [¹³C]triglyceride; 99.0 atom% ¹³C) (together with a test meal consisting of 100 g of toast, 0.25 g per kg body weight of butter to which the tracer has been added). Including the basal sample taken immediately before tracer intake 13 breath samples should be collected at 30 minute intervals. FANci2 or HeliFANplus, respectively, then displays the 6 h-cumulative ¹³C-excretion (CPDR)*. CPDR = 23.0 after 6 hours is considered to indicate normal pancreatic function. If CPDR < 23.0 after 6 hours, then a pancreatic malfunction must be assumed.

*The cumulative percent ¹³C-dose recovery (CPDR) is the total ¹³C-dose eliminated with breath during a certain time after tracer administration in % of the tracer intake:

$$\text{cumulative percent } ^{13}\text{C-dose recovery CPDR} = m \cdot (1 - e^{-kt})^\beta$$

with t = time, m = total cumulative percentage of the recovered dose and k and β to be determined by non-linear regression analysis

The data of the CPDR-curve are obtained from PDR-values by numerical integration.

Diagnostic Validity:

The lower limit value of the 6 h-cumulative ¹³CO₂ excretion for discriminating between normal and abnormal exocrine pancreatic function is 22%. Intraduodenal pancreatic lipolytic activity is impaired in approximately 24 % of patients with celiac disease.

References:

- Ghoos YF, Rutgeerts PJ, Vantrappen GR et al. (1981): A Mixed Triglyceride Breath Test for Intraluminal Fat Digestive Capacity. *Digestion* 22, 239 – 247
- Ghoos Y, Rutgeerts P, Hiele M et al. (1988): Use of Stable Isotopes in Gastroenterology: ¹³CO₂ Breath Tests. In: *Klinische Ernährung* 34. Use of Stable Isotopes in Clinical Research and Practice. International Workshop. Berlin, Zuckerschwerdt-Verlag, 52 – 61

- Vantrappen GR, Rutgeerts PJ, Ghoois YF et al. (1989): Mixed Triglyceride Breath Test: A Noninvasive Test of Lipase Activity in the Duodenum. *Gastroenterol* 96, 1126 – 1134
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- Ferri F, Pastore M, Vesta V et al. (1998): Intraduodenal Lipase Activity in Celiac Disease Assessed by Means of [¹³C]Mixed Triglyceride Breath Test. *J Paediatr Gastroenterol Nutr* 27, 407 – 410
- De-Boek K, Delbeke I, Eggermont E, Veereman-Wouters G, and Ghoois Y (1998): Lipid Digestion in Cystic Fibrosis: Comparison of Conventional and High-Lipase Enzyme Therapy Using the Mixed-Triglyceride Breath Test. *Journal of Paediatric Gastroenterology and Nutrition* 26, 408 – 411
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- Manson WG, Coward WA, Harding M and Weaver LT (1999): Development of Fat Digestion in Infancy. *Arch Dis Child Fetal Neonatal Ed* 80, F 183 – F187
- Pfaffenbach B, Luypaerts A, Geypens P, and Adamek B (1999): ¹³C Mixed Triglyceride Breath Test : Isotope Selective Non-Dispersive Infrared Spectrometry in Comparison with Isotope Ratio Mass Spectrometry in Volunteers and Patients with Chronic Pancreatitis. *Scandinavian Journal of Gastroenterology* 34, 1153 – 1156
- Kalivianakis M, Minnich DM, Bijeveld Ma, van Aalderen WMC, Stellard F, Laseur M, Vonk RJ and Verkade HJ (1999): Fat Malabsorption in Cystic Fibrosis Patients Receiving Enzyme Replacement Therapy Is Due to Impaired Intestinal Uptake of Long Chain Fatty Acids. *American Journal of Clinical Nutrition* 69, 127 – 134.
- Kalivianakis M, Elstrodt J, Havinga R, Kuipers F, Stellaard F, Sauer PJJ, Vonk RJ, and Verkade HJ, (1999): Validation in an Animal Model of the Carbon-13-Labelled Mixed Triglyceride Breath Test for the Detection of Intestinal Fat Malabsorption. *The Journal of Pediatrics* 135, 444 – 450
- Boedeker C, Goetze O, Pfaffenbach B, Luypaerts A, Geypens P, and Adamek B (1999): ¹³C Mixed Triglyceride Breath Test : Isotope Selective Non-Dispersive Infrared Spectrometry in Comparison with Isotope Ratio Mass Spectrometry in Volunteers and Patients with Chronic Pancreatitis. *Scandinavian Journal of Gastroenterology* 34, 1153 – 1156
- Ling SC, Amarri S, Slater C, Hollman AS, Preston T, and Weaver LT (2000): Liver Disease does not Affect Lipolysis as Measured with the ¹³C-Mixed Glycerol Breath Test in Children with Cystic Fibrosis. *Journal of Pediatric Gastroenterology and Nutrition* 30, 368 – 372
- Van Dijk-van Aalst K, Van Den Driessche M, Van Der Schoor S, Schiffelers S, Van't Westeinde T, Ghoois Y, and Veereman-Wouters G (2001): ¹³C-Mixed Triglyceride Breath Test: A Non-Invasive Method to Assess Lipase Activity in Children. *Journal of Pediatric Gastroenterology and Nutrition* 32, 579 – 585
- Watts GF, Chan DC, Berrett PH, Martins IJ, and Redgrave TG (2001): Preliminary Experience with a New Stable Isotope Breath Test for Chylomicron Remnant Metabolism: A Study in Central Obesity. *Clinical Science (London)* 101, 683 – 690

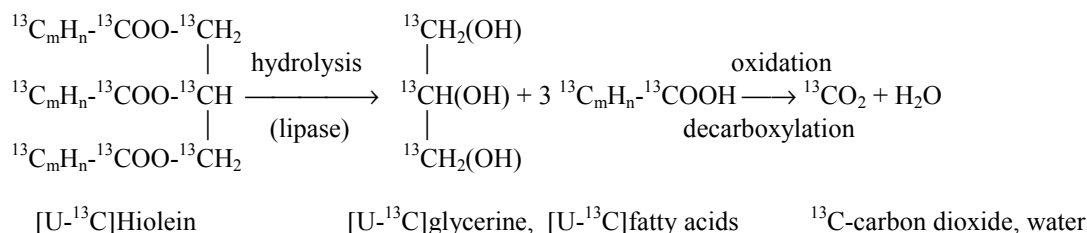
[U-¹³C]HIOLEIN BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

The breath test using [U-¹³C]Hiolein as substrate is suitable for evaluating exocrine pancreas function, for monitoring enzyme replacement therapy in cystic fibrosis patients and for diagnosing pancreatic steatorrhea, especially in insulin-treated diabetes mellitus patients. (Hiolein is a biosynthetic oil generated by microalgae grown in an illuminated bioreactor with ¹³CO₂ as their sole carbon source. It consists primarily of triglycerides (> 93%) with the following fatty acid composition: C_{16:0} 15%, C_{16:1} 3%, C_{16:2} 3%, C_{18:0} 2%, C_{18:1} 60%, C_{18:2} 15%, C_{18:3} 2% which is quite similar to the fatty acid composition of olive oil.)

Suitability for clinical diagnosis: satisfactory.

Metabolism of Substrate:



Procedure:

An oral dose of 2 mg [U-¹³C]Hiolein (98 % ¹³C) per kg body mass is given with breakfast which may consist of 1.5 g per kg body mass of rice cookies. Breath samples are collected at baseline and at 20 to 30 minute intervals for at least eight hours.

Diagnostic Validity:

The sensitivity for detecting steatorrhea is 92 % with a specificity of 86 %. Recovery of [¹³C]Hiolein as ¹³CO₂ is significantly reduced in gestational diabetes mellitus patients in comparison to controls in both the antepartum and postpartum periods.

References:

- Lembcke B, Braden B, and Caspary WF (1996): Exocrine Pancreatic Insufficiency: Accuracy and Clinical Value of the Uniformly Labelled ¹³C-Hiolein Breath Test. *GUT* 39, 668-674
- Hsu HW, Butte NF, Wong WW, Moon JK, Ellis KJ, Klein PD, and Moise KJ (1997): Oxidative Metabolism in Insulin-Treated Gestational Diabetes Mellitus. *Am J Physiol* 272, E 1099 – E 1107
- Lembcke B (1997): Atemtests bei Darmerkrankungen und in der gastroenterologischen Funktionsdiagnostik. *Schweiz Rundsch Med Praxis* 36, 25 – 26
- Braden B, Picard H, Caspary WF et al. (1997): Monitoring Pancreatin Supplementation in Cystic Fibrosis Patients with the [¹³C]Hiolein Breath Test: Evidence for Normalised Fat Assimilation with High Dose Pancreatic Therapy. *Z Gastroenterol* 35, 123 – 129
- Ashraf H, Hildebrand P, Meir R, Beglinger C, and Gyr N (2000): Induction of Artificial Fat Maldigestion by Tetrahydrolipstat Assessed by the ¹³C-Hiolein Breath test in Healthy Volunteers: A Double-Blind Controlled Pilot Study. *Digestion* 62, 159 – 163
- Sun DY, Jiang YB, Rong L, Jin SJ, and Xie WZ (2003): Clinical Application of ¹³C-Hiolein Breath Test in Assessing Pancreatic Exocrine Insufficiency. *Hepatobiliary Pancreat Dis Int* 449 – 452

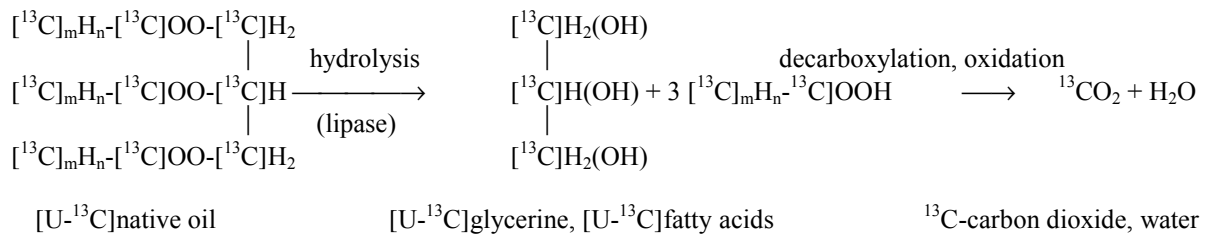
[¹³C]TRIGLYCERIDE BREATH TESTS WITH GENUINE PLANT OILS AND OTHER GENUINE TRIGLYCERIDES

Indication / Relevance to Medical Research and Diagnosis:

Sometimes plant oils like soy oil, maize oil or maize germ oil, respectively, naturally enriched or depleted in ¹³C, are used as substrates of ¹³C-breath tests. Like for ¹³C-breath tests with other triglycerides with long chain fatty acids as substrates such ¹³C-breath tests can be used for the investigation of fat malabsorption, particularly for the diagnosis of defects in lipolysis due to exocrine pancreatic insufficiency as well as for optimising enteral or parenteral nutrition of acutely ill patients after heavy injuries. In combination with ¹⁵N-glycine U-[¹³C]algae lipid mixtures can be applied for evaluating the interchanges between protein and fat metabolism.

Suitability for clinical diagnosis: satisfactory.

Metabolism of Substrate:



Procedure:

After an overnight fast adults take a dose of 70 g maize germ oil ($\delta^{13}\text{C} = -14.3$) together with a suitable diet. Breath samples are collected immediately before and every ten minutes during the first hour after tracer intake and then every 30 minutes for about six hours.

In North America with its population preferring C₄-plants (maize, cane sugar) in their nutrition patients and volunteers should be placed on a low ¹³C-diet for a period of two weeks before starting the ¹³C-breath test, while in Europe, where people prefer C₃-plants (wheat, potatoes, beet sugar, rice, cabbage) in their menus the inverse procedure is opportune.

Diagnostic Validity:

Genuine plant oils naturally enriched in ¹³C as ¹³C breath test substrates are a useful alternative to the application of ¹³C breath tests with triglycerides artificially labelled with this stable isotope.

References:

- Schoeller DA, Klein PD, MacLean WC et al. (1980): ¹³C-Abundances of Nutrients and the Effect of Variations in ¹³C-Abundances of Test Meals Formulated for ¹³CO₂ Breath Tests. *Am J Clin Nutr* 33, 2375 – 2385
- Schoeller DA, Klein PD, Watkins JN et al. (1980): ¹³C-Abundances of Nutrients and the Effect of Variations in ¹³C-Isotopic Abundances of Test Meals Formulated for ¹³CO₂ Breath Tests. *Am J Clin Nutr* 33, 2375 – 2385
- Wolfram C (1986): Medium Chain Triglycerides (MCT) for Total Parenteral Nutrition. *World J Surg* 10, 33 – 37
- Shulman RJ (1988): Measurement of Carbohydrate Absorption and Utilisation Using the Stable Isotope ¹³C. In: *Klinische Ernährung 34. Use of Stable Isotopes in Clinical Research and Practice. International Workshop. Berlin, Zuckerschwerdt-Verlag, 85 – 88*
- Paust H, Park W, Knoblach G, and Keles T (1988): Studies of Fatty Acid Metabolism by ¹³C-Triglyceride Infusion Technique in Children. In: *Klinische Ernährung 34. Use of Stable Isotopes in Clinical Research and Practice. International Workshop. Berlin, Zuckerschwerdt-Verlag, 127 – 140*
- Wolfram G and Metges C (1988): Fatty Acid Oxidation Following Enteral or Parenteral Application of ¹³C-Labelled Medium and Long Chain Triglycerides. In: *Klinische Ernährung 34. Use of Stable Isotopes in Clinical Research and Practice. International Workshop. Berlin, Zuckerschwerdt-Verlag, 89 - 92*
- Wutzke KD, Heine W, Köster D et al. (2000): HAY's Diet Increases Fat Oxidation (Abstract). *Biomed-SIGN (Stable Isotopes in Gastroenterology and Nutrition)-Meeting, University of Rostock, September 29-30, 11*

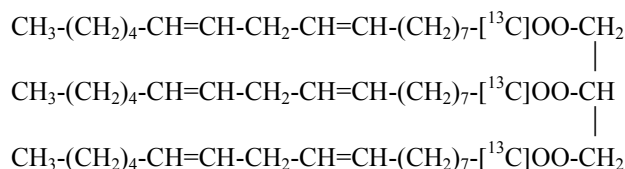
[1-¹³C]TRILINOLEATE BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

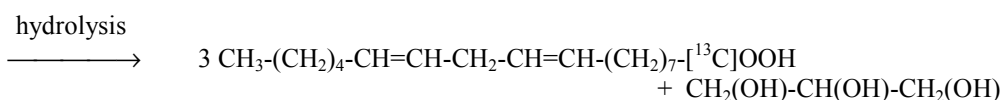
[1-¹³C]trilinoleate breath test can be used for studying the metabolism of multiply unsaturated long chain triglycerides. Beside [¹³C]tripalmitate and [¹³C]tristearate [¹³C]trilinoleate is applied for labelling long chain triglycerides.

Suitability for clinical diagnosis: controversial.

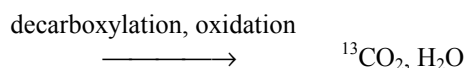
Metabolism of Substrate:



[¹³C]trilinoleate



[1-¹³C]linoleic acid, glycerine



¹³C-carbon dioxide, water

Procedure:

17 mg/kg body mass of 1-carboxyl-¹³C₃-trilinolein (80 – 99 % ¹³C) are given after a nocturnal fasting period. Breath samples are taken at 30 minute intervals over six hours. Substrate is administered either orally or by short term infusion together with a suitable fat emulsion.

Diagnostic Validity:

[¹³C]trilinoleate breath test can be assumed to be attractive for studying the metabolism of multiply unsaturated long chain triglycerides.

References:

Schmidt H-L and Metges C (1986): Variations of the Natural Isotope Abundance in Diet. Causes of Artefacts or the Basis of New Possibilities in Stable Tracer Work. In: Dietze G et al. (eds.), Clinical Nutrition and Metabolic Research. Karger, Basel, 56 – 168

Wolfram G and Metges C (1988): Fatty Acid Oxidation Following Enteral or Parenteral Application of ¹³C-Labelled Medium and Long Chain Triglycerides. In: Klinische Ernährung 34. Use of Stable Isotopes in Clinical Research and Practice. International Workshop. Berlin, Zuckerschwerdt-Verlag, 89 – 92

[1-¹³C₃]TRIOCTANOIN BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

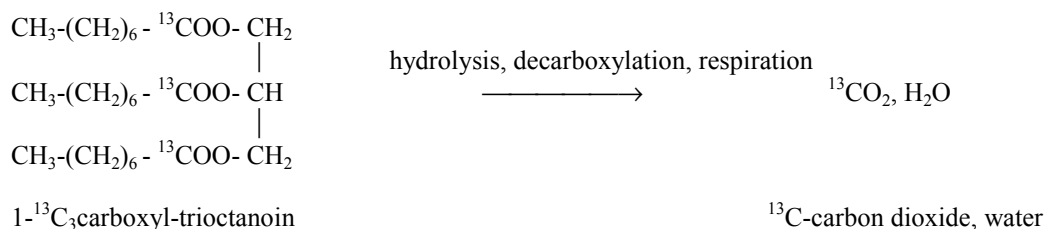
[¹³C₃]trioctanoin breath test is used for studying fat absorption, particularly with respect to medium chain fatty acids. (For investigating the metabolism of long chain fatty acids [¹³C]triolein breath test should be preferred.) Important fields of application are paediatrics, especially investigation of fat metabolism in preterm infants, diagnosis of cystic fibrosis and steatorrhea, the investigation of fat metabolism after surgical operations like pancreatoduodenectomy, duodenum-preserving pancreatic head resection and pancreatoduodenectomy with gastrectomy as well as studying the influence of exogenous pancreatic enzymes on fat absorption.

Evaluating the effect of valproic acid as a therapeutic agent for epileptic patients on their lipid metabolism is a further objective of [¹³C]trioctanoin breath test.

Combination of [¹³C]trioctanoin breath test with [¹³C]triolein or [¹³C]palmitic acid breath test enables additional insight into the mechanism of fat metabolism disorders (pancreatic insufficiency, mucosal disease, bile salt deficiency etc.).

Suitability for clinical diagnosis: good.

Metabolism of Substrate:



Procedure:

6.5 mg/kg body mass of 1-carboxyl-¹³C₃-trioctanoin (99 % ¹³C) are given orally in 5 g of butter after an overnight fast. Breath samples are obtained 15 minutes before and at 60 minute intervals over five or six hours after tracer intake, the investigated individuals resting quietly during the test. For some investigations the patients or volunteers, respectively, are supplied with Lipomul during the test, the fatty-acid content of which – according to the manufacturer (The Upjohn Co., Kalamazoo, Mich.) – is 8 % C₁₆ (palmitic), 1 % C_{16:1}, 2 % C₁₈ (stearic), 30 % C_{18:1} (oleic), 56 % C_{18:2} (linoleic) and 2 % polyunsaturated fatty acids; 20 g fat /30 ml.

Patients are hospitalised in some cases and ingest 3g fat per kg body mass and day for 3 days and during the test. Simultaneous intake of carbohydrates retards fat absorption. Except for sips of water no additional food or liquids should therefore be allowed for that time.

Neonates are given 7 – 10 mg/kg 1-carboxyl-¹³C₃-trioctanoin (99 % ¹³C) mixed with 0.5 ml of medium chain triglyceride oil. The mixture is injected into the stomach through an orogastric tube immediately before feeding. Breast or bottle feeding is performed every three hours. Breath is sampled just before tracer intake and then every 60 minutes for six hours by a facemask with a unidirectional valve.

For characterising fatty acid metabolism in children under clinical routine conditions we propose to take the results of Paust H, Park W, Knoblach G, and Keles T (1988) as a starting point and to proceed as follows: The individuals receive an intravenous dose of 10 mg of ¹³C-octanoin. Breath samples are collected immediately before and 40 minutes after tracer intake. For healthy children the DOB value in breath is then in the order of 43 %. The optimum cut-off value for distinguishing between healthy individuals and those with fat malabsorption depends on the particular reason for this phenomenon and therefore needs further investigations.

Diagnostic Validity:

The reliability with which the coefficient of fat absorption can be measured is ± 14 %. The greatest discriminatory values are obtained from the percent dose recovered between 2 and 5 hours.

References:

- Watkins JB, Schoeller DA, Klein PD et al. (1975): ¹³C-Trioctanoin: A Sensitive, Safe Test for Fat Malabsorption. Proceedings of the 2nd International Symposium on Stable Isotopes. Oak Brook, 274 – 281
- Watkins JB, Dale A, Schoeller DA et al. (1977): ¹³C-Octanoin: A Nonradioactive Breath Test to Detect Fat Malabsorption. J Lab Clin Med 90, 422 - 430

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- Watkins JB, Klein PD, Dale A et al. (1982): Diagnosis and Differentiation of Fat Malabsorption in Children Using ¹³C-Labelled Lipids: Trioktanoin, Triolein and Palmitic Acid Breath Test. *Gastroenterol* 82, 911 – 917
- Paust H, Park W, Knoblach G et al. (1988): Studies of Fatty Acid Metabolism by ¹³C-Triglyceride Infusion Technique in Children. In: *Klinische Ernährung 34. Use of Stable Isotopes in Clinical Research and Practice*. International Workshop. Berlin, Zuckerschwerdt-Verlag, 127 – 140
- Gilger MA, Klein PD, Klish WJ et al. (1988): Scoring the ¹³C-Trioktanoin Breath Test to Predict Coefficient of Fat Absorption. *Gastroenterol* 94, A 147
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- Kato H, Nakao A, Kishimoto W et al. (1993): ¹³C-Labelled Trioktanoin Breath Test for Exocrine Pancreatic Function Test in Patients after Pancreatoduodenectomy. *Am J Gastroenterol* 88, 64 – 69
- Miyakawa S, Hayakawa M, Horiguchi A, Mizuno K, Ishihara S, Niwamoto N and Miura K (1996): Estimation of Fat Absorption with the ¹³C-Trioktanoin Breath Test after Pancreato-Duodenectomy or Pancreatic Head Resection. *World J Surg* 20, 1028 – 1029
- McClellan P, Harding M, Coward WA, Prentice A, Austin S, and Weaver LT (1998): Bile Salt-Stimulated Lipase and Digestion of Non-Breast Milk Fat. *Journal of Gastroenterology and Nutrition* 26, 39 – 42
- Miyakawa S, Niwamoto N, Horiguchi A et al. (2000): Fat Absorption after Pylorus-Preserving Pancreatoduodenectomy Reconstructed with Billroth II Pancreaticojejunostomy or Billroth I Pancreaticogastrostomy. *Hepatogastroenterol* 47, 264 – 268
- Miyakawa S, Niwamoto N, Horiguchi A, Hanai T, Mizuno K, and Miura K (2000): Fat absorption after Pylorus-Preserving Pancreatoduodenectomy Reconstructed with Billroth II Pancreaticojejunostomy or Billroth I Pancreaticogastrostomy. *Hepatogastroenterology* 47, 264 – 268

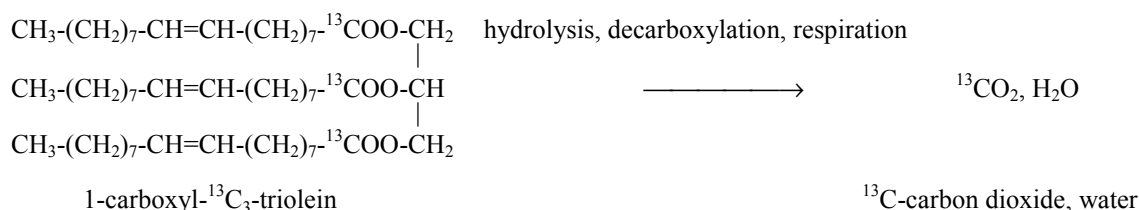
[1-¹³C]TRIOLEIN BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

[¹³C]triolein breath test is used for studying pancreatic lipase activity in cystic fibrosis, investigating Crohn's disease, fat malabsorption and aetiology of steatorrhea, particularly with respect to long chain fatty acids. (For investigating the metabolism of medium chain fatty acids [¹³C]trioctanoin breath test should be preferred.) Especially important fields of application are paediatrics, studying fat metabolism after surgical operations like pancreatoduodenectomy with or without gastrectomy and pancreatic head resection and investigation of interactions between fat and carbohydrate metabolism. Combination of [¹³C]triolein breath test with [¹³C]trioctanoin or [¹³C]palmitic acid breath test enables additional insight into the mechanism of fat metabolism disorders (pancreatic insufficiency, mucosal disease, bile salt deficiency etc.).

Suitability for clinical diagnosis: satisfactory.

Metabolism of Substrate:



Procedure:

17 mg/kg body mass of 1-carboxyl-¹³C₃-triolein (80 – 99 % ¹³C) are given after a nocturnal or 4 h (for under 1 year old individuals) fasting period. Breath samples are taken at 30 minute intervals over six hours. Substrate is administered either orally or by short term infusion together with 0.7 g/kg Lipomul the fatty-acid content of which – according to the manufacturer (The Upjohn Co., Kalamazoo, Mich.) – is 8 % C₁₆ (palmitic), 1 % C_{16:1}, 2 % C₁₈ (stearic), 30 % C_{18:1} (oleic), 56 % C_{18:2} (linoleic) and 2 % polyunsaturated fatty acids; 20 g fat / 30 ml.

Patients should be hospitalised and ingest 3g fat per kg body mass and day for 3 days and during the test. Simultaneous intake of carbohydrates retards fat absorption. Except for sips of water no additional food or liquids should therefore be allowed for that time.

A peak excretion rate of ¹³C of 2.7 % dose/h is recommended as cut-off value for distinguishing fat malabsorption from normal fat metabolism.

The software of FANci2 or HeliFANplus, respectively, leads the user according to the following guidelines: (After a nocturnal or 4 h (for under one year old individuals) fasting period) a dose of 17 mg per kg body weight [1-carboxyl-¹³C₃]triolein (99.0 atom% ¹³C) is taken in. Including the basal sample taken immediately before tracer intake seven breath samples should be collected at 60 minute intervals. FANci2 or HeliFANplus, respectively, then displays the percentage ¹³C-dose recovery per hour (PDR)* of the investigated individual.

* The percentage ¹³C-dose recovery per hour (PDR) is defined as the expired ¹³C-dose per hour in % of the administered ¹³C-dose:

$$\%^{13}\text{C dose/h PDR} = \left(\frac{^{13}\text{C - excess in breath}}{^{13}\text{C - excess administered}} \right) \times 100 \text{ in } \%$$

Diagnostic Validity:

In 10 normal and 17 patients with documented steatorrhea the sensitivity was 100 % and the specificity 89 %, if a peak excretion rate of ¹³C of 2.7 % dose/h is used as cut-off value.

References:

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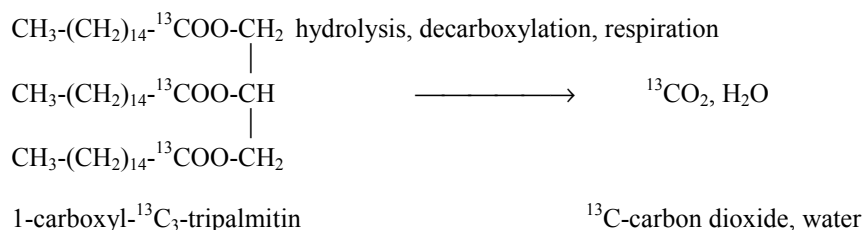
[¹³C]TRIPALMITIN BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

The [¹³C]tripalmitin breath test is used for studying fat malabsorption, pancreatic lipase activity in cystic fibrosis, Crohn's disease and aetiology of steatorrhea, particularly with respect to long chain fatty acids. (For investigating the metabolism of medium chain fatty acids [¹³C]trioctanoin breath test should be preferred.) Especially important fields of application are paediatrics, studying fat metabolism after surgical operations like pancreatoduodenectomy with or without gastrectomy and pancreatic head resection and investigation of interactions between fat and carbohydrate metabolism.

Suitability for clinical diagnosis: satisfactory.

Metabolism of Substrate:



Procedure:

Children ingest 4 mg [1,1,1-¹³C₃]glyceryl tripalmitate per kg body mass at 8 a.m. together with the framework of a special meal. Breath samples are collected in 15 to 30 minute intervals for 8 hours.

Diagnostic Validity:

The [¹³C]tripalmitin breath test can be applied for evaluating pancreatic lipase activity before and during enzyme supplementation.

References:

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4. ¹³C-Breath Tests for Investigating Liver Function and Diagnosing Liver Diseases

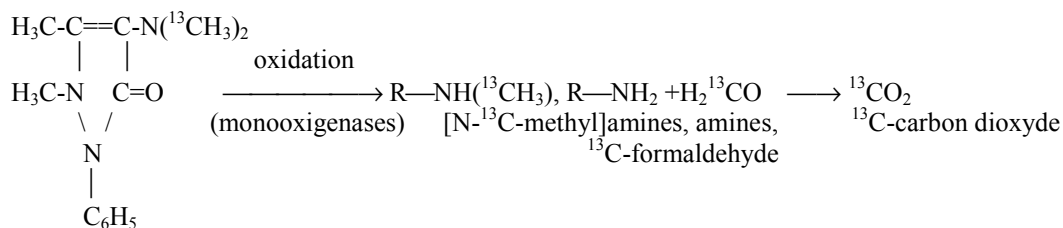
[¹³C₂]AMINOPYRINE BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

The N, N-dimethyl-¹³C-aminopyrine breath test is used for studying hepatic microsomal biotransformation, especially its demethylating and oxidative capacity, and for diagnosing and assessing therapy of liver diseases in which the activity of microsomal monooxygenases is diminished. It can be used to monitor inactivation of P450 enzymes in the liver. The test also detects altered liver metabolism caused by low-dose oral contraceptives. When the excretion of ¹³CO₂ in breath of patients with cirrhosis or chronic hepatitis diminishes in the course of time, the test predicts evolution to hepatic coma and death. Moreover the test is helpful for determining optimum liver transplantation time and for the preventive diagnosis of rejection reaction after liver transplantation.

Suitability for clinical diagnosis: excellent.

Metabolism of Substrate:



[N,N-dimethyl-¹³C]aminopyrine

Procedure:

After an overnight fast subjects ingest 2 mg per kg body mass [N,N-dimethyl-¹³C]-aminoantipyrine (¹³C₂-aminopyrine; 99.0 % ¹³C) dissolved in a glass of water. Breath samples are taken just before and at 30 minute intervals for two to three hours after tracer intake. Other authors use intravenous injection (150 mg over one minute) of the tracer.

For diagnosing and assessing therapy of liver diseases under clinical routine conditions we propose to take the results of Meyer-Wyss B, Renner E, Luo H et al. (1993) as a starting point and to proceed as follows: After an overnight fast the individuals receive an intravenous injection of 150 mg [N,N-dimethyl-¹³C]₂-aminoantipyrine over one minute. Breath samples are recommended to be taken immediately before and 10 minutes after tracer intake.

The software of FANci2 or HeliFANplus, respectively, leads the user according to the following guidelines: (After a nocturnal fasting period) an oral dose of 2.0 mg per kg body weight [¹³C₂]aminopyrine (99.0 atom% ¹³C) (dissolved in a glass of water) is ingested. Including the basal sample taken immediately before tracer intake 5 breath samples should be collected at 30 minute intervals. FANci2 or HeliFANplus, respectively, then displays the percentage ¹³C-dose recovery per hour (PDR)* of the investigated individual. If PDR ≥ 8 %, then the liver function is considered to be normal. If PDR < 8 %, then a malfunction of the liver must be taken into consideration.

* The percentage ¹³C-dose recovery per hour (PDR) is defined as the expired ¹³C-dose per hour in % of the administered ¹³C-dose:

$$\%^{13}\text{C dose/h PDR} = (\text{}^{13}\text{C - excess in breath} / \text{}^{13}\text{C - excess administered}) \times 100 \text{ in } \%$$

Diagnostic Validity:

In normals the height of the ¹³CO₂ peak excretion after one hour amounts to at least 8 δ-units, the 2-h cumulative ¹³CO₂ excretion to at least 9 % of the dose. Lower values indicate liver diseases. When 180 mg phenobarbital/day is administered, a 40 to 100 % increase in exhaled ¹³CO₂ is observed.

References:

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[¹³C]CAFFEINE BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

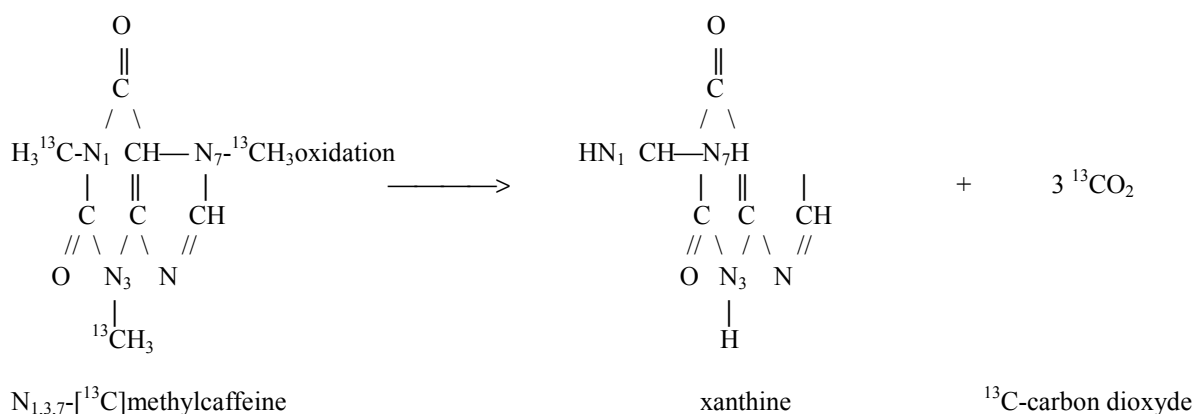
Rate of demethylation of [¹³C-methyl] caffeine strongly depends on hepatic cytochrome P-450 activity, the latter being influenced by disorders of microsomal hepatic biotransformation as well as by administering drugs like omeprazole. To a certain degree demethylation of [¹³C-methyl] caffeine and [¹³C-methyl] caffeine also depends on hepatic biotransformation. ¹³C-caffeine breath tests therefore are a good means of studying hepatic microsomal biotransformation. The test allows quantitative validation of liver function.

A new field of application can be seen to emerge in investigating the influence of environmental polychlorodibenzodioxins (PCDDs) and polychlorodibenzofurans (PCDFs) on hepatic monooxygenase activity of newborns and infants taken up by breast milk. The test is also used in studying drug elimination and exposure to polybrominated biphenyls.

Slow ¹³CO₂ exhalation indicates low cytochrome P4501A2 activity or disorders of microsomal hepatic biotransformation, respectively.

Suitability for clinical diagnosis: good.

Metabolism of Substrate:



Procedure:

3 mg/kg body mass -[¹³C-methyl]-caffeine dissolved in a glass of tap water are orally administered. Breath samples are taken immediately before and 30, 60, 90 and 120 minutes after tracer intake.

Other authors synthesised N_{1,3,7}-[¹³C]methylcaffeine by methylation of xanthine dissolved in 0.25 n NaOH, with ¹³C-methyl iodide (90 atom% ¹³C) at room temperature. The tracer is then extracted with dichloromethane and separated from dimethylxanthines chromatographically. 4 mg/kg body mass of this substrate dissolved in 50 ml of hot water with instant coffee is orally administered after an overnight fast. The subjects are investigated at rest in a sitting position. A significant increase of ¹³C in breath is observed already 5 minutes after tracer intake. A ¹³C maximum is attained within one hour. After 5 hours ¹³C decreases to return near the basal value after 24 hours. Healthy volunteers excrete 21 to 26 % of total ¹³C administration over 24 hours.

For characterising microsomal hepatic biotransformation under clinical routine conditions we propose to take the results of Arnaud MJ, Thelin-Doerner A, Ravussin E et al. (1980) as a starting point and to proceed as follows: After an overnight fast the individuals receive an oral dose of 4 mg/kg body mass [N-1,3,7-¹³C-methyl] caffeine (90 atom% ¹³C) dissolved in 10 ml of hot instant coffee followed by 100 ml of water. Breath samples are collected immediately before and one hour after tracer intake. The cut-off value for distinguishing between healthy individuals and those with disorders of microsomal hepatic biotransformation can then be supposed to be in the order of 8 ‰ delta over baseline (DOB) in that case.

Diagnostic Validity:

There is a close relation between hepatic cytochrome P-450 activity and ¹³CO₂ excretion.

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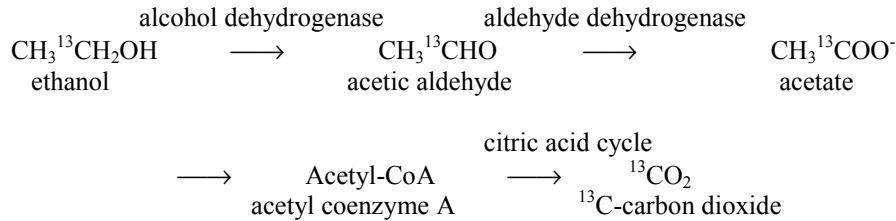
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[¹³C]ETHANOL BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

The ¹³C-ethanol breath test is a good means of diagnosing aldehyde dehydrogenase deficiency. Suitability for clinical diagnosis: satisfactory.

Metabolism of Substrate:



Procedure:

After a nocturnal fasting period 63 mg of [1-¹³C]ethanol (90% ¹³C) dissolved in about 50 ml of tap water followed by the same amount of tap water are orally given. During the four hours of test duration the individuals stay seated and neither physical activity, food intake nor smoking is allowed. Breath samples are taken just before and 30, 60, 120, 210 and 240 minutes after tracer intake. In order to avoid sampling of dead volume air before sampling aspiration is performed through the nose, breath sampling being started a few seconds after onset of the expiration process.

Diagnostic Validity:

The level of significance is p = 0.05 for the time interval between 1 h and 3 h and p = 0.08 for the 4 h time point.

References:

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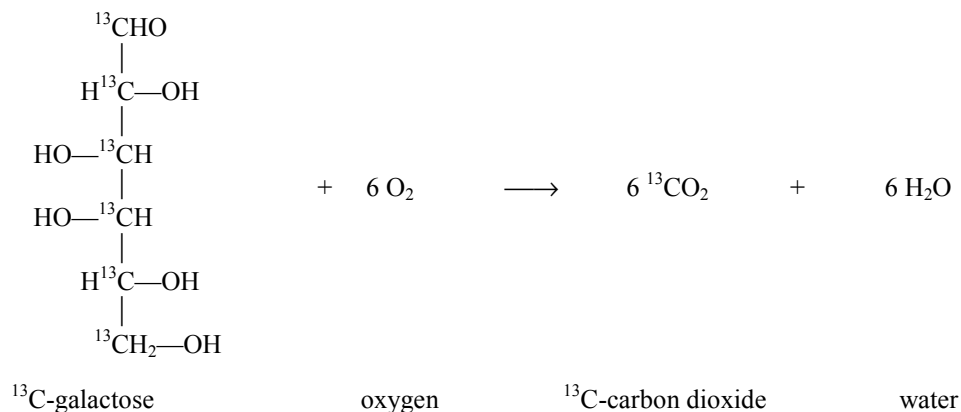
[¹³C]GALACTOSE BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

[U-¹³C]galactose breath test can be used for the diagnosis of liver diseases, particularly for the diagnosis of liver fibrosis in chronic hepatitis C, for recognising presence or degree of severity of alcoholic cirrhosis and for studying galactose oxidation in patients with galactose-1-phosphate uridylyltransferase deficiency. In experiments with rats the test was used for investigating effects of ethanol and diabetes on galactose oxidation in rats.

Suitability for clinical diagnosis: excellent.

Metabolism of Substrate:



Procedure:

After 150 g carbohydrate per day in the diet for three days and one overnight fast an aqueous solution of 10 g uniformly labelled ¹³C-galactose (1.0 atom% ¹³C-excess) per m² of body area is orally administered. Breath samples are collected immediately before and at 30 minute intervals over three to four hours. The largest differences of ¹³C in breath between normal and cirrhotic individuals are attained 90 minutes after tracer intake, the time of peak concentration of ¹³C in breath of cirrhotic patients (150 – 180 min) being later than that of normal subjects (90 to 120 min).

Instead of 10 g uniformly labelled ¹³C-galactose with 1.0 atom% ¹³C-excess 7 mg per kg body mass of [1-¹³C]galactose can be administered in the fasting state either orally or intravenously.

For assessing endogenous galactose production in vivo a primed continuous infusion approach can be applied. After an overnight fast adults intravenously receive a priming dose of 7 mg/kg body mass D-[¹³C]galactose and then a continuous intravenous infusion of 0.76 mg/kg body mass per hour of this tracer. Additionally, D-glucose is infused at a rate of 2 mg/kg body mass per minute. Samples of breath and intravenous EDTA-blood are collected prior to tracer administration and then in one hour intervals for six hours after priming.

For characterising galactose absorption and utilisation by alcoholic or diabetic liver under clinical routine conditions we recommend to take the investigations of Shreeve WW (1987) as a starting point and to proceed as follows: After an overnight fast the individuals receive an oral dose of 10 g [U-¹³C]galactose (1.0 atom% ¹³C-excess) dissolved in 93 ml orange juice per m² body area. The tracer can be prepared by photosynthesis from ¹³CO₂ using the red marine algae, *Gigantina corymbifera* (Shreeve WW, Shoop JD, Ott DG et al. (1976)). The highly enriched [¹³C]galactose (30-40 atom% ¹³C) obtained is diluted with galactose with natural ¹³C-abundance to a value of 2.1 atom% ¹³C, corresponding with 1.0 atom% excess. Breath samples should be collected immediately before and one hour after tracer intake. For normals the DOB value should attain about 64 ‰ under these conditions. Patients with liver disease caused by diabetes mellitus or alcohol abuse can be expected to achieve DOB-values of less than 56 ‰.

The software of FANci2 or HeliFANplus, respectively, leads the user according to the following guidelines: (After 150 g carbohydrate per day in the diet for three days and one overnight fast an aqueous solution of) 2.0 mg per kg body weight [1-¹³C]galactose (99.0 atom% ¹³C) is orally administered. Including the basal sample taken immediately before tracer intake 5 breath samples should be collected at 30 minute intervals for two hours. FANci2 or HeliFANplus, respectively, then displays the cumulative percent ¹³C-dose recovery (CPDR)* eliminated with breath during these two hours.

*The cumulative percent ¹³C-dose recovery (CPDR) is the total ¹³C-dose eliminated with breath during a certain time after tracer administration in % of the tracer intake:

$$\text{cumulative percent } ^{13}\text{C-dose recovery CPDR} = m \cdot (1 - e^{-kt})^\beta$$

with t = time, m = total cumulative percentage of the recovered dose and k and β to be determined by non-linear regression analysis

The data of the CPDR-curve are obtained from PDR-values by numerical integration.

Diagnostic Validity:

Like the [¹⁴C]galactose breath test the [¹³C]galactose breath test with respect to sensitivity and specificity appears superior to some other tests for recognizing liver cirrhosis like serum albumin alkaline phosphatase, total bilirubin and transaminase test. The [¹³C]galactose breath test is able to distinguish between class A and class B or C cirrhosis. In addition it a useful tool for distinguishing between healthy individuals and patients with liver cirrhosis and between cirrhotics with well compensated liver disease and those with decompensated liver disease. The test is also supposed to increase understanding of genotype-phenotype relationships in hereditary galactosemia.

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$$\text{cumulative percent } ^{13}\text{C-dose recovery CPDR} = m \cdot (1 - e^{-kt})^\beta$$

with t = time, m = total cumulative percentage of the recovered dose and k and β to be determined by non-linear regression analysis

The data of the CPDR-curve are obtained from PDR-values by numerical integration.

Diagnostic Validity:

L-[U- ^{13}C]glucose breath test curves from children with glucose-galactose malabsorption and from those with diarrhea are significantly different from breath test curves from healthy children and severely malnourished ones without diarrhoea.

Simultaneous absorption of substrate from the small and large intestine may limit the usefulness of ^{13}C -breath tests in the premature infant.

The test is suitable to study the digestibility of carbohydrates like glucose. In combination with ^{13}C -starch as substrate the ^{13}C -glucose breath test allows to measure starch hydrolysis rates.

References:

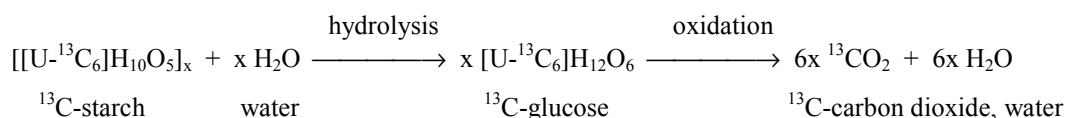
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Indication / Relevance to Medical Research and Diagnosis:

¹³C-breath tests with carbohydrates like starch, glycogen and other glucose polymers as substrates naturally enriched or depleted in ¹³C are used for studying carbohydrate absorption and metabolic degradation, particularly of weaning food for infants in developing countries. ¹³C-starch breath test is also useful for studying carbohydrate metabolism of patients with cystic fibrosis or exocrine pancreatic insufficiency, respectively.

A combination of ¹³C-starch and ¹³C-glucose as substrates of breath tests allows to measure starch hydrolysis rates.

Metabolism of Substrate:



Procedure:

A dose of 100 g dry starch naturally enriched in ¹³C together with a standard breakfast is applied after an overnight fast. If maize starch is used the $\delta^{13}C$ -value is about - 10 ‰. Breath samples are collected every 10 minutes during the first hour and then every 30 minutes for eight hours. In children the dose is 2 g/kg body mass. Breath samples are collected ten and five minutes before tracer intake and then every 30 minutes for four hours, the subjects remaining quietly seated during the test period.

For measuring the utilisation of dietary cereal by young infants the individuals receive a basal diet consisting of soy formula to which beet sucrose is added as the sole carbohydrate source to achieve a final concentration of 5 g/100 ml. These substances are naturally low in ¹³C. The test carbohydrates consist of glucose, glucose polymers and cereal, all derived from maize, a foodstuff naturally rich in ¹³C. The maize cereal is prepared from degerminated yellow maize flour, which is treated with malt enzymes, precooked and drum dried. On test days the carbohydrate to be tested is substituted for beet sucrose in one go at a dose of 1g/kg body mass. 225 minutes later the individuals receive the next feeding which again consists of the basal diet.

Diagnostic Validity:

¹³C-breath tests with starch and other glucose polymers as substrates naturally enriched or depleted in ¹³C are a good means of studying carbohydrate absorption and metabolic degradation.

In patients with pancreatic disease the ratio of the 6-h cumulative percentage of ¹³CO₂ excretion after starch intake to the 6-h cumulative percentage of ¹³CO₂ excretion after glucose intake is 0.51 ± 0.24 , whereas in normal individuals it amounts to 0.89 ± 0.24 , equal tracer amounts taken for granted. The ¹³C-starch breath test may be useful in evaluating the digestibility of various starch preparations in physiologic and pathological conditions.

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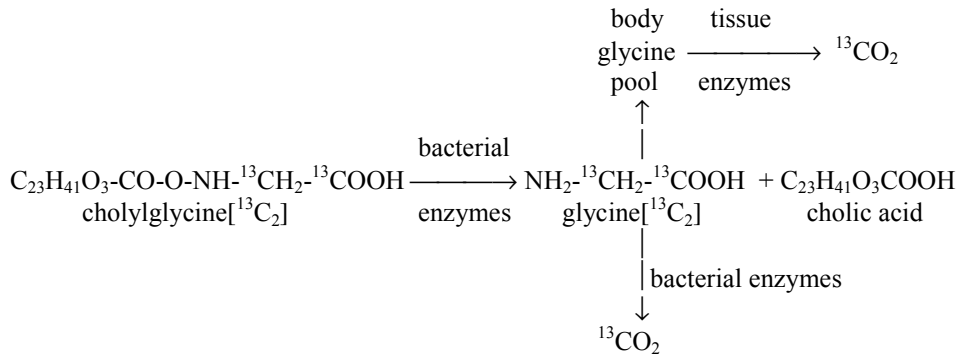
[¹³C]GLYCOCHOLIC ACID BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

¹³C-glycocholic acid breath test can be used for studying enterohepatic circulation of bile acids, bacterial overgrowth in the jejunum, bile acid loss by impaired ileac function etc. The diagnostic yield can be enhanced by simultaneously measuring ¹³C-excretion in faeces.

Suitability for clinical diagnosis: controversial.

Metabolism of Substrate:



Procedure:

After an overnight fast subjects ingest 4 mg per kg body mass [1,2-¹³C]glycocholate dissolved in water. The test meal may consist of toast and butter. Breath samples are taken just before and at 30 minute intervals for 6 hours after tracer intake.

The software of FANci2 or HeliFANplus, respectively, leads the user according to the following guidelines: (After an overnight fast) an oral dose of 4.0 mg per kg body weight [1,2-¹³C]glycocholic acid (99.0 atom% ¹³C), (dissolved in water) is administered (together with a meal consisting of toast and butter). Including the basal sample taken immediately before tracer intake 13 breath samples should be collected at 30 minute intervals. FANci2 or HeliFANplus, respectively, then displays the 6 h-cumulative percent ¹³C-dose recovery (CPDR)*. If CPDR > 3.0 after these 6 hours, then a malfunction must be assumed. (Faecal bile acid loss cannot be diagnosed by this test.)

*The cumulative percent ¹³C-dose recovery (CPDR) is the total ¹³C-dose eliminated with breath during a certain time after tracer administration in % of the tracer intake:

$$\text{cumulative percent } ^{13}\text{C-dose recovery CPDR} = m \cdot (1 - e^{-kt})^\beta$$

with t = time, m = total cumulative percentage of the recovered dose and k and β to be determined by non-linear regression analysis

The data of the CPDR-curve are obtained from PDR-values by numerical integration.

Diagnostic Validity:

The test is pathological if the 6-h cumulative ¹³CO₂ excretion is higher than 3% of the administered label. A low ¹³CO₂ excretion, however, does not discriminate between normals and patients with massive faecal bile acid loss. To complete the test faecal ¹³C excretion should therefore be measured simultaneously.

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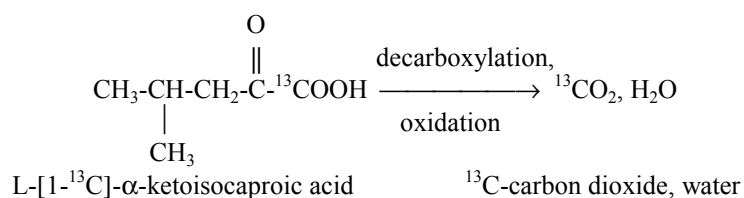
[1-¹³C]α-KETOISOCAPROIC ACID ([1-¹³C]α-KETOISOCAPROATE) BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

The [¹³C]α-ketoisocaproic acid breath test is a tool of diagnosing liver diseases and studying mitochondrial function, especially of assessing the effect of alcohol and xenobiotics like acetylsalicylic acid on mitochondrial function.

Suitability for clinical diagnosis: satisfactory.

Metabolism of Substrate:



Procedure:

After an overnight fast subjects receive an oral dose of 1 mg per kg body mass 2-keto-[1-¹³C]isocaproic acid (99 % ¹³C) together with 20 mg of L-leucine/kg body mass for inhibiting transamination of the ¹³C-labelled substrate to leucine. The subjects rest for 30 min before tracer intake and during the test. Breath is sampled just before tracer intake and then every 30 minutes for two hours. Concomitant administration of leucine increases the fraction of the administered tracer which is decarboxylated thus improving the discrimination between alcoholics and non-alcoholics.

Diagnostic Validity:

Probably the reliability of the [¹³C]α-ketoisocaproic acid breath test as a marker of excessive alcohol consumption and/or as a means of distinguishing alcoholic from nonalcoholic hepatic steatosis can be enhanced, if different cut-off values are applied for female and male patients, thus taking into account that the substrate in females is oxidized faster than in males.

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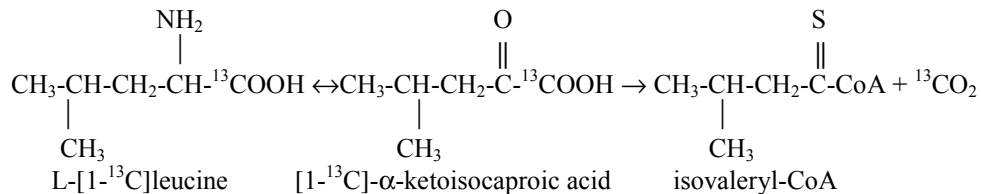
L-[1-¹³C]LEUCINE BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

Since leucine decarboxylation depends on carbohydrate and energy intake the L-[1-¹³C]leucine breath test is useful for evaluating nutritional concepts and dietetic products. The test is also used for studying amino acid and protein metabolism particularly in paediatric, postoperative, phenylketonuria and gestational diabetes mellitus patients. Like isoleucine and valine leucine is an amino acid with a branched carbon chain. The L-[1-¹³C]leucine breath test can therefore also be used for the diagnosis of maple syrup urine disease.

Suitability for clinical diagnosis: controversial.

Metabolism of Substrate:



Procedure:

Term newborn infants receive an intravenous dose of 4 mg of L-[1-¹³C]leucine per kg body mass on two consecutive days. For adults a bolus dose of 1 mg per kg body mass or an infusion of 0.64 mg per kg and h for a few hours are recommended. Breath samples can be collected at 0, 15, 30 and 60 minutes and then every full hour for at most ten hours.

To investigate adults suffering from maple syrup urine disease an oral dose of 38 µmol per kg body mass is ingested after an overnight fast. Breath samples can be collected in 6 min-intervals during the first hour after tracer intake and then every 30 min for the following 5 hours.

Diagnostic Validity:

The L-[1-¹³C]leucine breath test can be used for studying amino acid and protein metabolism and has proved to be an effective tool of evaluating nutritional concepts and dietetic products. The validity of the test for investigating maple syrup urine disease is dubious.

The software of FANci2 or HeliFANplus, respectively, leads the user according to the following guidelines: (After a nocturnal fasting period) a dose of 3.0 mg per kg body weight L-[1-¹³C]leucine (99.0 atom% ¹³C) is given. Including the basal sample taken immediately before tracer intake 6 breath samples should be collected at 60 minute intervals. FANci2 or HeliFANplus, respectively, then displays the percentage ¹³C-dose recovery per hour (PDR)* of the investigated individual.

* The percentage ¹³C-dose recovery per hour (PDR) is defined as the expired ¹³C-dose per hour in % of the administered ¹³C-dose:

$$\%^{13}\text{C dose/h PDR} = (\text{^{13}C - excess in breath} / \text{^{13}C - excess administered}) \times 100 \text{ in \%}$$

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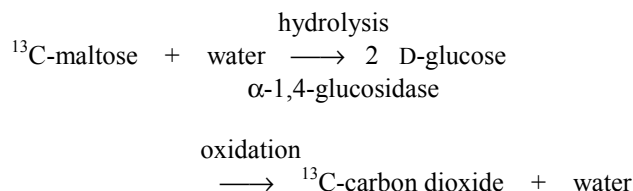
[¹³C]MALTOSE BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

[¹³C]maltose breath test can be used for studying carbohydrate metabolism, particularly the activity of α -1,4-glucosidases.

Suitability for clinical diagnosis: controversial.

Metabolism of Substrate:



Procedure:

A dose of some 10 g maltose naturally enriched in ¹³C is applied after an overnight fast. Breath samples are collected every 10 minutes during the first hour and then every 30 minutes for six to eight hours. The substrate can be synthesised by enzymatic degradation of maize starch. For investigating the activity of α -1,4-glucosidases in neonates 2 mg/kg of body mass of [¹³C₁₂]maltose (98.5 atom% ¹³C) are administered through an orogastric tube just before feeding. Breath samples are collected immediately before and then every 3 h until 12 h after tracer intake in this case.

Diagnostic Validity:

No significant differences neither between maltose and glucose oxidation nor between normal and preterm infants.

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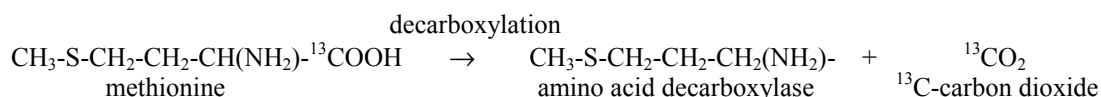
L-[1-¹³C]METHIONINE BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

The L-[1-¹³C]methionine breath test can be used for investigating liver mitochondrial function and for the diagnosis of liver cirrhosis.

Suitability for clinical diagnosis: Not yet clear, because too small a number of patients were investigated so far.

Metabolism of Substrate:



Procedure:

After an overnight fast an oral dose of 75 mg of L-[1-¹³C]methionine (99.8 atom%), dissolved in 100 ml of water, is administered. To avoid the unpleasant taste of L-methionine addition of aromatic tea is recommended. Breath samples can be collected immediately before and every ten minutes for one hour and 90, 120 and 180 minutes after tracer intake.

Diagnostic Validity:

The L-[1-¹³C]methionine breath test seems to be an effective tool of distinguishing between healthy individuals and cirrhotics. DOB-values at 40 minutes, dose per hour in % of the administered dose as well as cumulative dose after normalisation for body CO₂-production according to body surface area.

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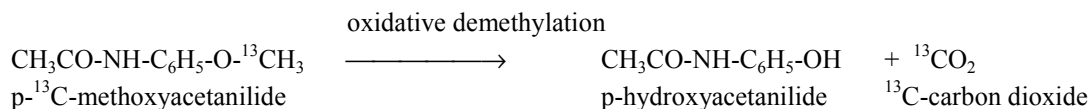
[¹³C]METHOXYACETANILIDE ([¹³C]METHACETIN) BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

The p-¹³C-methoxyacetanilide breath test is useful for investigating hepatic microsomal biotransformation, for quantitative cytochrome-P450-dependent liver function testing, for diagnosing liver diseases and for monitoring hypocaloric dietary management.

Suitability for clinical diagnosis: controversial.

Metabolism of Substrate:



Procedure:

After an overnight fast subjects ingest an oral dose of 2 mg per kg body mass of p-¹³C-methoxyacetanilide (80% ¹³C) dissolved in 50 ml of water or in 100 ml of tea. Breath is sampled immediately before tracer intake and then 5, 10, 15, 20, 30, 40, 50, 60, 80, 100, 120, 150 and 180 minutes thereafter. The individuals are asked to avoid any activity during the test period.

Babies ingest 0.5 mg p-¹³C-methoxyacetanilide per kg body mass. Breath is collected before tracer administration and then in 15 minute intervals for the first hour and in 45 minute intervals for 90 consecutive minutes after tracer intake.

The software of FANci2 or HeliFANplus, respectively, leads the user according to the following guidelines: (After a nocturnal fasting period) a dose of 2.0 mg per kg body weight p-¹³C-methoxyacetanilide (methacetin; 99.0 atom% ¹³C) is taken in. Including the basal sample taken immediately before tracer intake 15 breath samples should be collected at 15 minute intervals. FANci2 or HeliFANplus, respectively, then displays the percentage ¹³C-dose recovery per hour (PDR)* and the 2 h-cumulative percent ¹³C-dose recovery (CPDR)** of the investigated individual. If CPDR ≥ 20 after 120 min, then normal liver function can be assumed. CPDR < 20 points to a malfunction of this organ.

**The cumulative percent ¹³C-dose recovery (CPDR) is the total ¹³C-dose eliminated with breath during a certain time after tracer administration in % of the tracer intake:

$$\text{cumulative percent } ^{13}\text{C-dose recovery CPDR} = m \cdot (1 - e^{-kt})^\beta$$

with t = time, m = total cumulative percentage of the recovered dose and k and β to be determined by non-linear regression analysis

The data of the CPDR-curve are obtained from PDR-values by numerical integration.

Diagnostic Validity:

There is a good correspondence between ¹³C- and ¹⁴C-methacetin measurements. With a cut-off value of 25 % at 20 minutes sensitivity and specificity of discrimination between cirrhotic and non-cirrhotic individuals are 93.5 and 95%, respectively. Correlation with the Child-Pugh score is r = 0.67.

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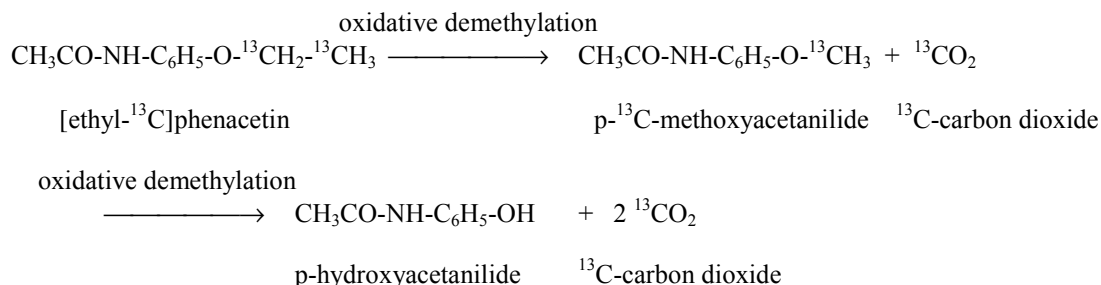
[ETHYL-1-¹³C]PHENACETIN BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

[Ethyl-1-¹³C]phenacetin breath test may be useful for investigating hepatic microsomal biotransformation and for diagnosing liver diseases.

Suitability for clinical diagnosis: controversial.

Metabolism of Substrate:



Procedure:

After an overnight fast subjects ingest an oral dose of 3.5 mg per kg body mass of [ethyl-1-¹³C]phenacetin dissolved in 50 ml of water. Breath is sampled immediately before tracer intake and 30 minutes thereafter. The individuals are asked to avoid any activity during the test period.

Diagnostic Validity:

The cut-off value for distinguishing patients with liver cirrhosis from healthy individuals is supposed to be 0.08 ¹³CO₂ % dose /min.

In patients with abnormal ¹³C-aminopyrine breath tests the results of ¹³C-phenacetin and ¹³C-aminopyrine breath test are highly correlated (r = 0.97) and the regression line passes through the origin, while there is no correlation between the two tests in healthy subjects (r = 0.07). Increasing tracer dose does not saturate p-dealkylation.

The low correlation in control subjects, the dose response data and the rapid ¹³CO₂ excretion suggest that p-dealkylation is not rate limiting and that ¹³CO₂ excretion rate reflects hydrogen carbonate kinetics. A larger dose of ¹³C-phenacetin may enhance the sensitivity of the ¹³C-phenacetin breath test towards the detection of mild liver disease.

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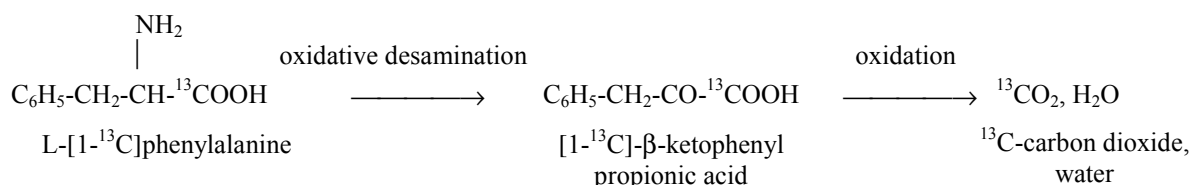
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Indication / Relevance to Medical Research and Diagnosis:

[¹³C]phenylalanine breath test is a means of evaluating liver function the isotopomer L-[1-¹³C]phenylalanine giving the best results. The test is also promising in measuring hepatocyte functional capacity in end-stage liver disease as well as cytosolic enzyme activity which can directly be correlated to liver disease severity. It is also a helpful predictor for postoperative complications in patients undergoing hepatectomy.

Suitability for clinical diagnosis: good.

Metabolism of Substrate:



Procedure:

After an overnight fast patients with end-stage liver disease under evaluation as potential liver transplantation candidates take an oral dose of 2 mg per kg body mass or altogether 100 mg, respectively, L-[1-¹³C]phenylalanine (99 atom% ¹³C) dissolved in a glass of water. Breath samples are taken immediately before tracer intake and then every 30 minutes for two hours or every ten minutes for one hour. Patients should be in a resting position during the test.

For diagnosing and assessing therapy of liver diseases under clinical routine conditions we propose to take the results of Burke PA, Stack JA, Wagner D et al. (1997) as a starting point and to proceed as follows: After an overnight fast the individuals which should remain in a resting position during the test, take an oral dose of 2 mg per kg body mass or altogether 100 mg, respectively,

L-[1-¹³C]phenylalanine (99% ¹³C) dissolved in a glass of water. Breath samples are taken immediately before and 20 or 30 minutes after tracer intake.

Diagnostic Validity:

With respect to its accuracy and the time needed for carrying out the L-[1-¹³C]phenylalanine breath test is superior to the [¹³C]phenacetin breath test in the diagnosis of liver disease. Results are closely correlated with those of the best clinical methods for diagnosing liver disease.

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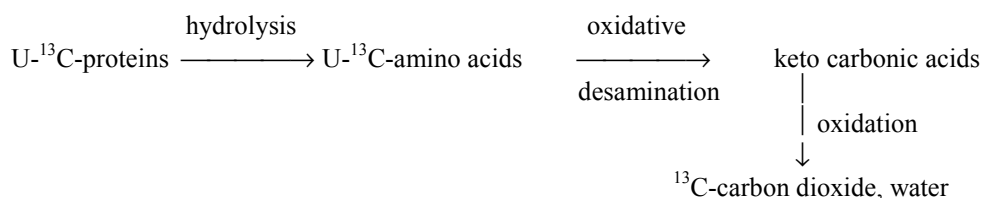
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Indication / Relevance to Medical Research and Diagnosis:

¹³C-breath tests with protein-rich substrates like casein and other milk products, algal biomass, whole egg, egg white or egg yolk, naturally or artificially enriched or depleted in ¹³C, are used for studying absorption and metabolic degradation of proteins. ¹³C-egg white breath test is applied for measuring pancreatic trypsin activity in the small intestine.

Suitability for clinical diagnosis: satisfactory.

Metabolism of Substrate:**Procedure:**

After an overnight fast subjects ingest a dose of 220 g (dry matter) whole egg ($\delta^{13}\text{C} = -15.7$) or 287 g (dry matter) egg white ($\delta^{13}\text{C} = -18.5$) together with a suitable diet. If egg white highly enriched in ¹³C is administered, the tracer dose can be reduced to 22 g.

Egg proteins labelled with [1-¹³C]L-leucine can be produced by feeding laying hens a 0.2 % leucin-deficient food supplemented with 0.2 % [1-¹³C]L-leucine (99 atom% ¹³C). The overall tracer recovery in egg proteins then amounts to 40 %.

Casein from milk of cows on a maize diet ($\delta^{13}\text{C} = -13.471$) is applied in a dose of 50 g in adults and in a dose of 2g/kg body mass in children.

Breath samples are collected ten and five minutes before tracer intake and then every 30 minutes for four hours, the subjects remaining quietly seated during the test period.

Diagnostic Validity:

¹³C-breath tests with protein-rich natural substances as substrates are useful for studying protein absorption and metabolic degradation.

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5. ¹³C–Breath Tests for Investigating Processes and Diagnosing Intestinal Diseases in Jejunum, Ileum, Caecum and Colon

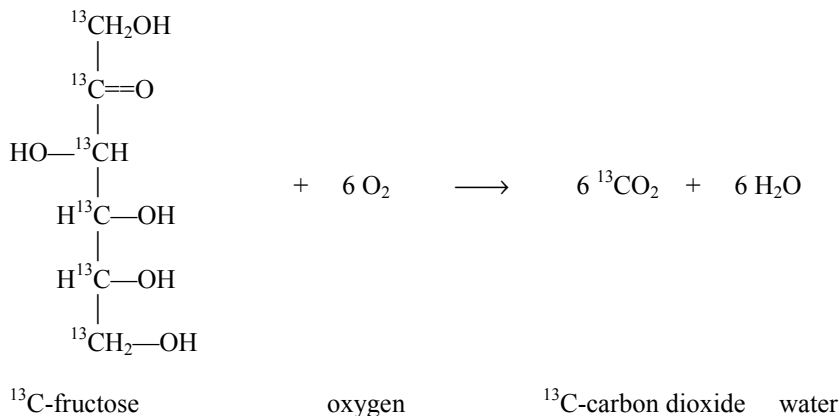
[¹³C]FRUCTOSE BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

The [¹³C]fructose breath test is used for studying small intestinal hexose absorption, especially for investigating fructose malabsorption which is supposed to be a cause of recurrent abdominal pain and chronic non-specific diarrhea in children. Moreover the effect of simultaneously ingested amino acids and glucose is studied by this test.

Suitability for clinical diagnosis: satisfactory.

Metabolism of Substrate:



Procedure:

Children from 12 to 16 years of age receive 25 g fructose labelled with 15 mg [6-¹³C]D-fructose, eventually together with equimolar doses of glucose or L-alanine. For children from 3 to 6 years of age a dose of 2 g fructose per kg body mass (maximum 37.5 g), alone or with an equimolar dose of L-alanine, is chosen. Breath samples for isotope analysis are taken every 10 min for 120 min.

Diagnostic Validity:

In contrast to older children L-alanine addition results in significantly lower increases of ¹³CO₂ in breath of younger ones.

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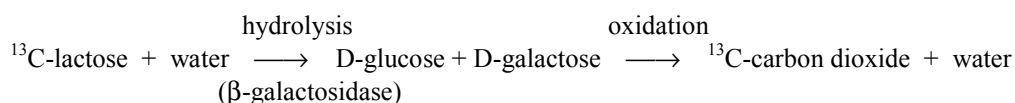
[¹³C]LACTOSE BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

[¹³C]lactose breath test either with L-[1-¹³C]lactose or with lactose naturally enriched in ¹³C is a means of investigating lactose assimilation, especially of detecting lactase deficiency in patients with gastrointestinal symptoms and of measuring lactase activity of brush border. The test enables to measure the hydrolysis rate of the disaccharide which is the rate limiting step in its metabolic pathway. In healthy patients in rest, glucose oxidation is the rate limiting step in lactose conversion into carbon dioxide. Increase of metabolism by exercise (bicycling, 50 Watt) shifts this step to intestinal hydrolysis of the substrate sensitizing the test with respect to diagnosing hypolactasia. For the diagnosis of hypolactasia at least in children the [¹³C]lactose breath test should be combined with the traditional H₂-breath test. Discordance in results of [¹³C]lactose breath test and H₂ lactose breath test indicates that a jejunal biopsy should be performed.

Suitability for clinical diagnosis: good.

Metabolism of Substrate:



Procedure:

Adults take a 50 g load of lactose naturally enriched in ¹³C together with a standard breakfast after an overnight fast. The substrate is prepared from milk of cows fed with silo maize for at least two weeks. The δ¹³C-value is then -13.293 ± 0.002. The tracer is dissolved in 250 ml of water. In children the dose is 2 g/kg body mass in 50 ml of water. Breath samples are collected ten to fifteen and five minutes before tracer intake and then every 30 minutes for four hours, the subjects remaining quietly seated during the test period.

For characterising lactose absorption and utilisation under clinical routine conditions we propose to apply either lactose naturally enriched in ¹³C in an oral dose according to the above mentioned procedure or to orally administer 475 mg [glucose-1-¹³C]-lactose / m² of body surface area (90 atom% ¹³C). Breath samples might be collected immediately before and 90 minutes after tracer intake which should be preceded by a fasting period of 5 hours for infants and 8 hours for children and adults.

The software of FANci2 or HeliFANplus, respectively, leads the user according to the following guidelines: After an overnight fast adults take an oral dose of 50 g [¹³C]lactose naturally enriched in ¹³C (1.09 atom% ¹³C) (together with a standard breakfast). Including the basal sample taken immediately before tracer intake 9 breath samples should be collected at 30 minute intervals. FANci2 or HeliFANplus, respectively, then displays the 3 h-cumulative percent ¹³C-dose recovery (CPDR)*. If CPDR < 8.0 after 3 hours, then a malfunction must be assumed.

*The cumulative percent ¹³C-dose recovery (CPDR) is the total ¹³C-dose eliminated with breath during a certain time after tracer administration in % of the tracer intake:

$$\text{cumulative percent } ^{13}\text{C-dose recovery CPDR} = m \cdot (1 - e^{-kt})^\beta$$

with

t = time, m = total cumulative percentage of the recovered dose and k and β to be determined by non-linear regression analysis.

The data of the CPDR-curve are obtained from PDR-values by numerical integration.

Diagnostic Validity:

With a cut-off level of 14.5% for the 4-hour cumulative ¹³CO₂ excretion, a sensitivity of 84% and a specificity of 96% [¹³C]lactose breath test exceeds corresponding values of H₂-breath test. The correlation coefficients of the regression of ¹³C on ¹⁴C excretion for seven adult subjects range from 0.950 to 0.997 with a mean value of 0.987. Simultaneous absorption of substrate from the small and large intestine may limit the usefulness of ¹³C-breath tests in the premature infant.

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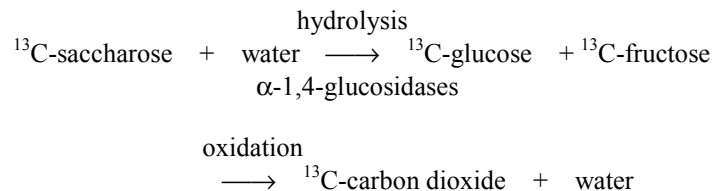
[U-¹³C]SACCHAROSE ([U-¹³C]SUCROSE) BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

[¹³C]saccharose breath test can be used for studying carbohydrate metabolism, particularly for studying sucrase activity in childhood and for investigating the effect of acarbose, a pseudotetrasaccharide of microbial origin which acts as an α -glucoside hydrolase inhibitor, on saccharose assimilation.

Suitability for clinical diagnosis: controversial.

Metabolism of Substrate:



Procedure:

Children drink an aqueous solution (20%) of 42.75 g of saccharose per m² of body area plus 2.85 mg per kg of body mass [U-¹³C]saccharose within 2 minutes between 8 and 9 a.m. after an overnight fast. During the test the children stay at rest and are not allowed to take up food. Immediately before the intake of saccharose the children receive a single dose of 50 mg of acarbose or a placebo, if the effect of this α -glucoside hydrolase inhibitor is to be studied. Breath samples are collected immediately before and at 30 to 60 minute intervals over five hours thereafter. ¹³C concentrations in breath rise to peak at about 2 1/4 hours after tracer ingestion without acarbose intake and at about 2 3/4 hours after tracer ingestion with acarbose ingestion.

Adults take a 50 g load of saccharose naturally enriched in ¹³C dissolved in 250 ml of water together with a standard breakfast after an overnight fast. The substrate is prepared from cane sugar ($\delta^{13}\text{C} = -10.600$). In children the dose is 2 g/kg body mass in 50 ml of water. Breath samples are collected ten and five minutes before tracer intake and then every 30 minutes for four hours, the subjects remaining quietly seated during the test period.

Diagnostic Validity:

The effect of acarbose on saccharose absorption can be assessed using the [U-¹³C]saccharose breath test. Acarbose delays peaking of ¹³C concentrations in breath and flattens the corresponding ¹³C curves significantly.

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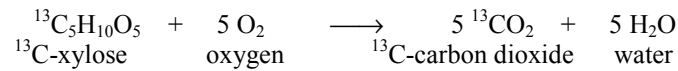
[¹³C]XYLOSE BREATH TEST

Indication / Relevance to Medical Research and Diagnosis:

[¹³C]xylose breath test is used for the diagnosis of small bowel bacterial overgrowth, including the evaluation of corresponding antibiotic therapy. The test can be applied also in paediatrics. Small bowel bacterial overgrowth reveals itself by accelerated ¹³CO₂-excretion.

Suitability for clinical diagnosis: satisfactory.

Metabolism of Substrate:



Procedure:

After an overnight fast of at least eight hours children to be investigated receive 50 mg of [¹³C]xylose dissolved in 30 ml of water, either capsuled or not, if the child is unable to swallow the capsule. Breath samples are taken immediately before tracer intake and every 30 min for four hours. The subjects are instructed to fast except for water and to engage in quiet play without strenuous activity.

Adults receive a 250 mg oral dose of xylose uniformly labelled with ¹³C dissolved in 50 ml of water after an overnight fast. Breath samples are collected immediately before tracer intake and thereafter every 30 minutes for at most four hours following tracer intake.

The software of FANci2 or HeliFANplus, respectively, leads the user according to the following guidelines: (After a nocturnal fasting period of at least eight hours) a dose of 2 mg of ¹³C-xylose (99.0 atom% ¹³C) per kg body weight are taken in. Breath samples are collected immediately before and 30, 60, 90 and 120 minutes after tracer intake. FANci2 and HeliFANplus then display the cumulated dose related to body weight.

Diagnostic Validity:

[¹³C]xylose breath test results reliably predict small bowel bacterial overgrowth both in children and adults. For adults the 180 minute breath samples show the largest differences in ¹³C between normal volunteers and patients with bacterial overgrowth.

References:

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6. The Future of ^{13}C -Breath Tests

^{13}C -breath tests are widely applied as a tool of investigating metabolic processes and infectious diseases, but most of them did not or did not yet enter into clinical routine application. No wonder that the procedures are preferentially directed to high cognitive yield rather than easy and reasonable implementation, e. g. with respect to the amount of the ^{13}C -labelled substance to be applied or the number of breath samples to be collected and analysed.

In order to promote the introduction of more ^{13}C -breath tests into clinical routine application we evaluated the tests so far known with respect to the following criteria (see Tab. 2):

1. Abundance and significance of the metabolic disorder or infection, respectively, to be investigated.
2. Want of attractive alternatives
3. Sensitivity and Specificity
4. Fastness
5. Price of substrate to be applied
6. Number of breath samples to be collected and analysed
7. Topicality

Each of these seven points of view is valued by one of three marks: 2 = favourable / 1 = satisfactory / 0 = unfavourable. The sum Σ of these marks, i. e. up to $7 \times 2 = 14$, is assumed as a measure of the total value of the respective ^{13}C -breath test. (To escape from being accused of arbitrariness we refrain from a differentiated weighing of the seven aspects mentioned above.)

As Tab. 2 reveals, we arrived at ten tests with a total value Σ of altogether ten or more points which seem to have an especially high potential to successfully enter into clinical practice. Among these the ^{13}C -urea breath test is already validated for clinical application by the appropriate governmental authority in a lot of countries. A few other ^{13}C -breath tests are already validated in someone or other country or validation is under way or in preparation, respectively.

Following the results presented in Tab. 2 we arrive at the conclusion that the following tests probably have the greatest chance to attain world-wide clinical routine application:

- ^{13}C -urea breath test ($\Sigma = 13$)
- ^{13}C -aminopyrine breath test ($\Sigma = 13$)
- ^{13}C -galactose breath test ($\Sigma = 13$)
- sodium[^{13}C]acetate breath test ($\Sigma = 12$)
- ^{13}C -caffeine breath test ($\Sigma = 11$)
- ^{13}C -octanoic acid breath test ($\Sigma = 11$)
- ^{13}C -phenylalanine breath test ($\Sigma = 11$)
- ^{13}C -trioctanoine breath test ($\Sigma = 10$)
- ^{13}C -glucose breath test ($\Sigma = 10$)
- ^{13}C -lactose breath test ($\Sigma = 10$)

In all we infer from the investigations summarised in table 2 that the efforts towards introduction into clinical practice for ^{13}C -breath tests like the ^{13}C -urea breath test, the sodium[^{13}C]acetate breath test and the ^{13}C -octanoic acid breath test should be continued and extended at least to the other above mentioned tests, particularly to those with the highest total values Σ like the ^{13}C -aminopyrine and the ^{13}C -galactose breath test ($\Sigma = 13$).

Such efforts should be preferentially directed towards the following aims:

1. Simplifying the procedure, especially diminishing the number of breath samples without noticeable loss of sensitivity and specificity. (This is probably true of nearly all above mentioned tests with the exception of the ^{13}C -urea breath test, for which the minimum of two samples is already attained).
2. Enhancement of sensitivity and specificity, especially by improving the attending circumstances of the test procedure (e.g. suitable diet before and during the test, particularly in order to keep a definite $\delta^{13}\text{C}$ -value of endogenic carbon reservoirs). According to our considerations this could be helpful for introducing ^{13}C -aminopyrine, ^{13}C -galactose, ^{13}C -caffeine, ^{13}C -phenylalanine, ^{13}C -octanoic acid, ^{13}C -cholesteryloctanoate, ^{13}C -glucose and ^{13}C -lactose breath test into clinical practice.
3. Minimising the tracer amount to be applied without noticeable loss of sensitivity and specificity.
4. Shortening the duration of the test. This could be preferentially advantageous for introducing ^{13}C -aminopyrine, ^{13}C -cholesteryloctanoate and ^{13}C -lactose breath test into clinical routine application.

5. Combination of any ^{13}C breath test with ^{13}C breath tests for measuring gastric emptying times in order to eliminate the effect of gastric emptying on metabolising the respective substrates. In this case the two tests would have to be carried out in succession or one of the two tests would have to be done with ^{14}C instead of ^{13}C . Also the combination with $^{99\text{m}}\text{Tc}$ -scintigraphy as a means of assessing gastric emptying kinetics has to be taken into account.

6. Combination of ^{13}C -breath tests with H_2 -breath tests.

7. Use of ^{13}C -breath tests to the diagnosis of severely ill patients, in view of the extremely low strain connected with the application of such tests. In this connection application in intensive care units has to be especially considered.

8. Inhalation of certain ^{13}C -labelled compounds in a gaseous and/or aerosolic state may offer a way towards breath tests for investigating pathophysiological changes in the pulmonary system.

9. A new field of application of ^{13}C -breath tests, particularly those with substrates like urea, bicarbonate and cholesteryl oleate, begins to emerge: the diagnosis of cardiovascular diseases.

In addition we are convinced that there will be found many other substrates for ^{13}C -breath tests in future which make use of the simple way of obtaining and measuring samples for characterising metabolic processes in the human organism.

Evaluation of ¹³C-Breath tests

2 = favourable
1 = satisfactory
0 = unfavourable

Substrate

Abundance and Significance
Want of Attractive Alternatives
Sensitivity and Specificity
Fastness
Price of Substrate
Number of Samples
Topicality
Total Value

¹³C-breath tests for investigating...

1 ... processes and diagnosing diseases in the gastric and duodenal area

Acetate	2	1	2	2	2	1	2	12 ☺
Bicarbonate	2	1	1	2	2	0	0	8
Glycine	2	2	0	0	2	0	1	7
Octanoic Acid	2	2	1	1	2	1	2	11 ☺
Urea	2	1	2	2	2	2	2	13 ☺

2 ... exocrine pancreatic function and diagnosing pancreatic diseases

Cholesteryl Octanoate	2	1	1	0	0	0	2	6
Oleic Acid	2	1	1	0	0	0	1	5
Palmitic Acid	2	1	1	1	0	2	1	8
Stearic Acid	2	1	1	0	0	0	1	5
Mixed Triglyceride	2	2	1	0	1	1	2	9 ☺
Hiolein	2	2	2	0	0	0	2	8
Genuine Plant Oils	2	2	1	0	2	0	0	7
Trilinoleate	1	1	1	0	0	1	0	4
Trioctanoin	2	2	1	2	1	1	1	10 ☺
Triolein	2	2	2	0	0	1	2	9 ☺
Tripalmitin	2	2	1	0	1	0	2	8

3 ... liver function and diagnosing liver diseases

Aminopyrine	2	2	2	2	1	2	2	13 ☺
Caffeine	2	1	2	2	0	2	2	11 ☺
Ethanol	1	1	1	1	2	2	0	8
Galactose	2	2	2	2	1	2	2	13 ☺
Glucose	2	2	2	0	2	1	1	10 ☺
Glucose Polymers	2	2	1	0	2	0	2	9 ☺
Glychocholic Acid	1	1	1	0	0	1	0	4
α-Ketoisocaproic Acid	1	1	0	2	1	2	1	8
Leucine	1	1	1	0	2	0	1	6
Maltose	1	0	0	0	2	0	0	3
Metacetin	1	1	2	0	1	0	1	6
Phenacetin	1	1	2	0	0	1	1	6
Phenylalanine	1	1	2	2	1	2	2	11 ☺
Protein-rich Genuine Substances	1	1	1	0	2	1	2	8

4 ... processes and diagnosing intestinal diseases in jejunum, ileum, caecum and colon

Fructose	1	2	1	2	1	0	1	8
Lactose	2	2	2	1	0	1	2	10 ☺
Lactose-Ureide	2	2	2	0	1	0	2	9 ☺
Saccharose	1	2	1	1	0	1	0	6
Xylose	1	2	2	1	0	1	1	8

Tab. 2. Evaluation of the ¹³C-Breath Tests (2 = favourable/ 1 = satisfactory/ 0 = unfavourable)