

Recent Results of the Development and Application of ^{13}C - Breath Tests

Klaus Wetzel and Heinz Fischer
Fischer ANalysen Instrumente GmbH (FAN), Leipzig

© Fischer Analysen Instrumente GmbH
Brahestraße 25-27 • 04347 Leipzig
Bundesrepublik Deutschland

Tel.: +49 341 24450 0 • Fax: +49 341 24450 22
Email: fan@fan-gmbh.de

The Authors' Addresses:

Prof. Dr. Dr. Klaus Wetzel
Schaddeler Dreieck 12
04668 Großbothen

Dr. Heinz Fischer
Brahestraße 25-27
04347 Leipzig

NOTE

Great care has been taken over the composition of the text, figures and tables of this booklet. The possibility of errors, however, cannot be excluded completely. Therefore Fischer Analysen Instrumente GmbH cannot accept any legal or other liability with respect to incorrect details and their consequences. The authors would be grateful for suggestions for improvements and information about errors.

The commercial exploitation of the models and projects shown in this book is not permitted. This book must not be photocopied, duplicated or translated into another language, neither partially nor completely, without the written agreement of Fischer Analysen Instrumente GmbH.

1st Edition
Printed in December 1999

© Copyright by Fischer Analysen Instrumente GmbH

Preface

Tracer technique using stable isotopes nowadays is widely used in medical research and diagnosis. In view of the crucial role of carbon in living matter it is no wonder that labelling just this element is of major interest. If the metabolic reaction to be investigated yields carbon dioxide, sampling for isotope analysis becomes particularly simple, because the patient has only to blow his breath into a bag or a sample cell. The number of publications on such ^{13}C -breath tests, nowadays amounting to more than hundred a year, is growing rapidly. The last summarising presentation of these tests was given in a monograph published in 1991. Therefore our pamphlet is intended to present the progress achieved during the last ten or twelve years, comprising the tools and the methodology of evaluating the data as well as the biochemical, medical and clinical aspects. We gratefully appreciate the cooperation of Prof. Dr. med. habil. Wolfgang Hartig, who reviewed the text and gave many stimulating suggestions.

The Authors

CONTENTS

1. Fundamentals	5
<i>1.1. Features of ¹³C–Breath Tests.....</i>	<i>12</i>
<i>1.2. Terminology of Tracer Investigations Using Stable Isotopes.....</i>	<i>16</i>
<i>1.3. Methods of Evaluating Tracer Investigations in Medicine</i>	<i>18</i>
2. Progress in Providing Substrates for ¹³C–Breath Tests.....	22
3. Progress in Analytical Techniques for ¹³C–Breath Tests.....	23
<i>3.1. Administering the Substrate, Sampling and Sample Processing.....</i>	<i>23</i>
<i>3.2. Mass Spectrometric Analysers</i>	<i>23</i>
<i>3.3. Optical Spectrometric Analysers</i>	<i>23</i>
<i>3.4. Automation</i>	<i>29</i>
4. Progress in the Application of ¹³C–Breath Tests in Medical Research and Diagnosis	30
<i>4.1. Methodological Progress</i>	<i>30</i>
4.1.1. General Progress in ¹³ C–Breath Tests.....	30
4.1.2. ¹³ C–Breath Tests with Novel Substrates	37
4.1.3. Novel Applications of ¹³ C–Breath Tests.....	38
4.1.4. Modified ¹³ C–Breath Tests.....	40
<i>4.2. Recent Results of the Application of ¹³C–Breath Tests</i>	<i>42</i>
5. The Future of ¹³C–Breath Tests.....	45
<i>5.1. Trends in Measuring Methods.....</i>	<i>45</i>
<i>5.2. Trends in Supplying Substrates</i>	<i>45</i>
<i>5.3. Trends in Administering Substrates.....</i>	<i>46</i>
<i>5.4. Trends in Sampling.....</i>	<i>46</i>
<i>5.5. Trends in Fields of Application.....</i>	<i>46</i>
References.....	48

1. Fundamentals

The majority of chemical elements consists of two or more kinds of atoms the nuclei of which do not differ from one another with respect to the number P of (positively charged) protons but relating to the number N of (uncharged) neutrons. Such kinds of atoms are called isotopes. The term is derived from Greek language: isos topos means „equal space“. Here one and the same space in the Periodic Table of Elements is meant, in which all kinds of atomic species with one and the same proton number P in their nuclei are attributed to one and the same space. E. g. the atomic nuclei of carbon additionally to the six protons obligatory upon this element contain three to ten neutrons with a mass nearly equal to that of protons.

The chemical and biochemical properties of a given chemical element are determined by the structure of the electron sheaths of its atoms, these again being dependent on the number Z of electrons, called atomic number, in the electron sheath. (For reasons of electric neutrality the number Z of electrons in the electron cloud is equal to the number P of protons in the nucleus.) From this follows that isotopes of one and the same element are distinguished by (at least nearly) equal chemical and biochemical properties.

This is, however, by no means true for a series of physical properties, particularly for nuclear properties and properties distinctly affected by the atomic mass of the chemical element in question, the differences in nuclear properties being the most conspicuous ones. Thus at a given proton number P it depends on the number N of neutrons in the atomic nucleus whether a particular isotope is stable or transforms into another chemical element emitting energy rich radiation. (The latter atomic species are named radioactive isotopes.)

Apart from technetium ($P = Z = 43$) and promethium ($P = Z = 61$) which do not occur in nature, all chemical elements up to bismuth ($P = Z = 83$) are represented in nature by one or more stable isotopes. If there are two or more, their quantitative proportions in nature for nearly all elements are (approximately) constant. Some of these elements exist in nature also in the form of radioactive isotopes, either because their radioactive decay proceeds so slowly that they only partly vanished during the billions of years since their synthesis in space or because they are continuously synthesised under the influence of cosmic radiation on the terrestrial atmosphere or on the earth's surface. (Some elements with atomic numbers Z above that of bismuth ($Z = 83$) also occur in nature, even if only as long lived radioactive isotopes, the most important one being uranium as the basis of recent nuclear energetics.)

Among the carbon isotopes already mentioned those with three, four, five, nine and ten neutrons in their atomic nuclei are irrelevant to medical research and diagnosis. These radioactive isotopes do not occur in nature and can therefore only be produced artificially and disintegrate within a fraction of a second or within a few minutes. The carbon isotope with eight neutrons in addition to the six protons characteristic of the chemical element carbon, however, is certainly also radioactive, but disintegrates only in a period of many thousands of years and is therefore relevant to medical research and diagnosis indeed, even if occurring in nature only in tiny concentrations. (Just these tiny concentrations are the basis of the famous radiocarbon method of age determination of geological, archaeological and similar samples.) It can easily be synthesised artificially, however, and transformed into any carbon compounds relevant to investigating human and animal metabolism. As already emphasised the addition of such a compound to the corresponding carbon compound of natural isotopic composition does not or does not noticeably change its chemical and biochemical properties. Thus we are enabled to follow the metabolic path of such a labelled compound using the easily detectable and measurable energy rich radiation emitted by the radioactive isotope, e. g. its resorption rate in the gastrointestinal system, its transition into the blood circuit, its metabolism by the

liver and the excretion of its metabolites via kidneys and urine, breath or faeces. Such kind of investigations are named tracer investigations or tracer experiments, the isotopes used and the substances synthesised from them are called tracers or markers. Frequently such investigations are also named labelling experiments (with labelled elements or substances, respectively).

Fortunately the addition of radioactive substances is not the only way to perform tracer investigations. Especially in human medical research and diagnosis they are scorned because of the exposition by ionising radiation involved. At infants and pregnant women they are strictly forbidden at all. The alternative consists of changing the quantitative proportions of the stable isotopes of the chemical element to be labelled or any of its chemical compounds, thus enabling to distinguish it from all other materials containing the element or compound in question without noticeably altering its chemical or biochemical properties. (As already mentioned, the isotopic ratio of most of the chemical elements in their natural occurrences is approximately constant.)

For some elements, especially for some light elements, such kind of labelling is sometimes already achievable utilizing the (commonly only) small variations of the quantitative proportions of the stable isotopes of such elements in their natural occurrences. This is true for elements like hydrogen, carbon, nitrogen, oxygen, sulphur and (due to the radioactive decay of uranium and thorium with lead isotopes as final products) also lead.

<i>Abundance</i>	<i>a [Atom%]</i>	<i>δ¹³C</i>
Limestone	1.1166 – 1.1058	+4.9 – -4.9
PDB-Standard	1.11123	±0.00000
Atmospheric CO ₂	1.1045 – 1.1011	-6.1 – -9.1
Maize, cane sugar and other C ₄ -plants	1.1009 – 1.0950	-9.2 – -14.6
Breath of Americans	1.0930 – 1.0879	-16.4 – -21.0
Breath of Europeans	1.0892 – 1.0842	-19.8 – -24.3
Potatoes, beet sugar, rice and other C ₃ -plants	1.0886 – 1.0713	-20.4 – -35.9
Brown and bituminous coal	1.0912 – 1.0646	-18 – -42
Natural oil and gas	1.0878 – 1.0346	-21 – -69

Table 1

¹³C-abundances and δ¹³C values of some carbon occurrences in nature. (PDB means calcium carbonate of the fossil *Belemnite* of the Cretaceous *Pee Dee* Formation in South Carolina, USA, used as international standard for carbon isotope analysis. C₄- and C₃-plants differ from one another by their mechanisms of photosynthesis.)

Tab. 1 presents the isotopic composition for some important occurrences of carbon in nature. In the first column the particular natural occurrences of carbon are given. The second column presents the abundance *a* of the (usually less abundant) isotope ¹³C (in atom%) and the last column the δ value

$$\delta^{13}\text{C} = [(a - a_0) / a_0] \times 1000 \text{‰},$$

where a_0 is the ^{13}C -abundance in a suitable standard. For carbon the so-called PDB standard is used, i. e. calcium carbonate of the fossil *Belemnitella* of the Cretaceous Pee Dee-Formation in South Carolina, USA. (Usually the sum of proton number P and neutron number N , the so-called mass number $M = P + N$, is added to the symbol of the particular element as a pre-superscript.)

As Tab. 1 reveals, the mean ^{13}C -abundance in natural carbonates exceeds the mean ^{13}C -abundance in breath of Europeans by 3 % or the mean ^{13}C -abundance in natural oil and gas even by 5 %. These are differences which can reliably be detected using modern stable isotope analysers. Thus carbon from carbonate deposits is labelled with respect to carbon in breath or natural oil and gas, labelled with the stable isotope ^{13}C , as we use to say. In the same sense carbon compounds from C_3 -plants are labelled with respect to carbon compounds from C_4 -plants. (C_3 - and C_4 -plants differ from one another with respect to the mechanisms of their photosynthesis, C_3 -plants following the so-called Calvin cycle and C_4 -plants the Hatch-Slack cycle.)

In most cases, however, artificially labelled compounds are favoured above naturally labelled ones. Labelling with stable isotopes then requires artificial enrichment of the less abundant stable isotope, or (if there are more than two stable isotopes) one of the less abundant stable isotopes.

Most of the methods for separating stable isotopes are multiplicative processes, i. e. the generally only small differences of isotopes and their chemical compounds in properties like boiling temperature, diffusion rate, reaction rate and position of chemical equilibrium are multiplied by means of the so-called countercurrent principle, until the desired degree of enrichment of the less abundant isotope is achieved. E. g. the preparation of any carbon compound labelled with ^{13}C since many years usually starts with low temperature countercurrent distillation of liquefied carbon monoxide, ^{13}CO because of its slightly higher boiling point in comparison to ^{12}CO being enriched at the sump of the countercurrent columns applied. Carbon monoxide is then oxidised to carbon dioxide which is used as source material for preparing a wide variety of ^{13}C -labelled organic and inorganic compounds using more or less modified methods of chemical and biochemical synthesis.

Not only the separation of the stable isotopes of one and the same chemical element, but also their analysis, i. e. the determination of the quantitative parts in the element in question, is based on the (often only small) differences in some of their physical or physicochemical properties.

The most common and oldest method of stable isotope analysis applicable to the isotopes of all elements of the periodic table is mass spectrometry which makes use of the dependence of the deflection of charged particles in electric and magnetic fields on their masses. The substance to be analysed with respect to the isotopic composition of any element has to be ionised in the so-called ion source of the mass spectrometer (Fig. 1). For gases and vapours this is usually performed by bombardment with fast electrons which push one (or more) electrons out of the electron clouds of the gas molecules thus inducing the generation of positively charged ions. The latter are withdrawn from the ion source and accelerated by an electric field and then pass either a magnetic field or a combination of a magnetic and an electric field.

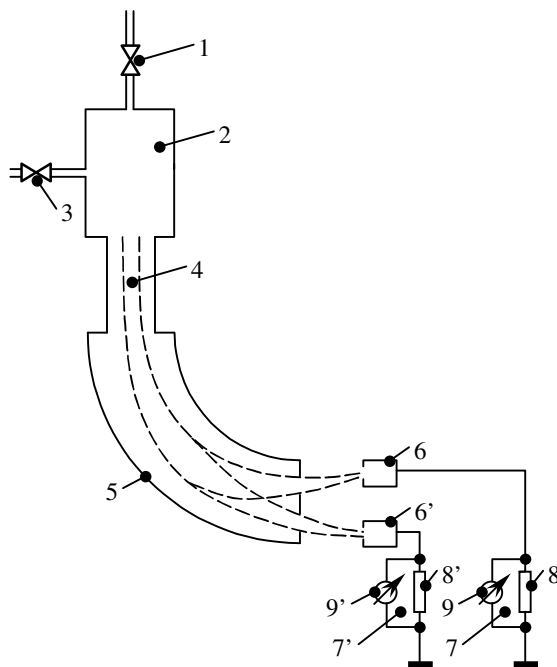


Figure 1

Outline of a mass spectrometer according to Nier. (For detailed explanation see text.)

- 1 valve for sample admission
- 2 ion source
- 3 valve for connecting vacuum pump
- 4 ion beam
- 5 magnetic field
- 6 receiver for the lighter ions
- 6' receiver for the heavier ions
- 7 device for measuring the electrical charge delivered at the receiver by the lighter ions. (Charge flows towards earth via an electrical resistor 8 and the potential difference at its ends is measured by the voltmeter 9.)
- 7' device for measuring the electrical charge delivered at the receiver by the heavier ions. (Charge flows towards earth via an electrical resistor 8' and the potential difference at its ends is measured by the voltmeter 9'.)
- 8, 8' electrical resistor
- 9, 9' voltmeter

In a magnetic field the ions are deflected into circular paths the radii r of which depend on the charge z to mass m ratio according to the equation

$$r = 1/B \sqrt{2m/zU}$$

in which B is the magnetic field strength and U the acceleration voltage. So the lighter ions are more deflected than the heavier ones and thus can be separately collected and measured at the receiver.

If the chemical compound, the isotopic composition of which has to be measured, is a component of a mixture with other substances, mass spectrometers are often coupled with a gas chromatograph which separates the component or the components to be subject to isotope analysis before their introduction into the ion source. This method is called gas chromatography–mass spectrometry (GC–MS).

Beside mass spectrometry optical spectroscopy is often used as a means of stable isotope

analysis. The frequency of a spectrum line or band in the optical spectrum of an atom or a molecule namely does not only depend on the structure of its electron cloud, but (to a certain degree) also on the mass of the atomic nuclei. (In case of spectrum lines due to change of the vibrational states of atoms within a molecule or of the rotatory state of the whole molecule this mass effect is evident. Spectrum lines due to electron transitions are affected by atomic masses, because – using the terminology of classical physics – electron and atomic nucleus circle around their joint centre of gravity.)

Usually optical spectrographs are equipped with an interferometer, a prism or a grating which disperses the electromagnetic radiation into its spectral components according to their frequencies or wavelengths, respectively. For the infrared range of the spectrum of electromagnetic waves, i. e. for wavelengths between 780 nm and 1 mm, an alternative method of optical spectroscopy has established which avoids spectral dispersion and therefore also interferometer, prism or grating, respectively, the so-called nondispersive infrared spectroscopy. Here the specificity to the single isotopes is achieved by using detectors filled with the isotopically pure gases for measuring radiation intensities, e. g. for the isotope analysis of carbon in carbon dioxide with $^{13}\text{CO}_2$ or $^{12}\text{CO}_2$, respectively. Thus the detectors become sensitive to those frequencies which correspond to lines or bands in the spectra of these gases. The absorption of infrared radiation causes the gases in the detectors to heat up. This gives rise to an increase in pressure which is used as a measure of radiation intensity just in that parts of the infrared spectrum in which the respective isotopically pure gas absorbs.

An outline of a device working on this principle is given by Fig. 2. The infrared sources 1 emit an infrared continuum with a constant and equal output. This radiation passes the cells 2 filled with the measuring gas and then the detector cells 3' and 3'' each loaded with one of the isotopically pure gaseous components to be measured. The gas in each of the detector cells absorbs in spectral ranges of the particular component in it and heats up to a degree which is proportional to the intensity of this radiation at its entrance window. This intensity in its turn does not only depend on the output of the lamps 1, but naturally also on the degree of radiation absorption in the measuring cells 2 and therefore also on the concentration or partial pressure, respectively, of the particular component in these measuring cells. According to Gay-Lussac's law the pressures in the detector cells 3' and 3'' enhance to a degree proportional to the increase in temperature due to radiation absorption. The membrane pressure gauge, the membrane of which is designed as one plate of a capacitor, indicates the difference of the pressures in the detector cells 3' and 3'' which is amplified and displayed by the amplifier 5. The rotating chopper 6 brings about a periodic interruption of the infrared beams so that the membrane of the pressure gauge 4 vibrates and an alternating voltage arrives at the amplifier which is proportional to the concentration difference of the components to be analysed in the measuring gas, in our example $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$.

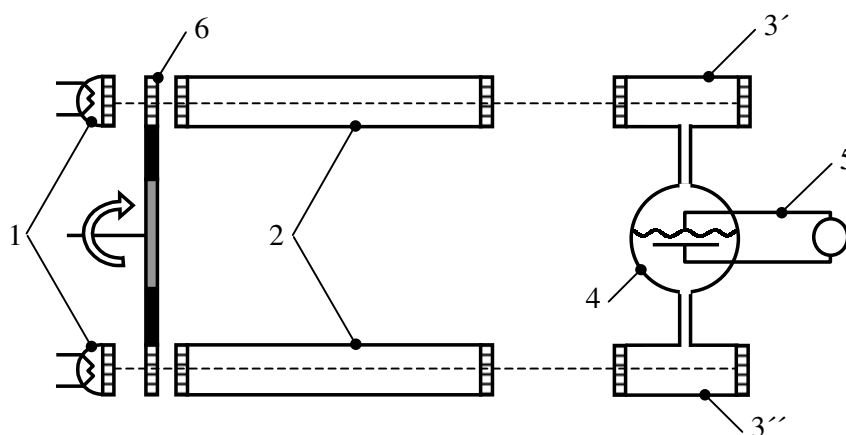


Figure 2

Outline of a simple nondispersive infrared spectrograph for measuring the isotopic composition of carbon in carbon dioxide.

- 1 infrared sources emitting a continuum in the infrared range
- 2 measuring cells filled with the gaseous sample to be analysed
- 3' detector cell filled with one of the isotopically pure gases
- 3'' detector cell filled with the other isotopically pure gas
- 4 membrane pressure gauge designed as a capacitor
- 5 signal detection
- 6 beam chopper
- infrared beam

While mass spectrometric ^{13}C -breath test devices usually need compressed air or helium (as a carrier gas for separating carbon dioxide from the other components of the breath) and liquid nitrogen or solid carbon dioxide (for separating carbon dioxide by condensation), ^{13}C -breath test devices using nondispersive infrared spectroscopy need neither auxiliary gases nor refrigerants. Measurement of $^{13}\text{C}/^{12}\text{C}$ ratios in exhaled carbon dioxide is directly performed in breath in this case.

It has to be emphasised that monoatomic gases, like noble gases, and gases the molecules of which consist of two equal atoms, like hydrogen (H_2), nitrogen (N_2) or oxygen (O_2), neither absorb nor emit electromagnetic radiation in the infrared range and are therefore not accessible to infrared spectroscopy. (All other gases, however, are infrared-active inasmuch as they do absorb and emit infrared radiation. If such gases are genuine or anthropogenic constituents of the earth's atmosphere, they act as greenhouse gases.)

Beside the methods of mass spectrometry and absorption spectroscopy emission spectrometry as well as nuclear magnetic resonance spectrometry, neutron activation and other nuclear methods are used for analysing stable isotopes. Especially for analysing the stable isotopes of hydrogen there is a series of additional methods based on the relatively large differences in the physical properties of hydrogen and deuterium and their chemical compounds (density, heat conductivity, refractive index etc.).

The method of labelling with stable isotopes is widespread in medical and biochemical research and in clinical diagnosis. In view of the crucial role of carbon in living matter it is not surprising that labelling just this chemical element is by far outstanding in this connection. The method is particularly attractive in those cases, where the metabolic process to be investigated yields a gaseous chemical compound which can directly be analysed by mass spectrometry or optical spectroscopy. Then sampling usually is very simple, because the

persons to be investigated have only to blow up a bag or a sample cell. In medical research and diagnosis the gaseous metabolite to be analysed in most cases is expired carbon dioxide. The nature of such ^{13}C -breath tests consists of applying a ^{13}C -labelled compound, the so-called substrate, to the test person or the patient and following the kinetics of the exhalation of [^{13}C]carbon dioxide with breath.

It is the subject of this booklet to characterise the methodology of ^{13}C -breath tests and to demonstrate its capabilities. The last summarising presentation of ^{13}C -breath tests was given in a monograph from the year 1991 (Krumbiegel P 1991). Our pamphlet is therefore intended to give a report on the progress in ^{13}C -breath tests achieved since the end of the eighties.

1.1. Features of ^{13}C -Breath Tests

Initially focussed on investigating infants and pregnant women with their particularly high health risks when exposed to energy rich radiation, ^{13}C -breath tests nowadays have replaced breath tests using the radioactive ^{14}C 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. (E. g. if the substrate is synthesised from C_4 -plants with their Hatch-Slack cycle of photosynthesis the diet should exclusively 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.

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 up the substrate and to draw the diagnostic conclusion from the increase of the ^{13}C -content during this period of time t .

Many other ^{13}C -breath tests are distinguished by measuring the ^{13}C -content for several or even many hours every ten to thirty minutes and plotting them into an $a(t)$ versus t diagram, as is schematically demonstrated in Fig. 3. 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. Corresponding earlier summarising presentations are given by Hofmann F and Lauterburg BH (1977), Barr RG, Perman JA, Schoeller DA et al. (1978), Gilger MA, Klein PD, Klish WJ et al. (1988), Krumbiegel P (1991) and others.

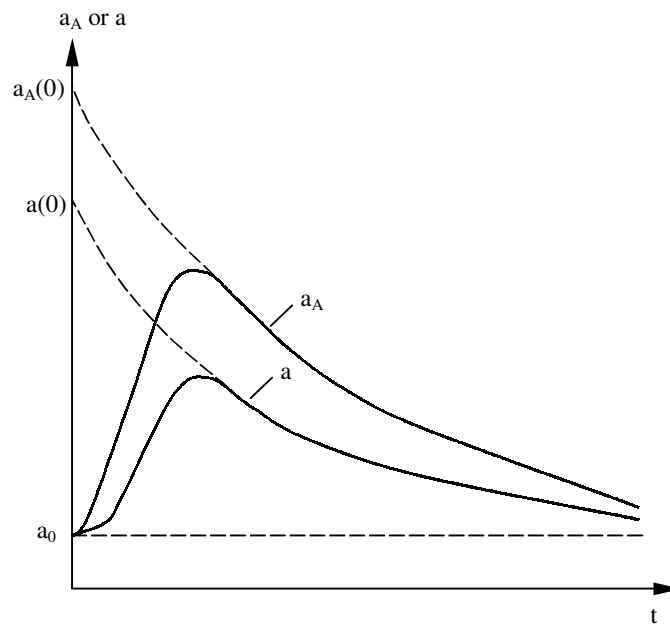
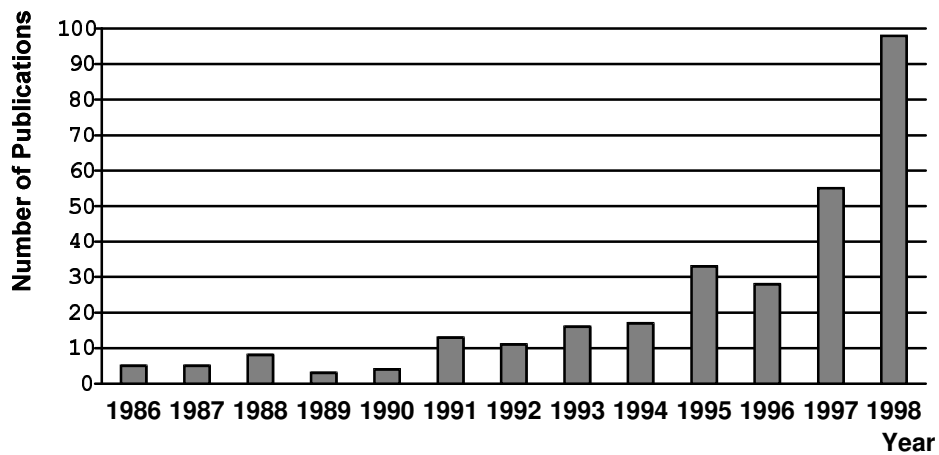


Figure 3

Outline of the dependence of the ^{13}C -content in breath (a) and in an endogenous pool intensively involved in metabolism (a_A) on the time t after administering ^{13}C -labelled substrate. (The initially slow increase in a corresponds with the lag-phase, a_0 representing the ^{13}C -content before taking up the substrate; the values $a(0)$ and $a_A(0)$ correspond with the extrapolation of the descending parts of the curves for a and a_A to $t=0$.)



As Fig. 4 reveals ^{13}C -breath tests develop with high dynamics.

Figure 4

Dynamics of the development and application of ^{13}C -breath tests.

Tab. 2 shows a survey on substrates of ^{13}C -breath tests and their application in clinical research and diagnosis so far investigated.

^{13}C -labelled substrate	diagnostic capability
1. Carbohydrates	
1.1. Monosaccharides	
$[^{13}\text{C}]$ glucose, $[\delta^{13}\text{C}]$ glucose	hexose absorption and utilisation, especially in diabetes mellitus; influence of body training on carbohydrate metabolism
$[^{13}\text{C}]$ fructose	hexose absorption and utilisation, especially in diabetes mellitus
$[^{13}\text{C}]$ galactose, $[\delta^{13}\text{C}]$ galactose	liver diseases; hepatic microsomal biotransformation; studying galactose-1-phosphate uridylyltransferase
$[^{13}\text{C}]$ xylose	intestinal bacterial overgrowth
1.2. Oligosaccharides	
$[^{13}\text{C}]$ sucrose, $[\delta^{13}\text{C}]$ sucrose	sucrase deficiency in children with gastrointestinal symptoms
$[^{13}\text{C}]$ lactose, $[\delta^{13}\text{C}]$ lactose	lactase deficiency in children with gastrointestinal symptoms
$[^{13}\text{C}]$ maltose	carbohydrate and fat metabolism
1.3. Polysaccharides	
$[\delta^{13}\text{C}]$ starch	digestion and resorption; pancreatic function; cystic fibrosis
$[\delta^{13}\text{C}]$ glucose polymers	digestion and metabolisation for energy production; studying liver glycogen metabolism
2. Proteins and amino acids	
2.1. Proteins	
$[\delta^{13}\text{C}]$ proteins	protein metabolism
$[\delta^{13}\text{C}]$ casein	steatorrhea; digestion and resorption of proteins
$[^{13}\text{C}]$ egg white	protein metabolism
^{13}C -labelled egg protein	evaluation of the true ileal digestibility of egg protein
^{13}C -labelled algal biomass	metabolism
2.2. Amino acids	
L-[1- ^{13}C]leucine	amino acid oxidation; studying phenylketonurea-diet-therapy
$[^{13}\text{C}_2]$ glycine	amino acid oxidation; gastric emptying rate
DL-[1- ^{13}C]phenylalanine	amino acid oxidation
L-[1- ^{13}C]phenylalanine	amino acid oxidation; evaluation of hepatic function
$[^{13}\text{C}]$ tyrosine	rate of tyrosine catabolism
2.3. Milk products	
$[\delta^{13}\text{C}]$ milk products	metabolism

Table 2

Survey on substrates of ^{13}C -breath tests and their application in clinical research and diagnosis so far investigated. (A δ before ^{13}C in square brackets means that the substrate is naturally enriched in ^{13}C . To be continued on the following page.)

¹³ C-labelled substrate	diagnostic capability
3. Lipids	
[δ ¹³ C]lipids	fat metabolism; fat malabsorption
[δ ¹³ C]corn oil	fat malabsorption
[δ ¹³ C]soy oil	fat malabsorption
[¹³ C] and [δ ¹³ C]triglycerides	defects in lipolysis due to exocrine pancreatic insufficiency
tri-[1- ¹³ C]octanoyl glycerol	fat malabsorption
1,3-distearyl-2-[1- ¹³ C]octanoyl-glycerol	fat malabsorption
[¹³ C]triolein	fat malabsorption
[¹³ C]tripalmitin	fat malabsorption
[¹³ C]trioctanoin	fat malabsorption
4. Carboxylic acids	
[¹³ C]fatty acids	myocardial metabolic studies
[¹³ C]acetates	gastric emptying rate; aldehyde dehydrogenase deficiency
[¹³ C]octanoic acid	gastric emptying rate
[¹³ C]ketoisocaproic acid	alcoholic hepatic steatosis
[¹³ C]glycocholic acid	malabsorption of bile acids; intestinal bacterial overgrowth; enterohepatic circulation of bile acids
[¹³ C]valproic acid	studying epileptic diseases
5. Carbonic acid and its derivatives	
sodium [¹³ C]hydrogen carbonate	gastrointestinal transit time; gastric emptying rate
[¹³ C]urea	Helicobacter (Campylobacter) pylori infection and gastrointestinal diseases, probably also corona diseases and migraine due to this infection
[¹³ C]glycosyl ureides	intestinal transit time
lactose[¹³ C]ureide	colonic fermentation; orocecal transit time; coecal retention time
lactose[¹³ C, ¹⁵ N]ureide	intestinal transit time; studying colonic fermentation
6. Alcohols	
[¹³ C]ethanol	aldehyde dehydrogenase deficiency
7. Miscellaneous	
[methyl- ¹³ C]methacetin	liver diseases; hepatic microsomal biotransformation
[1,3,7-methyl- ¹³ C]caffeine	liver diseases; hepatic microsomal biotransformation
[¹³ C ₂]aminopyrin	liver diseases; hepatic microsomal biotransformation
[¹³ C]erythromycin	liver diseases

Table 2 (Continued from the preceding page.)

Survey on substrates of ¹³C-breath tests and their application in clinical research and diagnosis so far investigated. (A δ before ¹³C in square brackets means that the substrate is naturally enriched in ¹³C.)

1.2. Terminology of Tracer Investigations Using Stable Isotopes

Understanding and comparability of results of clinical as well as any other investigations using stable isotopes is hampered by the lack of a commonly accepted, uniform measure of the content of a stable isotope like ^{13}C in any substances. On the contrary there are at least five such measures in use side by side (Krumbiegel P 1980). In the following the term ^{13}C -content will be used as a generic term for the different expressions for characterising the ^{13}C -share of the element carbon.

The (relative) abundance is defined as the ratio of the amount of the isotope in question (in mol), in our case ^{13}C , and the amount of the total element, comprising all naturally occurring isotopes of this element (in mol), in our case ^{12}C and ^{13}C . (The amount of the radioactive isotope ^{14}C also occurring in nature may be and is neglected in this connection.) The hundred-fold value is called isotope abundance in atom%.

The enrichment of an isotope, usually the less abundant one, in our case ^{13}C , is frequently given in terms of excess abundance in atom% excess (at.% exc.). That means the abundance of the enriched stable isotope minus its natural abundance in atom%.

If the relative abundance of a stable isotope only slightly deviates from its natural abundance, as it is true for tracer investigations at high isotope dilution as well as for investigations on variations of the isotopic composition of chemical elements in their natural occurrences, instead of the enrichment in atom% or atom% excess the δ value of the isotope in question is used which is defined as the relative deviation of the abundance a_{sample} of a given isotope in atom% from its abundance a_{standard} in a standard in per mill:

$$\delta = [(a_{\text{sample}} - a_{\text{standard}}) / a_{\text{standard}}] \times 1000 \text{ ‰}$$

For carbon usually the so-called PDB standard is mostly used which is defined as the calcium carbonate of the fossil Belemnitella of the Pee Dee formation in South Carolina with $a_{\text{standard}} = 1.11123$ atom% ^{13}C corresponding to $\delta^{13}\text{C} = \pm 0.00000$. From this follows:

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

for the conversion of δ values into relative abundances a in atom%.

Similar to the procedure of nominating isotope contents as excessive abundances δ values often are expressed in the form of their deviation from the δ value δ_{baseline} measured before the ^{13}C -labelled substrate has been taken up (DOB \equiv Delta Over Baseline):

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

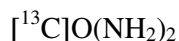
Especially if the chemical element in question consists of two stable isotopes, as it is true for carbon, the enrichment sometimes is expressed in the form of the isotope ratio. This is defined as the quotient of the amounts of the two isotopes (in mol), the less abundant isotope being either the numerator or the denominator.

Fortunately this variety of defining ^{13}C -contents usually is not an obstacle to interpreting results of ^{13}C -breath tests, because the labelling isotope ^{13}C in practice usually is by two orders of magnitudes less abundant than the other stable isotope of carbon ^{12}C . As long as the ^{13}C -contents do not considerably deviate from PDB standard the numerical values of the relative abundances of ^{13}C and the $^{13}\text{C}/^{12}\text{C}$ ratios therefore approximately go together.

Nuclides are atomic species the atomic nuclei of which are characterised by a definite number of protons and a definite number of neutrons. If two or more nuclides correspond regarding the number of protons in their nuclei, i. e. if they are nuclides of one and the same chemical element, they are usually called isotopes, especially if the affiliation to one and the same element is to be emphasised. (Unfortunately the term 'isotope' is often also used instead of the term 'nuclide'.) In 1978 and 1979 the International Union of Pure and Applied Chemistry (IUPAC) proposed some nomenclature rules in order to promote a uniform and unambiguous description of isotopic modifications of chemical compounds. According to these rules isotopically labelled compounds are mixtures of an isotopically unmodified compound and one or several analogous, isotopically substituted compounds.

If the position(s) and the number of the labelling nuclide(s) in the molecules are well defined, the corresponding chemical compound is named a specifically labelled one.

The structural formulae of specifically labelled compounds are written as usual but with the appropriate isotope symbol(s) enclosed in square brackets. E. g.

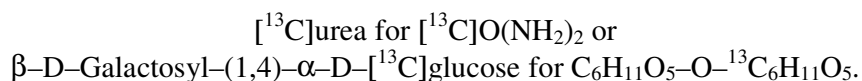


denotes urea enriched in ^{13}C . In case of pure nuclide substances, i. e. isotopically substituted compounds, the square brackets are omitted. E. g.

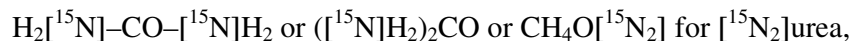


denotes urea with pure carbon-13. Since such compounds are in fact hypothetical, the notation without brackets is often also used for specifically labelled compounds.

The name of a specifically labelled compound has to be formed by inserting the nuclide symbol(s) in square brackets preceded by any necessary locant(s) like letters or numbers either before the name or the denomination of that part of the compound which is modified, as is demonstrated by the following examples:



Compounds multiply labelled with a single isotope are denoted as demonstrated here:



while mixed labelled compounds are written like this:



for urea labelled with ^{13}C and with ^{15}N in both NH_2 -groups.

Also some symbols recommended by the International Union of Pure and Applied Chemistry (IUPAC) are especially important for tracer technique with stable isotopes and will be presented here.

Like in classical chemistry the symbol

- m is used for mass of a substance in grams [g]
- M is used for molar mass in grams per mole [g/mol]
- n is used for amount of a substance in moles [mol]

Isotopic peculiarities are indicated by indexing these symbols in an appropriate manner, e. g.

- m_{13C} or $m(13C)$ is used for mass of ^{13}C in grams [g]
- M_{13C} or $M(13C)$ is used for molar mass of ^{13}C in grams per mole [g/mol]
- n_{13C} or $n(13C)$ is used for amount of ^{13}C in moles [mol]

Within the framework of the mentioned IUPAC recommendations only one physical quantity is distinguished by a meaning typical of stable isotope chemistry without any parallel in non-isotope chemistry, namely the already mentioned relative isotope abundance a , i. e. the quotient of the amount n_i divided by the sum Σn of the amounts of all isotopes of the respective chemical element: $a_i = n_i / \Sigma n$. According to the IUPAC recommendations the hundredfold value of the quantity a is given as atom% or at.%.

The symbol a_0 is used for the abundance of an isotope in the natural abundances of the respective element. As already mentioned, a_0 varies to some degree (see Tab. 1).

Isotope abundances and isotope amounts are often expressed as excess abundances a' or excess amounts n' , respectively, which is indicated by a prime: $a' = a - a_0$ or $n' = n - n_0$, respectively.

1.3. Methods of Evaluating Tracer Investigations in Medicine

Gravimetric analysis requires a complete separation of the component to be determined in a pure state. Using isotope dilution technique it is sufficient to partly separate the component in question. Particularly in biochemistry and medicine with their systems usually consisting of many components often occurring in low concentrations this is a highly important advantage. In isotope dilution analysis a known amount n_t of the tracer, i. e. the compound to be determined in a state labelled with an isotope, is added to the system to be analysed. Then the isotope balance equation

$$n a_0 + n_t a_t = (n + n_t) a$$

is valid, where n is the amount of the component to be determined, a_t the relative abundance of the labelling isotope in the tracer and a_0 the natural abundance of this isotope. After thorough homogenisation any part of the substance to be determined is separated from the mixture and subject to isotope analysis yielding the relative abundance a in the above mentioned isotope balance equation.

This equation can be rearranged to give the amount

$$n = n_t (a_t - a) / (a - a_0)$$

to be determined. Using excess abundances $a' = a - a_0$ and $a_t' = a_t - a_0$ we arrive at:

$$n = n_t (a_t' / a' - 1) .$$

As can easily be shown the advantage of isotope dilution analysis does not only consist in the possibility to abandon complete separation of the substance to be determined, but also in the opportunity of analysing low concentrations with high accuracy and sensibility. The

determination of blood volume and total body liquid exemplify applications of isotope dilution technique in medicine.

Deviating from this static isotope dilution analysis in many other investigations the parameter 'time' plays an important part. This kind of tracer applications is the subject of tracer kinetics or dynamic isotope dilution technique. Particularly in biochemistry and medicine the system to be investigated by tracer kinetics as a rule is a living organism or some part of a living organism. Thus tracer kinetics in medicine deals with models of human (or animal) metabolism, metabolic pools or compartments, material flows, rates of biochemical reactions, metabolic pathways etc.

Already in 1949 Sprinson and Rittenberg invented a model of human protein metabolism (Fig. 5) for evaluating ^{15}N -tracer studies consisting of a protein, a metabolic and an excretion pool (Sprinson DB and Rittenberg D 1949).

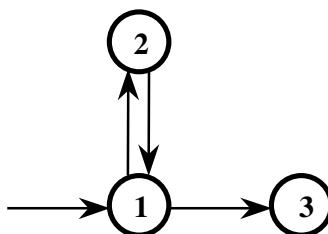


Figure 5

Three-pool model of human protein metabolism.

Pool 1 non-protein nitrogen (amino acids, amides, ammonium salts etc.)

Pool 2 protein nitrogen

Pool 3 total nitrogen in urine

The arrows symbolise flows of nitrogen intake, nitrogen excretion, protein synthesis (anabolic processes) and protein degradation (catabolic processes)

As early as 1962 Berman M, Shan E and Weiss MF (1962) presented a method of mathematical modelling of processes in biological systems which proved to be also valuable for tracer investigations. The concept of this so-called compartment theory defines certain material reservoirs within the system to be experimentally studied which are connected with each other and with the surroundings by certain material flows. Flows entering the system may be food or beverages or any part of them, pharmaceuticals or just isotopically labelled compounds. On the other hand substances like urine, faeces, breath and, e. g. in case of lactating animals, milk or any constituents of them have to be treated as flows leaving the system to be investigated.

Enterohepatic circulation, microsomal biotransformation, anabolic and catabolic processes, reductive amination of keto acids and oxidative desamination of amino acids, synthesis and breakdown of polysaccharides, carboxylation and decarboxylation and stages of metabolism of endogenous substances as well as xenobiotics can frequently be modelled as flows interconnecting certain compartments.

The mathematical formalism of compartment theory is founded on the assumption that compartments are material reservoirs distinguished by material (and in case of tracer investigations also) isotopic homogeneity, this homogeneity being brought about by rapid mixing, e. g. by circulation or by peristalsis, respectively, or simply by residing in one and the same relatively secluded organ like the liver or the bladder.

For each of these compartments one material balance is valid:

$$\boxed{\text{sum of flows entering the compartment}} - \boxed{\text{sum of flows leaving the compartment}} = \boxed{\text{change of the material amount in this compartment}}$$

If the material amount in the respective compartment is constant, the material balance equation reduces to:

$$\boxed{\text{sum of flows entering the compartment}} = \boxed{\text{sum of flows leaving the compartment}}$$

Thus we arrive at a number of balance equations which can be used for determining flows as well as sizes of compartments not accessible to direct experimental determination. It is the point of tracer experiments that each of these material balances is supplemented by a corresponding isotope balance thus supplying twice the number of equations for calculating flows or sizes of compartments. Therefore tracer investigations yield results decisively exceeding the capabilities of analogous classical investigations.

This important consideration is to be illustrated by an example: The result of studying problems of parenteral nutrition by merely balancing the masses of substances taken up and excreted and the body masses of the persons to be investigated will never be more than the difference between the rates of anabolism and catabolism, while isotopic labelling will yield the rates of both anabolism and catabolism themselves.

While in case of endogenous substances administered to living organisms the entrance into some compartment or compartments, respectively, will be a rule, xenobiotics like pharmaceuticals will usually not enter any compartment, because corresponding endogenous reservoirs do not exist at all. Evaluating results of such investigations including tracer experiments is then less difficult than described before. Formulated in the language of compartment theory the size of the respective compartment has to be set nil. (Note that authors abstaining from the strict mathematical formalism of compartment theory usually prefer the term 'pool' to the term 'compartment'.)

Results of tracer investigations with labelled endogenous substances can be presented as pool sizes (in mol), or, if material flows are to be characterised, as turnover rates (in mol/s) or turnover times (in s).

Mainly for labelled xenobiotics some additional terms are used. Probably the most important terms of this kind are:

- eliminated portion of tracer dose
- elimination half life of the labelled compound
- maximum elimination rate and time of maximum elimination rate

The interaction between absorption and elimination of labelled substances can be described by the classical Bateman function established for representing the radioactive decay of a nuclide which itself is generated by the radioactive decay of a mother nuclide. This function comprises the difference of two exponential functions the one describing the kinetics of tracer resorption and the other the dynamics of tracer elimination. Often the rate of tracer intake can be regarded as being high in comparison to the rate of excretion. Then the exponential function representing absorption is equal to zero and the Bateman function reduces to the simple equation

$$c_t = c_0 e^{-k t},$$

where c_0 and c_t are the tracer concentrations at the time of tracer intake and at the time t and k the elimination coefficient. From this follows for the elimination half life

$$t_{1/2} = \ln 2 / k .$$

As for further information on evaluating results of tracer investigations in dynamic systems see e. g. Krumbiegel P (1991 a), Wetzel K, Hübner G and Gebhardt O (1961), Winkler E, Faust H, Wetzel K et al. (1978), Wetzel K, Junghans P, Faust H et al. (1979), Gebhardt O, Wetzel K and Hübner G (1964), Wetzel K, Gebhardt O and Hübner G (1966), Wetzel K, Gebhardt O and Hübner G (1966 a), Wetzel K, Hübner G and Gebhardt O (1963), Wetzel K, Winkler E, Faust H et al. (1968), Pfeifer S, Pflegel P and Borchert HH (1984), Krumbiegel P, Teichmann B, Faust H et al. (1988) and Faust H, Junghans P, Matkowitz et al. (1981).

2. Progress in Providing Substrates for ^{13}C -Breath Tests

Recently Klein et al. (Klein PD 1998) proposed and tested a method of providing substrates of ^{13}C – breath tests for determining gastric emptying rates using the biomass of algae which are grown in an atmosphere containing [^{13}C]carbon dioxide instead of carbon dioxide with natural isotopic composition. The [^{13}C]biomass synthesised in this way is directly converted into foodstuffs like cake which immediately can be applied as substrates of ^{13}C – breath tests for determining gastric emptying rates. The main advantage of such substrates is that their digestive properties approximate those of common, particularly vegetarian foodstuffs, more closely than other substrates of ^{13}C -breath tests (e. g. [^{13}C]acetates or [^{13}C]octanoic acid) applied for determining gastric emptying rates do.

In this connection we refer to metabolic studies by Berthold HK, Crain PF, Gouni I et al. (1995) and Berthold HK, Jahoor F, Klein PD et al. (1995), in the framework of which laboratory animals or human beings, respectively, were supplied with ^{13}C -labelled algae produced by hydroponic culture in $^{13}\text{CO}_2$.

Wetzel and Fischer (Wetzel K and Fischer H 1998) pursued a different, novel path of synthesising ^{13}C -labelled substances: Like in the procedure described before algae are grown, though not employing artificially enriched [^{13}C]carbon dioxide, but starting from not too small a stock of carbon dioxide with natural isotopic composition in a semi-closed system to which (virtually) no further carbon dioxide is supplied and from which algal biomass is withdrawn in small doses or continuously up to a residue of carbon dioxide of the order of some percent or per mil. (This more or less continuous withdrawal minimises internal recycling of organic carbon to carbon dioxide by respiration.)

Algae are C_3 -plants, i. e. their photosynthesis follows the so-called Calvin cycle. At this mechanism ^{12}C is assimilated by about 2 % faster than ^{13}C does, thus being preferentially withdrawn from the system. On the other hand ^{13}C is enriched in the residual amount of carbon dioxide and thus can be obtained as a product. Also the algal biomass generated in this stage of the process is enriched in this isotope to a degree sufficient for use as substrate of ^{13}C -breath tests. From this biomass a great part of the wide range of ^{13}C -labelled organic substances used for ^{13}C -breath tests and other tracer investigations with ^{13}C -labelled substances can easily be prepared in one go: Dishes from plant (and indirectly also from animal) materials, proteins, fats, lipoids, carbohydrates, their hydrolytes, biologically active substances etc.

If instead of atmospheric carbon dioxide or carbon dioxide produced by combustion of fossil fuels carbon dioxide from limestone is used as source material, substrates with 1.2 to 1.3 atom% ^{13}C (instead of 1.08 atom% in C_3 -plants) can be produced without depending on the total synthesis from artificially enriched ^{13}C which is frequently troublesome and expensive particularly in case of complicated organic substances.

An advantageous procedure for synthesising [^{13}C]urea was given by Iida K, Chiyoda T and Kajiwara M (1996).

3. Progress in Analytical Techniques for ^{13}C -Breath Tests

3.1. Administering the Substrate, Sampling and Sample Processing

Kajiwara M, Katsumi J, Takatori K et al. (1997) proved ^{13}C -urea breath tests to be a highly sensitive, specific and accurate means of diagnosing *Helicobacter pylori* infection and recommended the following standard procedure: Immediately before administering 100 mg ^{13}C -urea dissolved in 100 ml of water the control $^{13}\text{C}/^{12}\text{C}$ ratio in breath is measured by infrared spectroscopy or gas chromatography / mass spectrometry (GC-MS). After taking up the substrate residual ^{13}C -urea is rinsed out of the mouth with water. 15 minutes after taking up the substrate again a breath sample is taken in a 250 ml aluminised bag and then measured. Under these conditions the cut-off level is supposed to be $\text{DOB} = 5$ per mil for the detection of *Helicobacter pylori* infection. It was shown that the breath samples in those 250 ml aluminised bags can be stored for one year.

3.2. Mass Spectrometric Analysers

Isotope ratio mass spectrometric analysers (IRMS) proved to be highly sensitive and accurate devices for performing ^{13}C -breath tests. But carbon dioxide purification by cooling with liquid nitrogen demands high expenditure of costs, time and qualification of staff. Substitution of carbon dioxide condensation by gas chromatographic purification lead to reliable and rugged devices (Braden B, Duan LP, Caspary WF et al. 1994).

In view of their high measuring capacity the devices are preferentially suitable for large hospitals and analytical centres. For supplying compact and rugged mass spectrometers for ^{13}C -breath tests quadrupole mass spectrometers as early as 1953 invented by Wolfgang Paul experienced a revival. In these devices the ion beam runs in the middle of and parallel to four bar electrodes arranged at the corners of a square which are connected to high frequency so that every two diametrically opposite electrodes have one and the same polarity. At a definite frequency and alternating voltage only ions of one definite mass are able to pass such an arrangement while all the other ions are excited to vibrations of increasing amplitudes and finally discharged at one of the electrodes.

For further information on mass spectrometric methods see Schröder E (1991), Johstone R (1996) and Leodolter A (1997).

3.3. Optical Spectrometric Analysers

Braden B, Haisch M, Duan LP et al. (1994) were the first to substitute mass spectrometry by nondispersive infrared spectroscopy as a means of measuring $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratios in breath. Koletzko S, Haisch M, Seeboth E et al. (1995) compared the efficiency of nondispersive infrared spectroscopy and conventional isotope ratio mass spectrometry for diagnosing *Helicobacter pylori* infection by the ^{13}C -urea breath test. They found a close correlation between the results of the two methods. All *Helicobacter pylori*-positive individuals, the status of which was confirmed by specific IgG antibody titres ($\text{SYN}^{\text{elias}}$) or by antral biopsies (urease test and histological examination), respectively, had DOB values above 11 ‰ in all samples taken 15 and 30 minutes after ingestion of 75 mg ^{13}C -urea dissolved in 150 ml 0.033 mol/l citric acid.

Promising results were also achieved by Ohara S, Kato M, Asaka M et al. (1989) comparing results of nondispersive infrared spectrometry (UBiT-100, Otsuka Electronics Co. Ltd, Hirakata, Japan) and mass spectrometry (ABCA-G, Europe Scientific, Creve, United Kingdom) for detecting *Helicobacter pylori* infection. 10, 20, 30, 45 and 60 minutes after ingesting 100 mg of ^{13}C -urea dissolved in water breath samples were taken and measured.

Subjects were asked to stop breathing for 10 seconds and then exhale in order to get breath samples that contain at least 2 vol-% carbon dioxide. As frequently usual 250 ml sample bags were applied. Regression plot from more than 2000 samples revealed an excellent correlation ($r = .992$) between results of nondispersive infrared spectrometry and mass spectrometry. The agreement proved to be best for samples with more than 2 vol-% carbon dioxide. Samples taken 20 minutes after ingesting the substrate proved to be suitable for *Helicobacter pylori* diagnosis. Results were available in 6 minutes. Thus the entire procedure lasts less than half an hour. Since nondispersive infrared analysers unlike mass spectrometers are cheap, compact and easy to operate, they are best suitable for the doctor's office and allow to perform the ^{13}C -urea breath test while the patient is still present in the office.

Higashi Y, Ohara H, Naruki Y et al. (1996) described a new tunable diode laser spectroscopic device for $^{13}\text{CO}_2/^{12}\text{CO}_2$ analysis and validated its applicability for ^{13}C -breath tests with [^{13}C]urea, [^{13}C]methacetin and [^{13}C]glycine as substrates by comparison with mass spectrometry. Results showed excellent correlation ($r = .996$) between $\delta^{13}\text{C}$ values obtained by the two methods.

Fischer H (1998) described a new method of calibrating nondispersive infrared spectrometers: Sample cell and reference cell are either evacuated or filled with an infrared-inactive gas and drifting is compensated by adjusting the electronic offset to zero point and then inserting a non-selective filter, a screen, a slit or a sieve into the infrared beam passing the sample cell. The signals then registered are adjusted to the values measured at preceding calibrations by changing electronic reinforcement.

A further innovation at calibrating nondispersive infrared spectrographic devices was proposed by Fischer and Kuhlmann (1998): At first the sample cell is filled with the gas or gas mixture, respectively, free of the gas to be measured, i. e. for ^{13}C -breath tests with air free from carbon dioxide, followed by measuring the signals for $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ at the detector cells which gives the coordinates of a first point of the calibration curve. Then a gas with known carbon dioxide concentration and known carbon isotopic concentration is added in certain doses. After having introduced every such dose the signals at the detector cells are again measured, thus yielding the coordinates of points of the calibration curve just as many as one likes.

Elektronik Mechanik Gerätebau GmbH, Westerdeich, Bremen, Bundesrepublik Deutschland (1997) claimed patent protection for a method of calibrating isotope analysers with respect to the influence of the concentration of the measuring gas in a gas mixture, e. g. of the carbon dioxide concentration in breath on the measuring signals for $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$, where at first the measuring cell is filled with a gas with a high, known concentration and a known isotope ratio of the measuring gas, so for ^{13}C -breath tests with air containing carbon dioxide in a concentration near the upper limit of the expected measuring range and a known $^{13}\text{C}/^{12}\text{C}$ ratio in this range. Registrating the signals then obtained yields the coordinates of a first point of the calibration curve ($^{13}\text{CO}_2/^{12}\text{CO}_2$ -ratios versus CO_2 -concentrations). All the other points of the calibration curve are obtained by gradual addition of gases free of measuring gas.

Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan (1995) proposed a method of calibrating nondispersive infrared analysers for correcting measured $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratios with respect to $^{12}\text{CO}_2$ concentration. Based on calibration measurements using gases with known $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ concentrations as a starting point normalised $^{13}\text{CO}_2/^{12}\text{CO}_2$ concentration ratios are defined by means of which the effect of $^{12}\text{CO}_2$ concentration on the measured $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratios of the samples can be eliminated.

Selectivity and sensitivity of nondispersive infrared isotope analysers can be considerably enhanced by an optical filter filled with the measuring gas with the more abundant isotope between the measuring cell and the detector for the less abundant isotope. E. g. for ^{13}C -breath test devices the filter is to be filled with $^{12}\text{CO}_2$ (Mannesmann AG, Düsseldorf, Bundesrepublik Deutschland 1993). The effect of absorbing those frequency ranges characteristic of the measuring gas with the more abundant isotope can also be brought about by arranging both detector cells one behind the other, namely the detector for the more abundant isotope in the direction of the infrared beam before that one for the less abundant isotope, thus simultaneously acting as an optical filter.

On June 05, 1997 the Patent Office of the German Federative Republic registered a patent design of a carbon isotope analyser (Fischer Analyseninstrumente GmbH 1997). The analyser consists of a nondispersive infrared spectrometric analyser, a gas management system, a digital input/output unit, a bus interface and an external PC unit with user software (see Figs. 6, 7 and 8).

The nondispersive infrared spectrometer 1 and the digital input/output unit 3 control a gas management system 2 integrated in a sample inlet system 7 and a sample supervising system 8. Infrared spectrometer 1, gas management system 2, digital input/output unit 3 and the bus interface 4 are arranged in a compact and rugged casing and cable linked with the external PC unit 5. All modules of the device are equipped with own micro controllers 9 which communicate with the external PC unit 5 via the bus interface 4. The PC unit 5 in connection with the user software 6 controls the entire measuring procedure and conducts the user on the display. The gas circuit system allows a rapid and complete change of the gas inside the measuring cuvettes.

New about the carbon isotope analyser FANci2 of Fischer Analysen Instrumente GmbH is that the nondispersive infrared spectrometer 1, the gas management system 2, the digital input/output system 3 and the bus interface 4 are arranged in a compact and robust casing and connected with the external PC unit 5, that every module is equipped with a separate microcontroller 9 and that the latter communicate with the external PC unit 5 by that bus interface 4.

The cross sensitivity of $^{13}\text{CO}_2$ to the (usually by a factor of about one hundred) higher $^{12}\text{CO}_2$ concentrations is diminished by inserting a $^{12}\text{CO}_2$ gas filter into the $^{13}\text{CO}_2$ channel and in addition by mathematical methods integrated in the software of FANci2. To achieve a high accuracy the system is hermetically closed and kept at a constant temperature. A CAN port can be inserted into the PC which together with the software realises the communication with the components of the measuring module. Additionally a special gas circulation system makes possible rapid and complete change of samples in the cuvettes. A sample identification system integrated in the hardware ensures simple and secure handling of the rugged device. Internal status checks guarantee failure diagnosis. Heated gas paths prevent residual moisture from condensation. Sample volume is 0.3 l. Eight sample ports for the breath bags are located in front of the device. Thus eight sample bags can concominantly be connected to the probe. Up to 40 samples per hour can be measured. The accuracy is better than 0.03 ‰ and so within the normal variations of the isotopic composition of carbon in breath. Thus FANci2 fulfils all requirements necessary of a ^{13}C -breath test device.

Braden B, Caspari WF and Lembcke B (1999) confirmed this statement by investigating 63 males and 79 females with dyspeptic symptoms by means of the ^{13}C -urea breath test for *Helicobacter pylori*-infection, the breath samples being measured both mass spectrometrically and by the nondispersive infrared spectrometer FANci2. Excellent agreement between the

diagnostic results of the two analytical methods was found. In reference to mass spectrometry as the golden standard FANci2 achieved a sensitivity of 97,8 % and a specificity of 98,9 % of the qualitative breath test results.

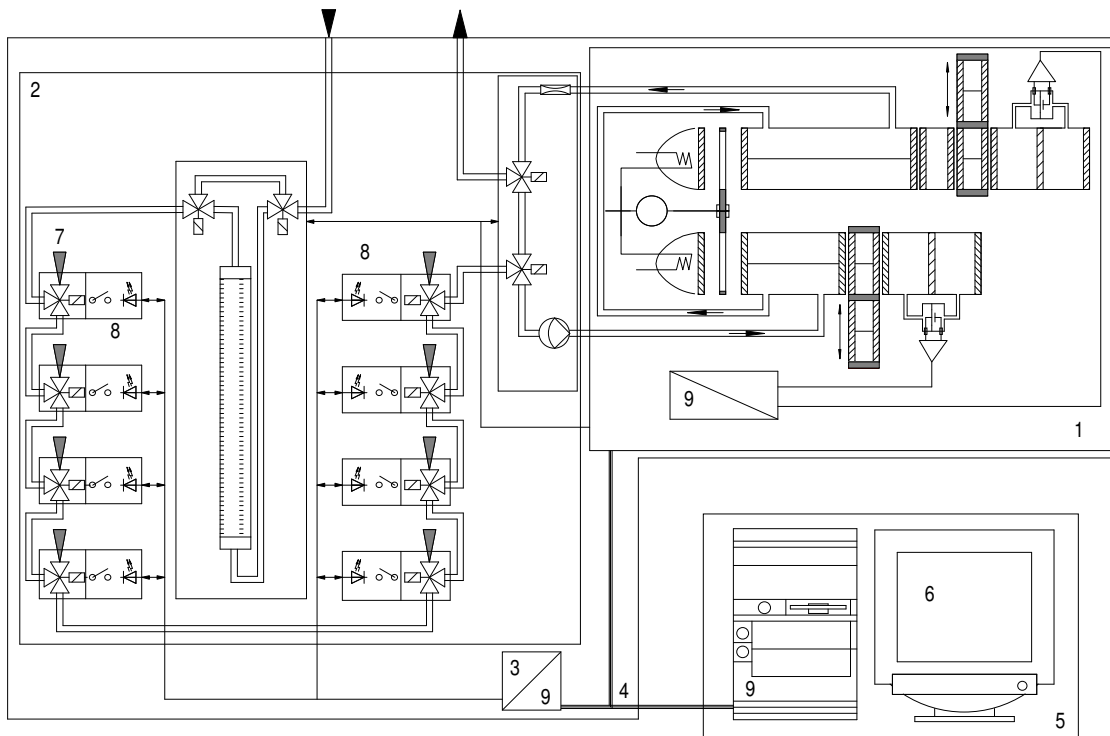


Figure 6

The carbon isotope analyser FANci2 of Fischer Analysen Instrumente GmbH, Leipzig.

- 1 nondispersive infrared spectrometer
- 2 gas management system
- 3 digital input/output unit
- 4 bus interface
- 5 external PC unit
- 6 user software
- 7 sample inlet system
- 8 sample supervising system
- 9 microcontroller

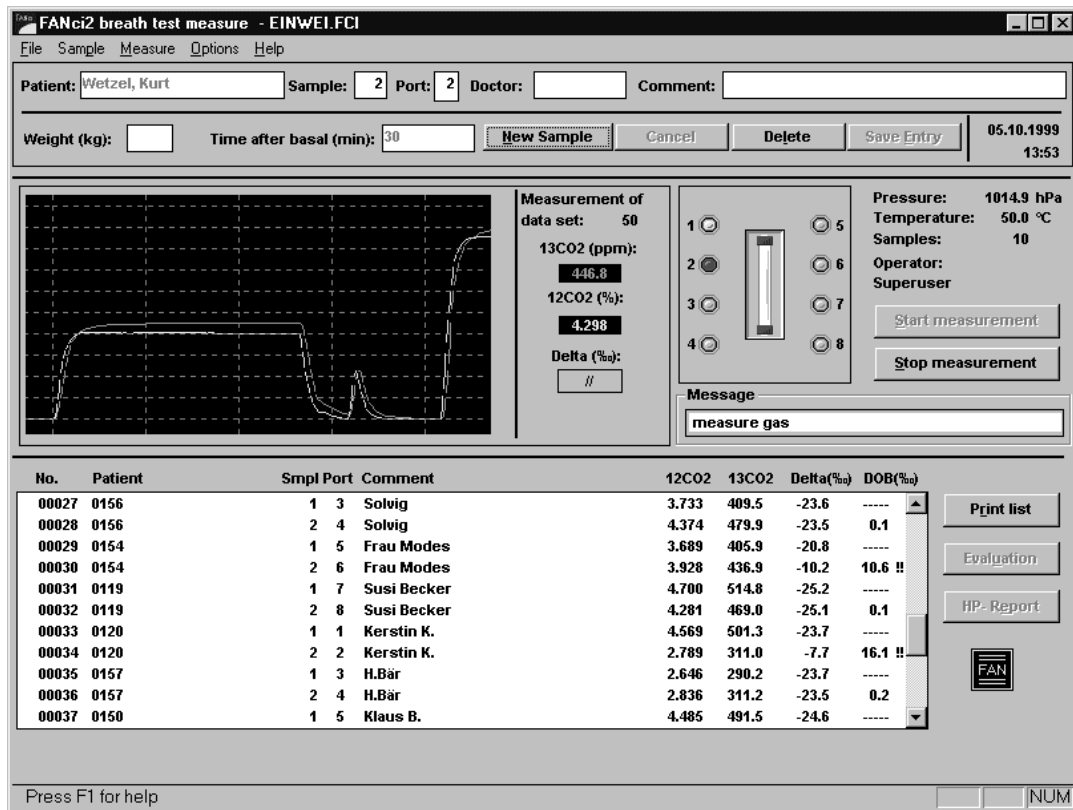


Figure 7

Display of the carbon isotope analyser FANci2 before measuring the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio.

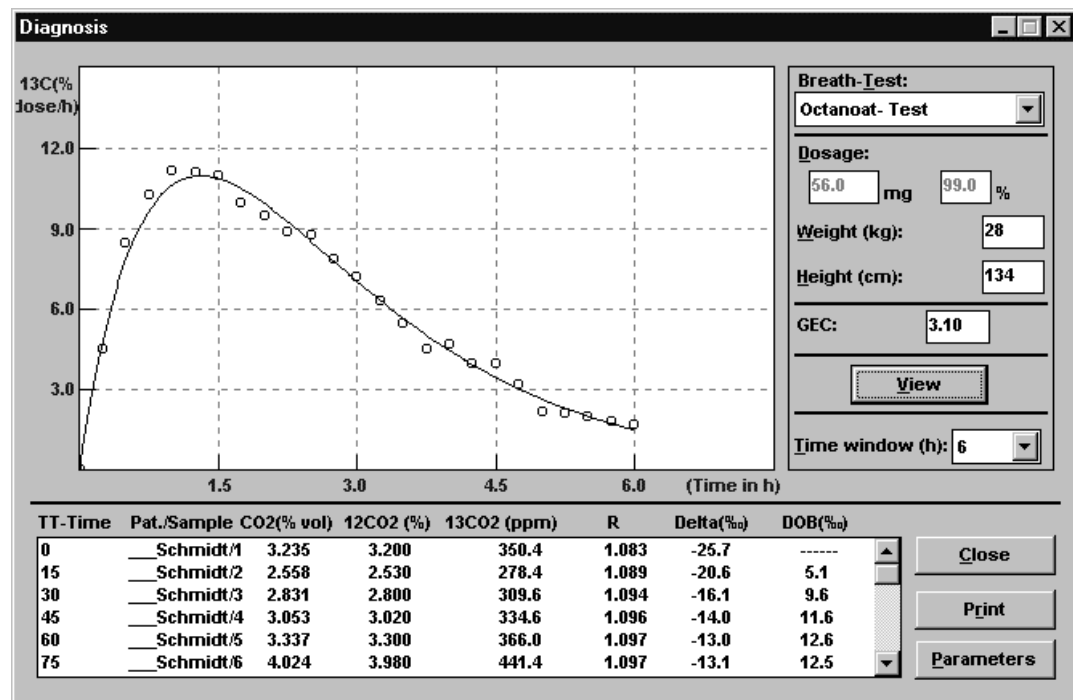


Figure 8

The carbon isotope analyser FANci2 in the state of presenting the results of a ^{13}C -breath test.

According to statements of MIC Medical Instrument Corporation, Solothurn, Schweiz (1994) measuring of $^{12}\text{CO}_2/^{13}\text{CO}_2$ ratios by nondispersive infrared absorption can be improved, if the two pulsed infrared beams at first pass cells filled with the two isotopically pure gases $^{13}\text{CO}_2$ or $^{12}\text{CO}_2$, respectively, and then enter a pair of cells filled with the gaseous sample which are connected with one another by a capacitor microphone. By varying the pressure in one of the two cells filled with isotopically pure carbon dioxide the microphone is tuned into the signal minimum and then related to a reference signal obtained in a corresponding way.

Finally we would like to point out some recent progress in common gas analysis which is or may become important also for $^{13}\text{CO}_2/^{12}\text{CO}_2$ analysis:

Thus Fabinski W (1992) gave a survey on methods of automatic adjustment of gas analysers using internal standards and atmospheric air instead of test gas bottles.

Palocz–Andresen M (1995) got a patent for a nondispersive infrared gas analyser distinguished by a chopper for periodically interrupting the infrared radiation with a cylindrical surface in the direction of a rotatory axis and a cylindrical opening vertical to it, the cross–section of which corresponds to the free cross–section of the sample cell with respect to diameter and form, thus being translucent in a first position and impervious to the infrared radiation in a position perpendicular to the first one.

Fuji Electric Co., Ltd., Kawasaki, Kanagawa, Japan (1995) got a patent for a nondispersive infrared gas analyser with two detector cells in separate infrared beams, the former being connected by a narrow tube with a set of thermoelectric sensors by means of which the gas flow rate in this tube is measured. It is the inventive idea that the distance between these thermoelectric sensors is so large that conductive coupling between them is negligible, while every single sensor consists of two thermocouples so tightly neighbouring that thermal coupling between them does exist.

A patent was granted to Siemens AG (1997) concerning a nondispersive infrared gas analyser with a unit for focussing the radiation entering the detector which consists of a mirror configuration with grazing reflection. Another patent by Siemens AG (1997a) regards a nondispersive infrared gas analyser with two choppers for modulating the infrared beam at different frequencies and/or phase sequences and a circuitry which splits the measuring signal into two parts corresponding to these modulations.

A new method of measuring very low concentrations of gases by infrared spectroscopy was claimed by Erno Raumfahrttechnik GmbH (1993): One part of an angled mirror reflects an infrared beam into a spherical measuring chamber with a mirrored inside surface, where it is multiply reflected, before it leaves the chamber via the other part of the mirror towards the detector. The long zigzag path of the infrared beam within the gas to be analysed guarantees high analytical sensitivity. A similar solution was found by Aktieselskabet Brüel & Kjaer, Naerum, DK (1989) with their photoacoustic gas analyser: The curved walls of the measuring chamber consist of a material with a high heat conductivity and are covered with a layer of high reflective power and the radiation enters the measuring chamber divergently in order to generate multiple reflection.

The Ingenieurhochschule Köthen, Bundesrepublik Deutschland (1988) took out a patent on a device for correcting static transmission effects of two–channel nondispersive absorption spectrometers distinguished by specially shaping the chopper disk and the circuitry as well as by linking the two signal lines by a multiplying unit. The device is also applicable in the infrared range and enables a reduction of intrinsic as well as extrinsic interferences.

The VEB Junkalor Dessau, Bundesrepublik Deutschland (1988) proposed an arrangement for zero point adjustment of measuring signals of nondispersive infrared analysers in terms of both numerical value and phase, distinguished by a plane optical deflecting unit arranged in the path of rays, adjustable in the direction of the optical axis and swivel about the latter.

Optogalvanic Effect Spectroscopy with laser light is distinguished by high resolving power. If two carbon dioxide lasers are used as light sources, one interacting with $^{13}\text{CO}_2$ and the other with $^{12}\text{CO}_2$, this method acts as a carbon isotope ratio analyser (Murnick PE and Peer BJ 1994). This principle is realised in the Laser Assisted Ratio Analyser (LARA) by Alimenterics Inc., Morris Plains, USA.

3.4. Automation

In view of the rapidly growing importance of ^{13}C -breath tests in medical research and diagnosis it is no wonder that more and more automated $^{13}\text{CO}_2/^{12}\text{CO}_2$ analysing systems come onto the market. There is a tendency, however, that automation is mainly focussed on ^{13}C -breath test devices equipped with mass spectrometers and preferentially used in large hospitals and centres for clinical analysis, where measuring capacity is of major interest, while for devices with nondispersive infrared spectrometers mainly applied in small hospitals or medical practices low costs and simple handling are more important than high capacity.

Thus Suehiro M, Kuroda A, Maeda M et al. (1987) described a micro-computer controlled ^{13}C -breath test device including carbon dioxide purification unit, mass spectrometric $^{13}\text{C}/^{12}\text{C}$ analyser, data processing and data filing unit. According to the procedure by Schoeller EA and Klein PD (1979) the carbon dioxide purification unit consists of a first trap of dry ice and acetone bath removing water cryogenically and a second trap cooled by liquid nitrogen which collects the carbon dioxide. After evacuating the other gases carbon dioxide is released at room temperature and then admitted to the inlet system of the isotope ratio mass spectrometer. The valve operating sequence is controlled by a penning gauge in connection with the micro-computer. Five to six samples per hour can be measured with the system. Since it is fully automated 120 to 144 samples corresponding to 12 to 18 subjects can be investigated a day. The standard deviation of results ranges from 0.13 to 0.01 per mil (at a confidence limit of 95 %).

4. Progress in the Application of ^{13}C -Breath Tests in Medical Research and Diagnosis

4.1. Methodological Progress

4.1.1. General Progress in ^{13}C -Breath Tests

In a review article Lembcke B (1997) assessed ^{13}C -, ^{14}C - and H_2 -breath tests as a means of gastroenterological research and diagnosis. Hydrogen which in cell metabolism is transformed into water, in the intestine is generated by bacterial metabolising of carbohydrates and excreted as molecular hydrogen not only with the intestinal gases, but via bloodstream also with breath. It is measured in the breath gas chromatographically or by means of electrochemical sensors with hydrogen-selective membranes just a few minutes after administering the substrate. Carbohydrates like D-xylose, glucose, lactose, lactulose, stachyose or raffinose are used as substrates for such H_2 -breath tests by means of which bacterial overgrowth of small bowel or malabsorption of carbohydrates can be diagnosed. Using lactulose as substrate the orocoecal transit time can be determined. But the glucose H_2 -breath tests did not turn out to be a reliable test for small bowel bacterial overgrowth, probably because of the establishment of a mixed bacterial flora with varying glucose decomposition activity. On the other hand the ^{13}C -xylose breath test seems to yield more reliable results in this field, at least in pediatric diagnosis (Dellert SF, Nowicki MJ, Farrell MK et al. 1997).

While ^{14}C -breath tests because of the implemented radiation risks scarcely play any role in gastroenterological research and diagnosis, according to Lembcke (1997) the following ^{13}C -breath tests gained acceptance with the following indications:

- diagnosis of *Helicobacter pylori* infection (^{13}C -urea breath test)
- determination of gastric emptying rate (^{13}C -acetate breath test for liquid, ^{13}C -octanoic acid breath test for solid food)
- diagnosis of small bowel bacterial overgrowth (^{13}C -xylose breath test)
- determination of the demethylation activity of the liver (^{13}C -aminopyrin breath test, ^{13}C -caffein breath test)
- diagnosis of the pancreas function and pancreatic steatorrhoe (^{13}C -hiolein breath test, ^{13}C -triolein breath test, ^{13}C -1,3-distearyl- ^{13}C octanoate breath test, cholestearyl- ^{13}C octanoate breath test)

It is a problem in interpreting results of ^{13}C -breath tests for medical research and diagnosis that the dependence of the ^{13}C -abundances in breath on the time after administering the ^{13}C -labelled substrate under certain circumstances (amount, galenism and ^{13}C -content of the substrate, preparation of the subject for the test, nutrition and conduct of the subject during the test) does not only depend on the topical metabolic state, but also on the physical constitution of the investigated individual.

This is due to one of the following two reasons. On the one hand the basal metabolic rate (BMR) which is dependent on body mass M, height H, age and sex, affects the time factor of $^{13}\text{CO}_2$ in breath. For the ^{13}C -urea breath test Klein PD, Malaty HM, Czinn SJ et al. (1998) introduced a method of eliminating these effects which on principle can be applied then and only then, if the labelled carbon of the substrate (like in case of ^{13}C -urea) is directly transformed into carbon dioxide and exhaled. Particularly for endogenous substrates like carbohydrates, proteins, amino acids, glycerides and carbonic acids this is, however, usually not true. The ^{13}C -labelled carbon of most of such substrates rather enters an endogenous pool, undergoes isotope dilution and not until then is metabolised and expired. The larger these endogenous pools are, the more noticeable will this isotope dilution be.

At this point we present a model of carbon metabolism by the help of which also for ^{13}C -breath tests with substrates entering endogenous carbon pools metabolic parameters can be calculated which contribute to deeper understanding carbon metabolism and in addition permit the elimination of effects of physical constitution of the investigated individuals on results measured by such ^{13}C -breath tests (Wetzel K and Fischer H 1998a).

This model (Fig. 9) differentiates between a carbon pool A of the amount M_A which rapidly exchanges with the ^{13}C -labelled substrate, with the substrate of natural isotopic composition eventually taken up with food and biochemical similar substances of natural isotopic composition a_0 taken up with the food, and a much larger carbon pool B of the amount M_B which exchanges with the pool A both chemically and isotopically. It is assumed that the exchange between the two pools A and B comes about by mutual transformation of substances to be attributed to these pools proceeding at the rates v_1 and v_2 , respectively, which are smaller than the rate w_A , at which substances to be assigned to pool A are taken up with food and excreted with breath, urine or faeces.

Quantity and chemical composition of pool A have to be assumed to depend on the nature of the substrate. For triglycerides used as substrates pool A probably will mainly be made up of mono-, di- and triglycerides, glycerole and fatty acids, possibly also of carbonic acids of lower molecular weight, α -hydroxy carbonic acids, α -ketocarbonic acids and perhaps some amino acids. If ^{13}C -labelled amino acids or proteins are used as substrates, pool A can be supposed to consist of amino acids, α -ketocarbonic acids, peptides and perhaps also some carbonic acids. ^{13}C -labelled mono-, di-, oligo- and polysaccharides as substrates can be assumed to enter a pool A preferentially consisting of mono-, di- and oligosaccharides, while ^{13}C -labelled carbonic acids used as substrates will find a pool A mainly made up of carbonic acids, α -hydroxy carbonic acids and α -ketocarbonic acids.

The flow v_1 comes about by processes like synthesis of triglycerides from glycerol and fatty acids, of proteins from amino acids and of polysaccharides from monosaccharides or their preliminary stages, respectively, while the flow v_2 is brought about by processes going into the opposite direction.

The flows w_A and w_B ingested with food are characterised by natural isotopic composition a_0 . Quantity and chemical composition of pool B have not to be considered here. It is sufficient to assume that pool B is much larger than pool A ($M_B \gg M_A$) and that it consists of chemical compounds which only slowly interchange with compounds in pool A. Then and only then we may assume that the flow v_2 from pool B to pool A as well as the flow w_B' leaving pool B and exhaled will not considerably exceed the natural isotopic composition a_0 during the test. The same should be true for the flow w_B'' leaving pool B via urine and faeces.

At the beginning of the ^{13}C -breath test, not represented in Fig. 9, the carbon amount M_S of the respective substrate with the ^{13}C -abundance a_S is administered to the individual to be investigated. Consecutively pool A will achieve an elevated ^{13}C -abundance a_A within a short time interval which will again decline to a_0 in dependence on how fast the ^{13}C -enriched carbon is excreted via breath (flow w_A'), urine and faeces (flow w_A'') or is transferred to pool B (flow v_1) and there (because of $M_B \gg M_A$) diluted to approximately the natural isotopic composition a_0 . Since there are no considerable isotope effects involved and in view of the assumed chemical homogeneity of pool A the carbon flows w_A' , w_A'' and v_1 can be assumed to have the ^{13}C -abundance a_A of pool A at any time t within the duration of the test.

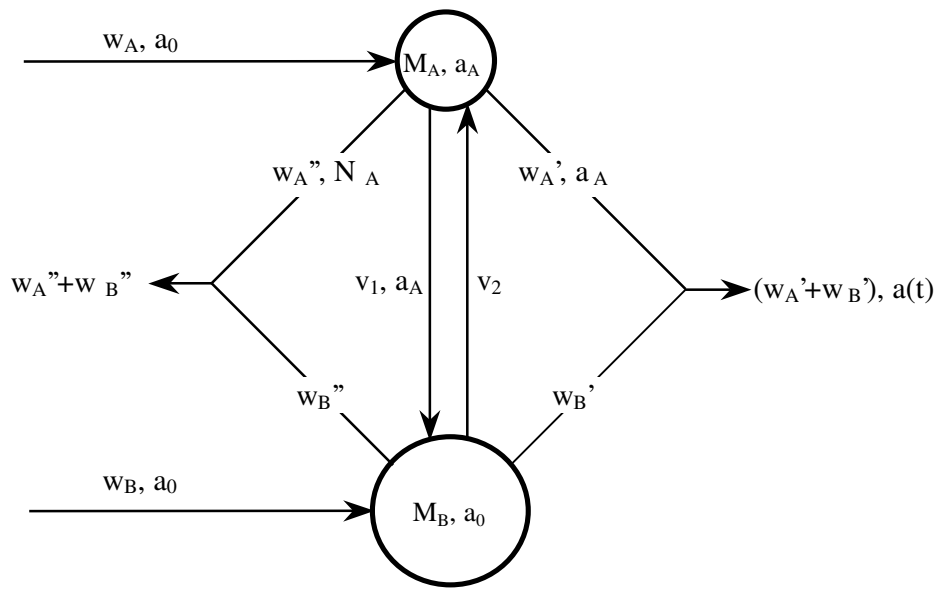


Figure 9

Model of carbon metabolism for evaluating ^{13}C -carbon breath tests.

M_A	amount of carbon compounds of pool A rapidly exchanging with substrate both chemically and isotopically
M_B	amount of carbon compounds of pool B slowly exchanging with substrate and compounds of pool A both chemically and isotopically
v_1, v_2	carbon flow from pool A to pool B or from pool B to pool A, respectively
w_A, w_B	carbon flow of compounds ingested with food and entering pool A or pool B, respectively
w_A', w_B'	carbon flow of compounds leaving pool A or pool B, respectively, and excreted with breath
w_A'', w_B''	carbon flow of compounds leaving pool A or pool B, respectively, and excreted with urine or faeces
a_0	natural relative abundance of ^{13}C
a_A	relative abundance of ^{13}C in pool A
a	relative abundance of ^{13}C in breath

The carbon dioxide expired by the investigated individual is made up of substances from the pools A (flow w_A' with the ^{13}C -abundance a_A) and B (flow w_B' with the approximate ^{13}C -abundance a_0) in unknown proportions. The ^{13}C -abundance a in breath can be expected to be between a_A and a_0 . As a rule among the substance amounts, flows and ^{13}C -abundances in Fig. 9 only the ^{13}C -abundance in breath is measured (as a function of time). In addition the natural ^{13}C -abundance a_0 , the amount M_S and the ^{13}C -abundance a_S of the substrate are known. That means we are left with not less than 12 unknown quantities, namely the carbon amounts M_A and M_B , the carbon flows $w_A, w_B, w_A', w_B', w_A'', w_B'', v_1$ and v_2 as well as the ^{13}C -abundances a_A and $a_A(0)$.

In order to make the model tractable for evaluating ^{13}C -breath tests, it has to be treated mathematically introducing some further simplifying assumptions. Thus it should be permitted to assume biochemically stationary conditions at least during the test (at most a few days). Then all flows w and v as well as pool sizes M_A and M_B are constant, disregarding fluctuations in the rhythm of food intake, and v_1 and v_2 become equal:

$$v_1 = v_2 = v \quad (1)$$

Furthermore we assume that the rate of absorption of the substrate is large compared to the

rate of its cleavage in the human organism. This is often assumed in evaluating breath tests and should be allowed if the curve in Fig. 3 in the short time period steeply ascends and then (after passing a maximum) in the long time period slowly approximates the value a_0 asymptotically. Under these frequently occurring circumstances for $t = 0$ the law of isotope dilution is valid:

$$M_S a_S + M_A a_0 = (M_S + M_A) a_A(0), \quad (2)$$

where $a_A(0)$ is the (hypothetical) ^{13}C -abundance in pool A which is established immediately after application of substrate, if its entry into this pool would instantly occur.

The flow

$$P = w_A' + w_B' \quad (3)$$

of exhaled carbon dioxide can be calculated from body mass W , height H , age and sex of the investigated individuals. In earlier investigations on the carbon dioxide excretion with breath only the body surface was taken into account (Haycock B, Schwartz GJ and Wisotski DH 1978), assuming a uniform value of 300 mmol per m^2 and hour (Shreeve WW, Cerasi E and Luft R 1970). Later research work (Schofield WN 1985; Klein PD, Malaty HM, Czinn SJ et al. 1998) revealed that human carbon dioxide exhalation is to a considerable extent not only affected by body surface but also by age and sex. Following these results the BMR (Basal Metabolic Rate) can be calculated using equations of the form:

$$\text{BMR} = \alpha W + \beta H + \gamma \quad (4)$$

where α , β and γ are coefficients presented in Tab. 3. (These values refer to the usual case that BMR is given in MJ/d, W in kg and H in m.)

Age [years]	male			female		
	α	β	γ	α	β	γ
3 – 10	0,082	0,542	1,736	0,071	0,677	1,533
>10 – 18	0,068	0,574	2,157	0,0035	1,948	0,837
>18 – 30	0,063	- 0,042	2,943	0,057	1,184	0,411
>30 – 60	0,048	- 0,011	3,670	0,0344	0,006	3,530

Table 3

Numerical values of the coefficients α , β and γ in eqn. 4 in dependence on age and sex (from Klein PD, Malaty HM, Czinn SJ et al. 1998).

The flow P of exhaled carbon dioxide can be calculated from BMR in eqn. 4 using the following formula:

$$P = 2,49 \text{ BMR mol/d}, \quad (5)$$

where BMR is again to be given in MJ/d (Klein PD, Malaty HM, Czinn SJ et al. 1998).

According to Fig. 9 for pool A the carbon balance

$$w_A + v_2 = w_A' + w_A'' + v_1 \quad (6)$$

and the ^{13}C -balance

$$- M_A da_A/dt = (w_A + v_2) a_0 - (w_A' + w_A'' + v_1) a_A \quad (7)$$

are valid; moreover the ^{13}C -balance

$$w_A' a_A + w_B' a_0 = (w_A' + w_B') a \quad (8)$$

can be made out.

Following the considerations reflected on eqn. 2 this ^{13}C -balance must hold also for $t = 0$:

$$w_A' a_A(0) + w_B' a_0 = (w_A' + w_B') a(0), \quad (9)$$

where $a(0)$ is that ^{13}C -abundance which would be established in breath after mixing the substrate with pool A in an infinitely short time. The numerical value of $a(0)$ can be determined by extrapolating the descending part of the curve in Fig. 3 to $t = 0$.

With the eqns. 1, 2, 3, 6, 7, 8 and 9 we arrive at seven equations for nine unknown quantities (M_A , w_A , w_A' , w_B' , w_A'' , w_B'' , v , a_A and $a_A(0)$). In the following the unknown quantities w_A' , w_B' , w_A'' , w_B'' and $a_A(0)$ will be eliminated. At first v_1 and v_2 are substituted by v according to eqn. 1 and w_A'' is eliminated by eqn. 6. Eqn. 7 then transforms into:

$$M_A da_A/dt = (w_A + v) (a_A - a_0) \quad (10)$$

The flow w_B' is eliminated by eqn. 3. Eqn. 8 then transforms into:

$$w_A' a_A + (P - w_A') a_0 = P a$$

or

$$w_A' (a_A - a_0) = P (a - a_0)$$

or

$$w_A' = P (a - a_0) / (a_A - a_0) \quad (11)$$

In an analogous way we obtain from eqn. 9:

$$w_A' = P (a(0) - a_0) / (a_A(0) - a_0) \quad (12)$$

Finally it follows from eqns. 11 and 12:

$$(a - a_0) / (a_A - a_0) = (a(0) - a_0) / (a_A(0) - a_0) \quad (13)$$

The quantity $a_A(0)$ in eqn. 13 can be eliminated by rearranging eqn. 2 to give:

$$a_A(0) = (M_S a_S + M_A a_0) / (M_S + M_A) \quad (14)$$

Introducing this expression into eqn. 13 finally gives:

$$(a - a_0) / (a_A - a_0) = (M_S + M_A) (a(0) - a_0) / M_S (a_S - a_0) \quad (15)$$

With the eqns. 10 and 15 we arrive at two equations for the following four unknown

quantities: M_A , a_A , w_A and v .

Further equations for determining these unknown quantities can scarcely be established. Deeper insights into metabolic mechanisms on the basis of the model presented here can therefore only be achieved by certain, commonly unusual measures in carrying out ^{13}C -breath tests. The following measures of this kind are obvious: Firstly the tests can be carried out in a fasting period which is sometimes anyhow done for reasons of reproducibility. In this case w_A is equal to zero and we are left with only three unknown quantities. The strain connected with this procedure can be reduced by administering vitamins, mineral salts, water and roughage like bran or gelatine with a negligible content of digestible components. Under the conditions of fasting, however, it may be doubtful that M_A will remain constant during the examination period.

Secondly the investigated individual can be supplied with a diet defined with respect to amount and content of digestible carbon compounds. If a clear decision is possible which components of such a diet have to be attributed to w_A , then w_A again transforms into a known quantity. For clarity we would like to recommend to administer that substance as an energy and, if possible, also as a nitrogen source which is applied as tracer, e. g. for the ^{13}C -glycine breath test glycine of natural isotopic composition and for the ^{13}C -glucose breath test glucose of natural isotopic composition etc. Coupling of such diets with roughage, vitamins, mineral salts and water is of course possible.

Modelling is always connected with simplifications, in our case mainly the following:

- 1.) Only two carbon pools are taken into account, one pool A with high metabolic activity and one pool B with a lower metabolic activity. In reality, however, there are many carbon pools in the human (as well as in the animal) organism, the metabolic activities of which range from the high activity we attribute to pool A to a very low one. In borderline cases carbon is stored for a period of time comparable to the life time of the individual itself, like it is true for collagen or calcium carbonate of the skeleton. It is supposed to be difficult to estimate the influence of this simplification on M_A and v without experimental investigations.
- 2.) The flow v_2 is supposed to keep its natural ^{13}C -abundance a_0 for several hours or even some days. This is often taken for granted in tracer investigations in medicine and biochemistry and is the more correct the larger M_B in comparison to M_A is.
- 3.) We assume that the rate of absorption of the substrate is large compared with its cleavage. Whether or not this hypothesis is true, can be estimated in the light of the shape of the curve in the $a(t)$ - t -diagram of Fig. 3 (steep ascent / flat descent of $a(t)$ with ascending t).
- 4.) Though food ingestion and digestion are rhythmic the model assumes these processes to be continuous. The influence of this simplification on M_A and v can be reduced by fasting or by food intake in small portions and short, equal intervals.

Regardless of these sources of error the model presented here permits the estimation of metabolic parameters like M_A and v for substrates of ^{13}C -breath tests entering endogenous pools of the human body before the products of their cleavage are expired. Thus insights into the human metabolism become available which otherwise cannot be obtained. Obviously such information is not only interesting from a scientific point of view, but also for enhancing selectivity and sensitivity of ^{13}C -breath tests. Two possible ways of using the model for

clinical diagnosis will be described in the following, namely calculating the quotient of that carbon flow w_A' which leaves pool A and is then exhaled and the total flow of carbon dioxide in breath, and standardising measured ^{13}C -abundances by means of the size M_A of this pool calculated on the basis of the model.

It is characteristic of both ways that the diagnostic result is not as usual derived from the experimental data themselves but from the numerical values which are calculated from certain model conceptions using these measured data. As far as examples of such kind of approach are concerned we refer to publications by Irving CS, Schoeller DA, Nakamura K et al. (1982), Ghos YF (1996) and Klein PD (1998).

For calculating the flow w_A' we substitute $a_A(0)$ in eqn. 12 using eqn. 14 and obtain:

$$w_A' = P (M_S + M_A) (a(0) - a_0) / [M_S (a_S - a_0)] \quad (16)$$

Amount M_S and ^{13}C -abundance a_S of the substrate as well as the natural ^{13}C -abundance a_0 in eqn. 16 are known. The flow P of exhaled carbon dioxide can be calculated using eqns. 4 and 5, if body mass, height, age and sex of the patient are known. The quantity $a(0)$ can be estimated by extrapolating the descending part of the curve in Fig. 3 to $t = 0$, while M_A is calculated by applying eqn. 10 using the descending part of this curve.

The quantity w_A' as well as the quantity a contain two factors, namely the normal physical constitution expressed by the quantity P as well as the state of health of the examined individual. It seems obvious to eliminate the effect of the physical constitution which is irrelevant for diagnosis by calculating the quotient $Q = w_A'/P$ and to use just this quotient instead of the ^{13}C -abundance in breath itself for diagnosis. This quotient can be obtained by rearranging eqn. 16:

$$Q = w_A'/P = (M_S + M_A) (a(0) - a_0) / [M_S (a_S - a_0)] \quad (17)$$

We therefore propose the quotient $Q = w_A'/P$ instead of the ^{13}C -abundance in breath as a diagnostic criterion which can be expected to be to some extent independent on the physical constitution of the investigated individual.

In the following we present another method by the help of which the effects of the physical constitution on measuring results obtained by ^{13}C -breath tests can be more or less eliminated. This method is founded on the standardisation of the ^{13}C -abundances in breath and the corresponding time t after administering the substrate. It is the aim of this standardisation to transform the measured values (^{13}C -abundances a and times t) into numbers without a physical dimension in order to obtain a - t diagrams being commensurable from individual to individual largely independent on their physical constitution.

For standardising ^{13}C -abundances a on the ordinate of the a - t diagram (see Fig. 3) the measured ^{13}C -values are referred to a^* of a homogeneous (with respect to both mass and ^{13}C -content) mixture of substrate and pool A of metabolically active carbon compounds. This value a^* can be calculated using eqn. 2 and taking $a_A = a^*$ into account:

$$a^* = (M_S a_S + M_A a_0) / (M_S + M_A) \quad (18)$$

The first standardisation step is replacing a in our a - t diagram by the quotient a/a^* using eqn. 18. That means the ^{13}C -abundance a in the breath after administering the substrate is divided by the ^{13}C -abundance a^* of a homogeneous mixture of the ingested substrate with the carbon amount M_A . In other words the quotient a/a^* is a multiple of the ^{13}C -content of this mixture.

The standardisation of a in the a - t diagram resembles the frequently applied practice to relate the amount of the ingested substrate to the body mass of the investigated individual or, as described by Leodolter A (1997), to standardise the measured δ values in correspondence to the applied amount of the substrate and the body mass. This procedure cannot be transferred to the t -axis of the a - t diagram, however. In addition it is not differentiated between pools of high and low metabolic activity in this way.

The times t in the a - t diagram are proposed to be standardised by relating them to that time t^* during which the carbon amount M_A is just once exhaled in the form of carbon dioxide. This time (in days) can be calculated by dividing M_A in moles by the carbon flow P of exhaled carbon dioxide in moles per day:

$$t^* = M_A [\text{mol}] / P[\text{mol/d}] = (M_A / P) [\text{d}] \quad (19)$$

Thus the second standardisation step consists of replacing the quantity t in the a - t diagram of a ^{13}C -breath test by the quotient t/t^* which is a multiple of that time during which the amount M_A of rapidly exchanging carbon is just once expired.

In this way a - t diagrams of ^{13}C -breath tests are transformed into a/a^* - t/t^* diagrams. The curves in these diagrams can be supposed to be largely independent on the physical constitution of the investigated individuals. Diagnostic conclusions drawn from such diagrams should therefore be superior to those drawn from the usual a - t diagrams with respect to sensitivity and specificity.

4.1.2. ^{13}C -Breath Tests with Novel Substrates

It is known since the middle of the eighties that *Helicobacter pylori*, at that time called *Campylobacter pyloridis* and later *Campylobacter pylori*, has a high urease activity, i. e. it cleaves urea to form ammonia and carbon dioxide. This gramnegative microaerophil or anaerobic microorganism settles in the gastrointestinal tract, the oral cavity and the genitals and brings on diseases like gastritis, gastric and duodenal ulcers, dyspepsia and, as was discovered later, even gastric cancer (Hazell SL and Lee H 1986). Today it is assumed that the majority of these diseases is induced by *Helicobacter pylori* infection. Only a few years later Graham DY, Evans D, Alpert L et al. (1987) presented the ^{13}C -urea breath test for the diagnosis of this infection, making use of the high urease activity of these microorganisms. Topical survey on the microbiological and clinical aspects of the *Helicobacter pylori* infection is given by Hinsen U (1998), Leodolter A (1997), Klein PD, Logan RP, Amarri S et al. (1998) as well as by Graham KS and Graham DY (1998).

As Heine WE, Berthold HK and Klein PD (1996) showed, ^{13}C -labelled glycosyl ureides are specific markers for the action of colonic microbial flora. Oral administration of lactose- or cellobiose- ^{13}C -ureide resulted in bacterial cleavage of the N-glycosyl bond and subsequent urea hydrolysis. $^{13}\text{CO}_2$ first appeared in breath after 5 to 6 hours, peak excretion occurred between 8 and 14 hours, and the signal returned to baseline after 18 to 24 hours. Metoclopramide (10 mg) administered one hour before taking up the substrate advanced the onset of the signal by 2 to 3 hours and the time of peak response by about one hour, while loperamide (16 mg) broadened the peak and delayed the time of breath signal onset by about one hour and peak excretion by approximately 4 hours. Thus the ^{13}C -glycosyl ureide breath test should be useful in the diagnosis of a variety of gastrointestinal motility disorders and in the development of drugs that affect gastrointestinal motility.

Alcoholic hepatic steatosis is supposed to be distinguishable from non-alcoholic steatosis by a new ^{13}C -breath test, the ^{13}C -ketoisocaproic acid breath test, measuring the rate of

decarboxylation of this ketocarboxylic acid which is decreased in subjects with alcoholic hepatic steatosis (Mion F, Rousseau M, Brazier J-L et al. 1995).

Ishii T, Takatori K, Iida K et al. (1998) examined the optimum ¹³C-labelling position in phenylalanine for use in a ¹³C-breath test for evaluating liver function. They found that the breath test with L-[1-¹³C]phenylalanine gave the best results and is superior to the ¹³C-phenacetin breath test.

If the metabolism of two substrates is to be studied simultaneously, combined ¹⁴C- and ¹³C-breath tests may be applied. Thus Maes BD, Ghoos YF, Geypens BJ et al. (1995) investigated the effect of octreotide, a synthetic peptide consisting of eight amino acid units, on the gastric emptying of both solids and liquids by means of a ¹⁴C-octanoic acid-¹³C-glycine breath test. It was demonstrated that the peptide which is injected subcutaneously, retards gastric emptying of both solids and liquids in normal healthy volunteers. The combined test is especially suitable for such kind of effects, because it turned out that octreotide does not affect absorption and metabolism of both octanoic acid and glycine. Another example of a combined ¹⁴C- and ¹³C-breath test was given by Evenepoel P, Claus D, Geypens P et al. (1998), who investigated protein assimilation under the influence of gastric acid suppression therapy using a ¹⁴C-octanoic acid / ¹³C-egg white breath test and showed that gastric acid suppression hampers protein assimilation. ¹³C-labelled egg protein is the basis of a ¹³C-breath test for evaluating the true ileal digestibility of egg protein (Evenepoel P, Geypens P, Luypaerts A et al. 1998).

4.1.3. Novel Applications of ¹³C-Breath Tests

Since their description by Marshall and Warren JR (1984) a series of tests have emerged for detecting *Helicobacter pylori* infection, invasive, needing endoscopy with biopsy, and non invasive ones. Dehesa Violante M (1993) compiled and compared them with respect to sensitivity and specificity and achieved results summarised in Tab. 4.

<i>Test</i>	<i>sensitivity</i>	<i>false negative</i>	<i>specificity</i>	<i>false positive</i>
<u>Invasive Tests</u>				
Culture	83 – 90	10 – 17	93	7
Histology	95	5	91 – 97	3 – 9
Rapid Urease Test	92	8	85 – 90	10 – 15
24 h Urease Test	67 – 77	23 – 33	93	7
<u>Non-invasive Tests</u>				
¹³ C Urea Breath Test	96 – 99	1 – 4	96 – 98	2 – 4
¹⁴ C Urea Breath Test	96	4	83 – 98	2 – 17
Serology	96	4	88 – 92	8 – 12

Table 4

Comparison of different methods for the diagnosis of *Helicobacter pylori* infection with respect to sensitivity and specificity (after Dehesa Violante M 1993). All data in %.

Tab. 4 clearly demonstrates the superiority of the ¹³C urea breath test. In view of the insignificant strain on the individuals to be examined the ¹³C urea breath test is particularly recommended for evaluating therapeutic efficiency (Dehesa Violante M 1993).

Klatt S, Taut C, Mayer D et al. (1997) demonstrated the superiority of the ¹³C-methacetin breath test for quantitative liver function testing in comparison to the Child-Pugh score and other quantitative liver function tests like MEGX-test and indocyanine green clearance. Oral

administration of 2 mg ^{13}C -methacetin per kg body mass to 31 patients with histologically proven liver cirrhosis of different aetiology and severity, ten patients with chronic viral hepatitis and ten healthy volunteers revealed DOB values with a superior correlation ($r = 0.67$) with the Child-Pugh score in comparison to MEGX-test ($r = 0.39$) and indocyanine green clearance ($r = 0.43$). With a cut-off value of 25 % at 20 minutes sensitivity and specificity of discrimination between cirrhotic and non-cirrhotic individuals were 93.5 and 95 %, respectively.

Opekun AR Jr, Klein PD and Graham DY (1995) showed that the ^{13}C -aminopyrine breath test detects altered liver metabolism caused by low-dose oral contraceptives.

As GhosYF, Maes BD, Geypens BJ et al. (1993) demonstrated, the ^{13}C -octanoic acid breath test is not only a tool of optimising clinical nutrition, particularly of differentiating between medium- and long-chain oxidation (Sauer PJJ, Lafeber HN and Sulkers EJ 1988), but also a good means of measuring the gastric emptying of solids. But half hour measurement intervals are not sufficient for obtaining a good reproducibility with healthy individuals and a satisfactory correlation with gastric emptying scintigraphy with diabetics (Pfaffenbach B, Wegener M, Adamek RJ et al. 1995). Gastric emptying rate of liquids in semisolid and liquid-solid meals can reliably be measured using the ^{13}C -acetate breath test (Braden B, Adams S, Duan LP et al. 1995; Pfaffenbach B, Schaffstein J, Adamek RJ et al. 1996). Since radioactive labelling, e. g. with ^{51}Cr and $^{199\text{m}}\text{Tc}$, is the usual alternative so far (Griffith GH, Owen GM, Kirkman S et al. 1966, Wegener M; Schaffstein J and Börsch G 1988), these results are particularly important.

Lifschitz CA, Boutton TW, Carazza TF et al. (1988) investigated glucose absorption and utilisation by means of the ^{13}C -glucose breath test. Five children with congenital glucose-galactose-malabsorption and five with severe small bowel villous atrophy and chronic diarrhoea were compared with each other and with three healthy and four children with severe malnutrition without diarrhoea. The breath test curves from the children with glucose-galactose-malabsorption and those with chronic diarrhoea were significantly different from those of the other two groups. Thus the ^{13}C -glucose breath test may be used for the diagnosis of at least severe glucose-galactose-malabsorption.

Tanis AA, van den Berg JW, Kroneman R et al. (1998) used a ^{13}C -breath test with naturally ^{13}C -enriched carbohydrates as a substrate for studying liver glycogen metabolism.

Conventional methods for investigating small bowel bacterial overgrowth are either invasive or need sophisticated laboratory procedures for analysis or are marked by inadequate sensitivity and specificity. Preliminary experiments by Lim, Wagner DA, Tosces PP et al. (1993) indicated that the ^{13}C -xylose breath test may be an attractive alternative. Dellert SF, Nowicki MJ, Farrell MK et al. (1997) demonstrated the clinical utility of this test for the diagnosis of small bowel bacterial overgrowth also in children.

In future ^{13}C -breath tests probably will play an important part in investigating the effect of anthropogenic air pollutants on the human organism. For obvious reasons such investigations have to apply methods of epidemiology and will be preceded by experiments with animals, where ^{14}C -breath tests will successfully compete with ^{13}C -breath tests, not only because radiation damaging of the objects to be investigated is hardly important, but also in view of the small amounts of breath samples to be expected (Martin JG and Redgrave TG 1998). Investigations by Krüger N, Neubert B, Helge H et al. (1990) and Krüger N, Helge H and Norbert D (1991), respectively, on the effect of PCDD and PCDF in mother's milk on the infant's metabolism may be regarded to be exemplary for this trend. Particularly as far as

lipophile xenobiotics like PCDD and PCDF are concerned, enrichment in the mother's milk has to be taken into account.

4.1.4. Modified ^{13}C -Breath Tests

Much research work on ^{13}C -breath tests is dedicated to simplifying and further developing such tests, to enhance their sensitivity and specificity and to have them validated by governmental authorities. This is particularly true for the ^{13}C -urea breath test for the diagnosis of *Helicobacter pylori* infection which is widespread nowadays in spite of being introduced not until 1988.

For this test Braden B, Duan LP, Caspary WF et al. (1994) recommended first breath sampling immediately before intake of 75 mg ^{13}C -urea dissolved in 200 ml 0.1 n aqueous citric acid solution and second breath sampling after 20 or 30 minutes (without intermediate intake of food).

As far as simplification is concerned Lotterer E, Ramaker J, Ludtke FE et al. (1991) could demonstrate that the isotopic analysis of a single breath sample after ^{13}C -urea intake is suitable for detection of *Helicobacter pylori* status in man. Moreover Malaty HM, el Zimaity HM, Ganta RM et al. (1996) arrived at the conclusion that omission of a meal administered together with the substrate and shortening the duration of the test to 20 minutes does not diminish specificity and selectivity (approximately 95% each) of the ^{13}C -urea breath test. According to Kato M, Asaka M, Kudo T et al. (1998) this time interval may be shortened even to 10 minutes, if 100 mg ^{13}C -urea are applied. Variation of the meal ingested together with the ^{13}C -urea and simultaneous intake of β -(3,4-dihydroxyphenyl)-ethylamine (dopamin) for delaying gastric emptying do not significantly affect the results of ^{13}C -urea breath tests, too (Casellas F, Lopez J, Borruel N et al. 1999).

Hamlet AK, Erlandsson KIM, Olbe L et al. (1995) proved the use of capsuled ^{14}C -urea instead of dissolved substrate to enhance the reliability of urea breath test, probably because contact with urease-producing bacteria other than *Helicobacter pylori* in the oral and oropharynx cavities is reduced. If so this result obviously also applies to the ^{13}C -urea breath test. Using gelatine capsules it could moreover be shown that capsule-based modifications of the ^{13}C -urea breath test enable to considerably reduce the amount of ^{13}C -urea to be administered (Bielanski W and Konturek SJ (1996).

Klein PD, Malaty HM, Martin RF et al. (1996) studied the ^{13}C -urea breath test for the detection of *Helicobacter pylori* infection on a large number of infected and non-infected subjects, particularly on duodenal ulcer patients before and after antimicrobial therapy, using varying tracer amounts (125 and 250 mg ^{13}C -urea) and postdose sampling time intervals (30 and 40 minutes). Results were compared with those of histological examination of gastric biopsies. The ^{13}C -urea breath test proved to be highly reliable with respect to patients both before and after antimicrobial treatment, provided the post-therapeutic testing is done not earlier than four weeks after ending therapy (Börsch GM and Graham DY 1991; Graham DY and Genta RM 1994; Graham DY 1994). The investigations confirm that a 125 mg dosage of ^{13}C -urea and one 30 minutes postdose sampling time ensure reliable non-invasive detection of *Helicobacter pylori* infection.

Applying ^{13}C -urea breath test for children with their higher endogenous carbon dioxide production evidently a lower cut-off value (3,5 instead of 5 ‰ at 2 mg per kg body mass and less than 100 mg ^{13}C -urea) has to be assumed (Cadranel S, Corvaglia L, Botems P et al. 1998).

Comparison of the kinetics of $^{13}\text{CO}_2$ exhalation after intake of either ^{13}C -urea or $\text{NaH}^{13}\text{CO}_3$ together with 200 ml 0.1 n citric acid revealed that individuals with *Helicobacter pylori* infection hydrolyse 15 to 60 % of administered urea to form carbon dioxide in breath (Eggers RH, Kulp A, Lüdtkke FE et al. 1990).

^{13}C -urea dissolved in 200 ml 0.1 n citric acid with 25 mg saccharine as sweetener proved to be superior to previously proposed semiliquid test meals (250 ml Meritene®, Wander Pharma or Ensure® (Abott), respectively, plus 50 ml Calogen® (SHS Pharma)) with respect to tracer amount and time involved (Dominguez-Munoz JE, Leodolter A, Sauerbruch T et al. 1997). This also supports the results mentioned before, according to which special meals for preventing false negative results due to too fast a gastric emptying of the substrate, can be abandoned.

Posture of individuals to be investigated during ^{13}C -urea breath test significantly affects results within the first five to ten minutes after tracer intake. Probably the most reliable results are obtained, if the fasting patients remain in a sitting position during the time from tracer intake to breath sampling (Miwa H, Murai T, Ohkura R et al. 1997).

Recently ^{13}C -breath tests begin to establish themselves as a means of not only qualitative, but also quantitative diagnosis. It is by no means surprising that this tendency can be primarily recognised for the most frequently applied and therefore most sophisticated ^{13}C -breath test, namely the ^{13}C -urea breath test (Ellenrieder V, Glasbrenner B, Stoffels C et al. 1997; Perry F, Clemente R, Pastore M et al. 1998).

As for additional investigations aimed to simplifying and optimising ^{13}C -urea breath test see Logan RPH, Polsen RJ, Misiewicz JJ et al. (1991), Logan RPH, Dill S, Bauer FE et al. (1991), Klein PD and Graham DY (1993), Braden B, Duan LP, Caspary WF et al. (1994), Eggers RH, Kulp A, Tegeler R et al. (1990) and Lotterer E, Ramaker J, Lüdtkke FE et al. (1991).

An excellent survey on *Helicobacter pylori* testing including profiles of many relevant companies is given by Bonney RC (1997). A comprehensive and rather up to date presentation of the ^{13}C -urea breath test for the diagnosis of *Helicobacter pylori* infection was given by Leodolter (1997). A survey of the medical aspects of *Helicobacter pylori*-associated diseases was presented by Graham KS and Graham DY (1998).

Choi M-G, Camillery M, Burton DD et al. (1998) could demonstrate that with ^{13}C -octanoic acid breath test eleven breath sampling times over an extended time of six rather than four hours after tracer intake yields reproducibilities of gastric emptying times of solids equal to those of scintigraphy with its inherent radiation damaging of the individuals to be investigated.

Beginning in the middle of the nineties mass spectrometric analysis of ^{13}C -breath test samples was supplemented by nondispersive infrared spectrometric and infrared spectrophotometric analysis, respectively (Koletzko S, Haisch M, Seeboth E et al. 1995; Taniguchi Y, Kimura K, Sato K et al. 1995; Braden B, Haisch M, Duan LP et al. 1994; Hildebrand P and Beglinger C 1997; Fischer Analysen Instrumente GmbH 1997; Wetzel K and Gerstenberger H 1978). Those devices proved to achieve sufficient reliability and reproducibility and surpass mass spectrometry with respect to their low price and their high serviceability.

4.2. Recent Results of the Application of ¹³C–Breath Tests

Like most of the methods of clinical diagnosis ¹³C–breath tests compete with alternative methods. Moreover usually ¹³C–breath tests are combined with alternative methods in medical research and diagnosis for mutual control. Therefore it is not always easily possible to clearly attribute any medical findings to a certain method of diagnosis. Selection of medical discoveries to be mentioned in this chapter therefore should primarily not depend on whether or not ¹³C–breath tests were included, but whether or not the fact that such tests do not imply any radiation, toxigenic or traumatic risk played a decisive role in establishing the result. No wonder that findings from epidemiological and preventive investigations, from investigations, in which many healthy or presumed healthy individuals have to be included, and from control of therapeutic measures will play a dominant role in the present chapter.

In 1996 the European Helicobacter Pylori Study Group (1997) issued a Consensus Report on the management of Helicobacter pylori infection which summarises discussions at a Meeting at Maastricht with 63 participants from 19 European countries and observers from Canada, Japan and USA.

According to this so-called Maastricht Consensus Report 1996 at least (active or chronic) peptic ulcer disease, bleeding peptic ulcer, low grade gastric mucosa associated lymphoid tissue (MALT) lymphoma, gastritis with severe abnormalities and physical conditions following early resection for gastric cancer are considered to be strong indications for Helicobacter pylori eradication therapy. Also for functional dyspepsia, family history of gastric cancer, long term treatment with protein pump inhibitors, for gastro-oesophageal reflux disease, planned or existing therapy with non-steroidal anti-inflammatory drugs as well as after gastric surgery for peptic ulcer Helicobacter pylori eradication therapy is recommended by the European Helicobacter Pylori Study Group (EHPSG).

As far as the treatment regimens are concerned, seven treatment days are advised using a standard dose proton pump inhibitor twice daily (20 mg omeprazole, 30 mg lansoprazole or 40 mg pantoprazole) and

- metronidazole (400 mg twice daily) / tinidazole (500 mg twice daily) plus clarithromycin (250 mg twice daily) or
- amoxycillin (1000 mg twice daily) plus clarithromycin (500 mg twice daily), advisable when metronidazole resistance is likely, or
- amoxycillin (500 mg three times daily) plus metronidazole (400 mg three times daily), advisable when clarithromycin resistance is likely.

The Food and Drug Administration of the United States of America (FDA) approved the following regimens for treatment of Helicobacter pylori infection:

- omeprazole plus clarithromycin
- ranitidine bismuth citrate plus clarithromycin
- bismuth subsalicylate plus metronidazole plus tetracycline hydrochloride
- lansoprazole plus amoxycillin plus clarithromycin
- lansoprazole plus amoxycillin,

each therapy lasting 14 days (Graham KS and Graham DY 1998).

Dual therapy of Helicobacter pylori infection with omeprazole plus clarithromycin proved to be well tolerable and effective against metronidazole resistant Helicobacter pylori and may therefore be an alternative to standard triple therapy (Logan RPH, Gummert PH, Schaufelberger HD et al. 1994; Labenz J, Gyenes E, Rhul GH et al. 1991). This result is supported by investigations by Lind T, Megraud F, Unge P et al. (1999), who showed that

addition of omeprazole achieves high eradication rates, decreases the impact of primary resistance and may reduce the risk of secondary resistance, when compared with regimens containing only two antibiotics. Adamek RH and Bethke TD (1998) demonstrated that a one-week triple therapy with pantoprazole, clarithromycin and metronidazole is a significantly superior strategy in comparison to a two-week dual therapy with pantoprazole and clarithromycin. Ellenrieder V, Fensterer H, Waurick M et al. (1998) showed that a decrease in clarithromycin dose by a factor of two within the scope of a triple therapy with clarithromycin, pantoprazole and metronidazole does not interfere with the efficacy of this therapy. This offers the opportunity of improving tolerance and lowering costs.

As Rothenbacher D, Bode G, Adler G et al. (1997) could show by questioning and investigating the infection status with the ^{13}C -urea breath test almost 500 adult outpatients in a suburban community near Ulm, a city in South Germany, that the use of commonly prescribed antibiotics is not associated with a prevalence of *Helicobacter pylori* infection. Evidently there is also no correlation between *Helicobacter pylori* infection of pre-school children and pets in their households (Bode G, Rothenbacher D, Brenner H et al. 1998).

Investigating more than 300 Italian children by means of the ^{13}C -urea breath test Perri F, Pastore M, Clemente R et al. (1998a) found some evidence that, at least during childhood, *Helicobacter pylori* infection may be a fluctuating disease with spontaneous eradication and possible recurrence.

Rothenbacher D, Bode G, Berg G et al. (1999) found some evidence of a parent-child transmission of *Helicobacter pylori* in this region. Investigations in a village in Linqu County, China, the inhabitants of which are exposed to a high risk of gastric cancer, yielded analogous results (Ma JL, Jou WC, Gail MH et al. 1998). Investigations of Brenner H, Rothenbacher D, Bode G et al. (1999) supported the hypothesis of a major role of spouse-to-spouse transmission of this infection.

Investigating almost 500 asymptomatic volunteers in the Houston metropolitan area, Texas, USA, using an enzyme-linked immunosorbent assay for Anti-*Helicobacter pylori* Immunoglobulin G (ELISA) and the ^{13}C -urea breath test revealed important epidemiological findings on *Helicobacter pylori* infection (Graham DY, Malaty HM, Evans DG et al. 1991). *Helicobacter pylori* infection was present in 52 %, the prevalence increasing rapidly with age (at 1 %/yr) for the overall population. The frequency of infection was higher in blacks (70 %) than in whites (34 %), this difference remaining after adjustments were made for age, gender, educational level, income and use of tobacco and alcohol. Significant inverse correlations were found between age-adjusted frequency of *Helicobacter pylori* infection and income on one hand and educational level on the other hand. No association was stated with consumption of alcohol and tobacco and intake of anti-inflammatory drugs. Having pets was correlated with a lower abundance of *Helicobacter pylori* infection, but this in its turn was associated with higher socio-economic status. The results are consistent with faecal-oral transmission as one important pathway of the infection.

^{13}C -urea breath test investigations by Rothenbacher D, Bode G, Berg G et al. (1998) revealed a large variation in prevalence of *Helicobacter pylori* infection in children living in the same geographic area according to their nationality, probably in dependence on living conditions in their early childhood.

Assessing more than 400 persons in Southern Germany Brenner H, Rothenbacher D, Bode G et al. (1999a) found a negative correlation between alcohol consumption and active infection with *Helicobacter pylori*.

There is growing evidence that *Helicobacter pylori* infection is correlated with gastric carcinoma (American Joint Committee on Cancer 1993; Blaser MJ, Kobayashi K, Cover TI et al. 1993; Breuer T, Sudhop T, Hoch J et al. 1996; Correa P 1991; Eurogast Study Group 1993; Forman D 1995; Hansson LE, Engstrand L, Nyren O et al. 1993; Hanson LE, Nyren O, Hsing AW et al. 1996; Kikuchi S, Wada O, Nakajima T et al. 1995; Nomura A, Stemmermann GN, Chiou PH et al. 1991; Nomura A and Stemmermann GN 1993; Parsonnet J, Friedman G, Vandersteen DP et al. 1991; Sipponen P 1994; Sipponen P, Kosunen TU, Valle S et al. 1992; Sipponen P, Riihelä M, Hyvarinen H et al. 1994; Ma JL, Jou WC, Gail MH et al. 1998).

Gastric cancer is likely to be multifactorial. Nevertheless in 1994 the International Agency for Cancer Research recognised a relationship between *Helicobacter pylori* infection and gastric cancer and classified *Helicobacter pylori* as a group I carcinogen.

Some results of ^{13}C -breath test investigations indicate that there is probably a correlation also between *Helicobacter pylori* infection and diseases other than those of the gastrointestinal tract. Thus chronic infection with *Helicobacter pylori*, often acquired in childhood, was linked to the risk of corona heart disease in adults (Patel P, Mendall MA, Carrington D et al. 1995). Perhaps the link between childhood poverty and adult corona heart disease is due to *Helicobacter pylori* infection (Mendall M, Goggin PM, Molineaux N et al. 1994). Recently Mendall MA, Patel B, Ballam B et al. (1996) demonstrated a strong association between C-reactive protein concentrations, sensitive markers of systemic inflammation, and corona heart disease, and speculated on that the body response to inflammation may play an important role in influencing the progression of arteriosclerosis.

There is also some evidence of a correlation between *Helicobacter pylori* infection and migraine. Eradication of the bacterium causes a significant decrease in attacks of migraine (Gasbarrini A, De-Luca A, Fiore G et al. 1998).

Deviating from results of earlier investigations Lionetti P, Amarri S, Silenzi F et al. (1999) came to the conclusion that the prevalence of *Helicobacter pylori* infection in HIV-1 perinatally infected children is comparable with the prevalence in a normal population of the same age.

Using the ^{13}C -trioctanoin breath test Shuishi Miyakawa MD, Makoto Hayakawa MD, Abihiko Horiguchi MD et al. (1996) showed that pancreatoduodenectomy or pancreatic head resection, respectively, reduces fat resorption less than does simultaneous resection of pancreas and stomach.

The same ^{13}C -breath test was used to evaluate a new medicament for activating gastric peristalsis by investigating 30 healthy volunteers. The effect of the motilide derived from erythromycin EM 574 on both lag-phase and gastric half emptying time proved to be dose dependent up to 20 mg with minimum side effects (Choi M-G, Camilleri M, Burton DD et al. 1998a).

Using a combination of the ^{13}C -acetate breath test and the ^{13}C -lactose/ ^{13}C -ureide breath test Biskup H, Heine WE and Wutzke KD (1999) demonstrated that the gastrointestinal transit time of liquid diets is not noticeably altered, if the osmolality and energy content of the formulas are limited to 460 mosmol/l and 150 kcal/100 ml.

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

In view of their simplicity and tolerability ^{13}C -breath tests will become an indispensable means of clinical research and diagnosis, particularly of nutritional research, diagnosis of gastrointestinal diseases and metabolic disorders and investigating post-operative and parenteral nutrition. In connection with these trends the range of substrates will continue to grow rapidly, including combinations of ^{13}C -labelled substrates with each other and ^{13}C -labelled substrates in connection with ^{14}C -labelled ones.

5.1. Trends in Measuring Methods

Progress in analysing stable isotopes during the last decades guarantees that ^{13}C -breath tests will continue to play an important and rapidly growing part in clinical research and diagnosis.

Beside the substitution of the radioactive isotope ^{14}C by the stable isotope ^{13}C the following trends are perceptible:

- Further development of nondispersive infrared spectroscopic ^{13}C -breath test devices which are much cheaper and easier to operate than mass spectrometric devices
- Transition to nondispersive infrared spectrometers with only one measuring cell and detector cells filled with pure $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$, respectively, arranged behind this measuring cell (see chapter 3.3.)
- Further development of mass spectrometers specifically orientated to carbon isotope analysis of carbon dioxide refraining from otherwise desired universality of mass spectrometric devices

We suppose that nondispersive infrared spectrometric ^{13}C -breath tests will largely replace mass spectrometric ones. But ^{13}C -labelling with substrates naturally enriched in ^{13}C could well remain a domain of mass spectrometry. Also for centres of clinical chemistry which have to measure numerous samples per day and therefore attach importance to large sample storage capacity and automated operation rather than to low price of the probe and to instant availability of the clinical data, mass spectrometric ^{13}C -breath test devices could maintain advantages. The ability of mass spectrometry to manage small amounts of samples scarcely plays a role for ^{13}C -breath tests.

Whether or not rapidly developing laser spectroscopic methods will compete with nondispersive infrared spectroscopy for ^{13}C -breath tests one day, remains an open question.

5.2. Trends in Supplying Substrates

One disadvantage of ^{13}C -breath tests in comparison to the earlier developed ^{14}C -breath tests is the high price of many substrates. In such cases we suppose that biosynthesis using artificially enriched ^{13}C will be an alternative, particularly since it allows to synthesise a large number of ^{13}C -labelled organic substances suitable for ^{13}C -breath tests in one go. Moreover in this way ^{13}C -labelled biomass can be produced which itself or after fractionation into fractions like proteins, lipids, carbohydrates and the like, can be applied as substrate similar to natural foodstuffs (Klein PD 1998).

As already mentioned in chapter 2 an alternative to utilise plant biosynthesis for producing ^{13}C -labelled organic compounds makes use of the fact that assimilation of carbon dioxide prefers $^{12}\text{CO}_2$ to $^{13}\text{CO}_2$ (Wetzel K and Fischer H 1998).

In both cases also the synthesis of special biologically active, ^{13}C -labelled substances is conceivable which are generated on the tide of secondary metabolism (alkaloids, essential oils etc.), particularly in view of the perspectives of genetic engineering.

Of course also animal biosynthesis can be and will increasingly be utilised for generating ^{13}C -labelled organic material. As for examples see Evenepoel P, Claus D, Geypens P et al. (1998) or Evenepoel P, Geypens P, Luypaerts A et al. (1998).

5.3. Trends in Administering Substrates

Substrates of ^{13}C -breath tests usually are administered orally. This is presumably due to the fact that relatively large tracer amounts have to be applied in order to achieve an effect in breath which can be measured at a sufficient accuracy. Not least therefore most of ^{13}C -breath tests are directed to the diagnosis of gastro-intestinal diseases. By enclosing the substrate in capsules made of materials persisting gastric enzymes and hydrochloric acid but not the enzymes excreted into the duodenum duodenal resorption could be investigated independently of gastric processes (Hartig W 1999).

Beyond that there is no reason to refrain from developing ^{13}C -breath tests the substrates of which are or have to be administered by infusion, injection or rectally. As an example of supplying the substrate by injection we point to investigations by Berry GT, Nissim I, Mazur AT et al. (1995) on the in vivo oxidation of [^{13}C]galactose in patients with galactose-1-phosphate uridylyltransferase deficiency. A necessity to do so may be the administration of a substrate which is instable in a gastric environment. It should also become possible in this way to get a deeper understanding of the biochemical and physiological mechanisms of parenteral nutrition or to carry out ^{13}C -breath tests with patients who can only be nourished parenterally. As for rectal administration of the substrate we point to a ^{14}C -butyrate breath test described by Den-Hond E, Hiele M, Evenepoel P et al. (1998) for investigating ulcerative colitis which if requested evidently can be transferred into a ^{13}C -butyrate breath test.

5.4. Trends in Sampling

Instead of measuring ^{13}C excretion with breath the metabolic cleavage of the substrate can also be investigated by determining ^{13}C abundances in components of faeces (bile acids etc.) or urine (urea, creatine, uric acid etc.) or in proteins and amino acids of plasma. Frequently in these cases methods of gas chromatography / mass spectrometry (GC-MS) or nuclear magnetic resonance spectroscopy (NMR) are applied (Tofts PS and Wray S 1988; Mathews DF 1986). In all these cases, however, the field of ^{13}C -breath tests in the true sense of the word with their simple sampling and measuring is left. In view of the high expenditure for isotope analysis such methods will scarcely become established in clinical routine.

5.5. Trends in Fields of Application

At present ^{13}C -urea breath test for the diagnosis of *Helicobacter pylori* infection dominates all other ^{13}C -breath tests by far, especially because of the fact that this infection can bring on such severe diseases like peptic ulcera, gastric cancer and malignant gastric lymphoma. In spite of the fact that *Helicobacter pylori* is demonstrably able to become resistant to antibiotics this kind of infection will decrease in a long period of time, since the ^{13}C -urea breath test will rapidly gain currency and medical research will probably soon succeed in developing vaccines against *Helicobacter pylori* (Nedrud JG and Czinn SJ 1997; Czinn SJ and Nedrud JG 1991; Chen M, Lee A and Hazell SL 1992; Lee A and Fox JG 1997; Lee A, O'Rourke J, De Ungria MC et al. 1997; Kreiss C, Buclin T, Cosma M et al. 1996; Doidge C, Crust I, Lee A et al. 1994). Therefore new fields of application of ^{13}C -breath tests can and will be opened in the near future.

Since many *Helicobacter pylori* infected persons proved to have abnormal findings in the upper gastrointestinal tract (Hollenz M, Stolte M and Labenz J 1999), there is evidently a need for a non-invasive *Helicobacter pylori* screening test in asymptomatic persons as a public health measure. In addition possibilities of epidemiological studies within the framework of school entrance examinations have to be taken into account (Gonser T,

Rothenbacher T, Bode G et al. 1998). We are convinced that the ^{13}C -urea breath test would be suitable for these purposes.

If the presumption that *Helicobacter pylori* infection is able to bring on also diseases other than gastrointestinal ones will prove to be true (see chapter 4.2.), ^{13}C -urea breath test has a great future ahead.

Because of the high tolerability of ^{13}C -breath tests, their application to investigating epidemiological and ecological problems will play a rapidly growing part, e. g. the exploration of the reasons for *Helicobacter pylori* infection in dependence on factors like age, race, sex and standard of life, the elucidation of the modes of transmission of pathogenic microorganisms as well as the investigation of the impacts of xenobiotics, particularly of those which present themselves as pollutants in the environment or are supposed to enter the food chain, respectively. In the latter cases we have also to think of implicating animal experiments, especially since sampling like for infants is easily possible using face masks.

As for epidemiological applications of ^{13}C -breath tests we also point to investigations by Klein PD, Gilman RH, Leon-Barus R et al. (1994) on the epidemiology of *Helicobacter pylori* in Peruvian children.

In connection with investigations on infants a new field of application of ^{13}C -breath tests will develop directed at exploring the physiological effects of breast-feeding including the influences of lipophil toxic substances like polyhalogenated dioxins and furans which enter the food chain to an increasing extent.

There is also a great potential in the application of ^{13}C -breath tests to (semi-)quantitative diagnosis. Evaluating the clinical state of individuals on the basis of cut-off values is certainly the simplest, but also the most primitive kind of valuing results of clinical tests. As exemplary for quantitative diagnosis by ^{13}C -breath tests we refer to a publication by Gisbert JP, Pajares JM, Garcia-Valriberes R et al. (1998) on the recurrence of *Helicobacter pylori* infection after eradication, an investigation on the acceleration of N-3-demethylation of caffeine by omeprazol using the ^{13}C -[N-3-methyl]-caffeine breath test (Rost KL and Roots I 1994), to research work on the quantitative assessment of whole body galactose metabolism in galactosemic patients using the [1- ^{13}C]galactose breath test (Berry GT, Nissim I, Gibson JB et al. 1997) and to a study for characterising the liver function in case of liver cirrhosis by means of the [^{13}C]methacetin-breath test (Pfaffenbach B, Gotze O, Szymanski C et al. 1998).

Hsu HW, Butte NF, Wong WW et al. (1997) used a combination of several ^{13}C -breath tests (^{13}C -leucin-, ^{13}C -glucose- and ^{13}C -hiolein breath test) to investigate, whether or not insulin-treatment normalises protein, carbohydrate and fat metabolism of gestational diabetes mellitus.

Further new or developing fields of ^{13}C - breath test applications were already mentioned in other connections. For completing our concise presentation of trends in applying ^{13}C -breath tests within the present chapter we summarise them as follows:

- Application of substrates similar to natural foodstuffs prepared from biomass, especially from algal biomass (see chapter 5.2.)
- Administration of substrates by infusion or injection, respectively, particularly for exploring parenteral nutrition (see chapter 5.3.)
- Exploration of the metabolism of ^{13}C -labelled compounds by measuring ^{13}C -excretion with faeces or urine or by examining ^{13}C -abundance in plasma components (see chapter 5.4.)

References

- Adamek RJ and Bethke TD (1998): Cure of *Helicobacter pylori* Infection and Healing of Duodenal Ulcer: Comparison of Pantoprazole–Based One–Week Modified Triple Therapy versus Two–Week Dual Therapy. The International Pantoprazole HP Study Group. *Am J Gastroenterol* 93, 1919 – 1924
- Aktieselskabet Brüel & Kjaer, Naerum, DK (1989): Photoacoustic Gas Analyser. *DE* 37 16 763
- American Joint Committee on Cancer (1993): Manual for Staging of Cancer (4th ed.). J. B. Lippincott, Philadelphia, Pennsylvania, USA
- Barr RG, Perman JA, Schoeller DA et al. (1978): Breath Tests in Pediatric Gastrointestinal Disorders: New Diagnostic Opportunities. *Pediatrics* 62, 393 – 401
- Berman M, Shan E and Weiss MF (1962): The Routine Fitting of Data to Models. *Biophys J* 2, 275 – 285
- Berry GT, Nissim I, Mazur AT et al. (1995): In Vivo Oxidation of [¹³C]Galactose in Patients with Galactose–1–phosphate Uridyltransferase Deficiency. *Biochem Mol Med* 56, 158 – 165
- Berthold HK, Crain PF, Gouni I et al. (1995): Evidence for Incorporation of Intact Dietary Pyrimidine (but not Purine) Nucleosides into Hepatic RNA. *Proc Natl Acad Sci USA* 92, 10123 – 10127
- Berthold HK, Jahoor F, Klein PD et al. (1995): Estimates of the Effect of Feeding on Whole Body Protein Degradation in Women Vary with the Amino Acid Used as Tracer. *J Nutr* 125, 2516 – 2527
- Bielanski W and Konturek SJ (1996): New Approach to ¹³C–Urea Breath Test: Capsule–based Modification with Low Dose of ¹³C–Urea in the Diagnosis of *Helicobacter pylori* Infection. *J Physiol Pharmacol* 47, 545 – 553
- Biskup H, Heine WE and Wutzke KD (1999): Magenentleerung und intestinale Transitzeit von hoch– und niederkalorischen Sondernahrungen. *Akt. Ernähr.–Med.* 24, 238 – 241
- Blaser MJ, Kobayashi K, Cover TI et al. (1993): *Helicobacter pylori* Infection in Japanese Patients with Adenocarcinoma of the Stomach. *Int J Cancer* 55, 799 – 802
- Bode G, Rothenbacher D, Brenner H et al. (1998): Pets Are not a Risk Factor for *Helicobacter pylori* Infection in Young Children: Results of a Population–Based Study in Southern Germany. *Pediatr Infect Dis J* 17, 909 – 912
- Bonney RC (1997): The Developing Market for *Helicobacter pylori* Testing. In: *Clinical Reports* PJB Publications Ltd., Richmond, Surrey, UK
- Börsch GM and Graham DY (1991): *Helicobacter pylori*. In: Collen MJ and Benjamin SB (eds.): *Pharmacology of Peptic Ulcer Disease*. In: *Handbook of Experimental Pharmacology*, Vol. 99, Springer–Verlag, Berlin, 107 – 148

- Braden B, Duan LP, Caspary WF et al. (1994): More Convenient ^{13}C -Urea Breath Test Modifications still Meet the Criteria for Valid Diagnosis of *Helicobacter pylori* Infection. *Z Gastroenterol* 32, 198 – 202
- Braden B, Haisch M, Duan LP et al. (1994): Clinically Feasible Stable Isotope Technique at a Reasonable Price: Analysis of $^{13}\text{C}/^{12}\text{C}$ -Abundance in Breath Samples with a New Isotope Selective Nondispersive Infrared Spectrometer. *Z Gastroenterol* 32, 675 – 678
- Braden B, Adams S, Duan LP et al. (1995): The [^{13}C]Acetate Breath Test Accurately Reflects Gastric Emptying of Liquids in Both Liquid and Semisolid Test Meals. *Gastroenterol* 108, 1048 – 1055
- Braden B, Caspary WF and Lembcke B (1999): Nondispersive Infrared Spectrometry for $^{13}\text{CO}_2/^{12}\text{CO}_2$ -Measurements: A Clinically Feasible Analyser for Stable Isotope Breath Tests in Gastroenterology. *Z Gastroenterol* 37, 477 – 481
- Brenner H, Rothenbacher D, Bode G et al. (1999): Active Infection with *Helicobacter pylori* in Healthy Couples. *Epidemiol Infect* 122, 91 – 95
- Brenner H, Rothenbacher D, Bode G et al. (1999a): Inverse Graded Relation between Alcohol Consumption and Active Infection with *Helicobacter pylori*. *Am J Epidemiol* 149, 571 – 576
- Breuer T, Sudhop T, Hoch J et al. (1996): Prevalence and Risk Factors for *Helicobacter pylori* Infection in the Western Part of Germany. *Europ J Gastroenterol Hepatol* 8, 47 – 52
- Cadranel S, Corvaglia L, Botems P et al. (1998): Detection of *Helicobacter pylori* Infection in Children with a Standardised and Simplified ^{13}C -Urea Breath Test. *J Pediatr Gastroenterol Nutr* 27, 275 – 280
- Casellas F, Lopez J, Borrueal N et al. (1999): The Impact of Delaying Gastric Emptying by either Meal Substrate or Drug on the [^{13}C]Urea Breath Test. *Am J Gastroenterol* 94, 369 – 373
- Chen M, Lee A and Hazell SL (1992): Immunisation against Gastric *Helicobacter* Infection in a Mouse / *Helicobacter Felis* Model. *Lancet* 339, 1120 – 1121
- Choi M-G, Camillery M, Burton DD et al. (1998): Reproducibility and Simplification of ^{13}C -Octanoic Acid Breath Test for Gastric Emptying of Solids. *Am J Gastroenterol* 93, 92 – 98
- Choi M-G, Camillery M, Burton DD et al. (1998a): Dose-related Effects of N-dimethyl-N-isopropyl-8,9-anhydroerythromycin-A-6,9-hemiacetal on Gastric Emptying of Solids in Healthy Human Volunteers. *J Pharmacol Exp Ther* 285, 37 – 40
- Correa P (1991): Is Gastric Carcinoma an Infectious Disease? *N Engl J Med* 325, 1170 – 1171
- Czinn SJ and Nedrud JG (1991): Oral Immunisation against *Helicobacter pylori*. *Infect Immun* 59, 2359 – 2363
- Dehesa Violante M (1993): Metodos de diagnostico en infection por *Helicobacter pylori*. *Rev Gastroenterol Mex* 58, 87 – 95

Dellert SF, Nowicki MJ, Farrell MK et al. (1997): The ^{13}C -Xylose Breath Test for the Diagnosis of Small Bowel Bacterial Overgrowth in Children. *J Pediatr Gastroenterol Nutr* 25, 153 – 158

Den-Hond E, Hiele M, Evenepoel P et al. (1998): In Vivo Butyrate Metabolism and Colonic Permeability in Extensive Colonic Ulceritis. *Gastroenterol* 115, 584 – 590

Doidge C, Crust I, Lee A et al. (1994): Therapeutic Immunisation against Helicobacter Infection. *Lancet* 343, 914 – 915

Dominguez-Munoz JE, Leodolter A, Sauerbruch T et al. (1997): A Citric Acid Solution is an Optimal Test Drink in the ^{13}C -Urea Breath Test for the Diagnosis of Helicobacter pylori Infection. *Gut* 40, 459 – 462

Eggers RH, Kulp A, Lüdtke FE et al. (1990): Characterisation of the ^{13}C -Urea Breath Test for Diagnosis of Campylobacter pylori Infections. In: Chapman TE et al. (eds.): *Stable Isotopes in Pediatric Nutritional and Metabolic Research*. Intercept Ltd., Andover, Hampshire, UK

Eggers RH, Kulp A, Tegeler R et al. (1990): A Methodological Analysis of the ^{13}C -Urea Breath Test for Detection of Campylobacter pylori Infections. *Eur J Gastroenterol Hepatol* 2, 437 – 444

Elektronik Mechanik Gerätebau GmbH, Westerdeich, Bremen, Bundesrepublik Deutschland (1997): Stable Isotope Analyser. WO 97/14952

Ellenrieder V, Glasbrenner B, Stoffels C et al. (1997): Qualitative and Semi-Quantitative Value of a Modified ^{13}C -Urea Breath Test for Identification of Helicobacter pylori Infection. *Eur J Gastroenterol Hepatol* 9, 1085 – 1089

Ellenrieder V, Fensterer H, Waurick M et al. (1998): Influence of Clarythromycin Dosage on Pantoprazole Combined Triple Therapy for Eradication of Helicobacter pylori. *Aliment Pharmacol Ther* 12, 613 – 618

Erno Raumfahrttechnik GmbH (1993): IR Spectroscopic Gas Analysis Appts. – Has Internal Reflective Hollow Spherical Sample Chamber. DE 42 03 166

Eurogast Study Group (1993): An International Association between Helicobacter pylori Infection and Gastric Cancer. *Lancet* 341, 1359 – 1362

European Helicobacter Pylori Study Group (1997): Current European Concepts in the Management of Helicobacter pylori Infection. The Maastricht Consensus Report. *Gut* 41, 8 – 13

Evenepoel P, Claus D, Geypens P et al. (1998): Evidence for Impaired Assimilation and Increased Colonic Fermentation of Protein, Related to Gastric Acid Suppression Therapy. *Aliment Pharmacol Ther* 12, 1011 – 1019

Evenepoel P, Geypens P, Luypaerts A et al. (1998): Digestibility of Cooked and Raw Egg Protein as Assessed by Stable Isotope Techniques. *J Nutr* 128, 1716 – 1722

Fabinski W (1992): On-line-Analysatoren – Automatischer Abgleich mit internen Standards. *Staub – Reinhaltung der Luft* 52, 149 – 155

- Faust H, Junghans P, Matkowitz et al. (1981): Estimation of Protein Turnover in Patients with Liver Diseases Using ^{15}N -labelled Glycine. Proc. 4th Int. Conf. on Stable Isotopes, Jülich, 319 – 324
- Fischer Analyseninstrumente GmbH (1997): Kohlenstoffisotopenanalysator. DE 297 06 668.4
- Fischer H (1998): Verfahren zur Kalibrierung nichtdispersiver Infrarot-Spektralanalysatoren. DE 198 47 351.6
- Fischer H and Kuhlmann B (1998): Verfahren zur Kalibrierung von Isotopenanalysatoren. DE 197 31 889
- Forman D (1995): The Prevalence of Helicobacter pylori Infection in Gastric Cancer. Aliment Pharmacol Ther 9 (Suppl. 2) 71 – 76
- Fuji Electric Co., Ltd., Kawasaki, Kanagawa, Japan (1995): Infrarot-Gasanalysator. DE 44 32 940.7
- Gasbarrini A, De-Luca A, Fiore G et al. (1998): Beneficial Effects of Helicobacter pylori Eradication on Migraine. Hepatogastroenterology 45, 765 – 770
- Gebhardt O, Wetzel K and Hübner G (1964): Über die Verwertung organischen Stickstoffs für die Synthese von Milch- und Körpereiwweiß beim laktierenden Rind. Arch Tierernährg 14, 157 – 178
- Ghoos YF, Maes PD, Geypens PJ et al. (1993): Measurement of Gastric Emptying of Solids by Means of Carbon-Labelled Octanoic Acid Breath Test. Gastroenterol 104, 1640 – 1647
- Ghoos YF (1996): ^{13}C -Urea Breath Tests at the Laboratory „Digestion – Absorption“. University Hospital Gathuisberg, Leuven, Belgium
- Gilger MA, Klein PD, Klish WJ et al. (1988): Scoring the ^{13}C -Trioctanoine Breath Test to Predict Coefficient of Fat Absorption. Gastroenterol 94 A, 147
- Gisbert JP, Pajares JM, Garcia-Valriberes R et al. (1998): Recurrence of Helicobacter pylori Infection after Eradication: Incidence and Variables Influencing It. Scand J Gastroenterol 33, 1144 – 1151
- Gonser T, Rothenbacher T, Bode G et al. (1998): Möglichkeiten epidemiologischer Erhebungen im Rahmen von Einschulungsuntersuchungen am Beispiel der Ulmer Helicobacter pylori-Studien. Gesundheitswesen 60 (Suppl. 1), 24 – 29
- Graham DY, Evans D, Alpert L et al. (1987): Campylobacter pylori Detected Non-invasively by the ^{13}C -Urea Breath Test. Lancet 1987, 1174 – 1177
- Graham DY, Malaty HM, Evans DG et al. (1991): Epidemiology of Helicobacter pylori in an Asymptomatic Population in the United States. Gastroenterol 100, 1495 – 1501
- Graham DY (1994): Determinants of Antimicrobial Effectiveness in H. pylori Gastritis. In: Hunt RH und Tytgat GNJ (eds.): Helicobacter pylori. Basic Mechanisms to Clinical Cure. Kluwer Academic Publishers, Dordrecht, The Netherlands, 531 – 537

Graham DY and Genta RM (1994): Reinfection with *H. pylori*. In: Hunt RH und Tytgat GNJ (eds.): *Helicobacter pylori*. Basic Mechanisms to Clinical Cure. Kluwer Academic Publishers, Dordrecht, The Netherlands, 113 – 120

Graham KS and Graham DY (1998): Contemporary Diagnosis and Management of *Helicobacter pylori*-Associated Gastrointestinal Diseases. Handbooks in Health Care Co., Newtown, Pennsylvania, USA

Griffith GH, Owen GM, Kirkman S et al. (1966): Measurement of Gastric Emptying Using Chromium-51. *Lancet* 1966, 1244 – 1245

Hamlet AK, Erlandsson KIM, Olbe L et al. (1995): A Simple, Rapid and Highly Reliable Capsule-Based ¹⁴C-Urea Breath Test for Diagnosis of *Helicobacter pylori* Infection. *Scand J Gastroenterol* 30, 1058 – 1063

Hansson LE, Engstrand L, Nyren O et al. (1993): *Helicobacter pylori* Infection: Independent Risk Factor of Gastric Adenocarcinoma. *Gastroenterol* 105, 1098 – 1103

Hanson LE, Nyren O, Hsing AW et al. (1996): The Risk of Stomach Cancer in Patients with Gastric or Duodenal Ulcer Disease. *N Engl J Med* 335, 242 – 249

Hartig W (1999): Personal Communication

Haycock B, Schwartz GJ and Wisotski DH (1978): A Geometric Method for Measuring Body Surface Area: A Weight Height Formula Validated in Infants, Children and Adults. *J Pediatrics* 93, 62 – 66

Hazell SL and Lee H (1986): *Campylobacter pyloridis*, Urease, Hydrogen in Batch Diffusion and Gastric Ulcers. *Lancet* 1986, 15 – 17

Heine WE, Berthold HK and Klein PD (1996): A Novel Stable Isotope Breath Test: ¹³C-Labelled Glycosyl Ureides Used as Non-invasive Markers of Intestinal Transit Time. *Am J Gastroenterol* 90, 93 – 98

Higashi Y, Ohara H, Naruki Y et al. (1996): Application of Laser Spectroscopy for ¹³C-Breath Tests. *Kaku Igaku* 33, 415 – 421

Hildebrand P and Beglinger C (1997): Nondispersive Infrared Spectrometry: A New Method for the Detection of *Helicobacter pylori* Infection with the ¹³C-Breath Test. *Clinical Infections Diseases* 25, 1003 – 1005

Hinsen U (1998): Diagnostik bei *Helicobacter pylori*-Infektion – Eine Langzeitstudie unter Berücksichtigung des Kostenaspekts. Dissertation der Hohen Medizinischen Fakultät der Westfälischen Wilhelms-Universität Münster

Hofmann F and Lauterburg BH (1977): Breath Tests With Isotopes of Carbon: Progress and Potential. *J Lab Clin Med* 90, 405 – 411

Hollenz M, Stolte M and Labenz J (1999): *Helicobacter pylori*-Screening in einer allgemeinärztlichen Praxis. *Dtsch Med Wochenschr* 124, 171 – 175

- Hsu HW, Butte NF, Wong WW et al. (1997): Oxidative Metabolism in Insulin-Treated Gestational Diabetes mellitus. *Am J Physiol* 272 (6 Pt 1) E 1099 – 1107
- Iida K, Chiyoda T and Kajiwara M (1996): *J Label Compds Radiopharm* 38, 1133 – 1138
- Ingenieurhochschule Köthen, Bundesrepublik Deutschland (1988): Anordnung zur Identifikation und Korrektur des statischen Übertragungsverhaltens von zweikanaligen nichtdispersiven Absorptionsfotometern. DD 300 325
- Irving CS, Schoeller DA, Nakamura K et al. (1982): The Aminopyrine Breath Test as a Measure of Liver Function. A Quantitative Description of Its Metabolic Basis in Normal Subjects. *J Lab Clin Med* 100, 356 – 373
- Ishii T, Takatori K, Iida K et al. (1998): Optimum Conditions for the ^{13}C -Phenylalanin Breath Test. *Chem Pharm Bull Tokyo* 46, 1330 – 1332
- IUPAC (1978 and 1979): Nomenclature of Organic Chemistry, Section H. Isotopically Modified Compounds, issued by the Commission on Nomenclature of Organic Chemistry of the International Union of Pure and Applied Chemistry. Approved Recommendations. *Pure Appl Chem* 51 (1979) 353–380; *Europ J Biochem* 86 (1978) 9–25 and 102 (1979) 315–316.
- Johstone R (1996): *Mass Spectrometry for Chemists and Biochemists*. Cambridge University Press, Cambridge
- Kajiwara M, Katsumi J, Takatori K et al. (1997): Validity of the ^{13}C -Urea Breath Test for the Diagnosis of Helicobacter pylori Infection. *Chem Pharm Bull Tokyo* 45, 741 – 743
- Kato M, Asaka M, Kudo T et al. (1998): Ten Minutes ^{13}C -Urea Breath Test for the Diagnosis of Helicobacter pylori Infection. *J Gastroenterol* 33 (Suppl. 10), 40 – 43
- Kikuchi S, Wada O, Nakajima T et al. (1995): Serum Anti-Helicobacter pylori Antibody and Gastric Carcinoma among Young Adults. *Cancer* 75, 2789 – 2793
- Klatt S, Taut C, Mayer D et al. (1997): Evaluation of the ^{13}C -Methacetin Breath Test for Quantitative Liver Function Testing. *Z Gastroenterol* 35, 609 – 615
- Klein PD and Graham DY (1993): Minimum Analysis Requirements for the Detection of Helicobacter pylori Infection by the ^{13}C -Urea Breath Test. *Am J Gastroenterol* 88, 1865 – 1869
- Klein PD, Gilman RH, Leon-Barus R et al. (1994): The Epidemiology of Helicobacter pylori in Peruvian Children between 6 and 30 Months of Age. *Am J Gastroenterol* 89, 2196 – 2200
- Klein PD, Malaty HM, Martin RF et al. (1996): Non-invasive Detection of Helicobacter pylori Infection in Clinical Practice. *Am J Gastroenterol* 91, 690 – 694
- Klein PD (1998): Personal Communication
- Klein PD, Logan RP, Amarri S et al. (1998): Update on Helicobacter pylori Research and Diagnosis. *Eur J Gastroenterol Hepatol* 9, 617 – 618

Klein PD, Malaty HM, Czinn SJ et al. (1998): Urea Hydrolysis Rate Calculation Normalises the ^{13}C -Urea Breath Test for Age, Sex, Weight and Height. To be published in J Gastroenterol

Koletzko S, Haisch M, Seeboth E et al. (1995): Isotope-selective Nondispersive Infrared Spectrometry for Detection of Helicobacter pylori Infection with ^{13}C -Urea Breath Test. Lancet 345, 961 – 962

Kreiss C, Buclin T, Cosma M et al. (1996): Safety of Oral Immunisation with Recombinant Urease in Patients with Helicobacter pylori Infection. Lancet 347, 1630 – 1631

Krüger N, Neubert B, Helge H et al. (1990): Induction of Caffein-Demethylations by 2,3,7,8-TCDD in Marmoset Monkeys Measured with a $^{14}\text{CO}_2$ -Breath Test. Chemosphere 20, 1173 – 1176

Krüger N, Helge H and Norbert D (1991): Bedeutung von PCDDs/PCDFs („Dioxinen“) in der Pädiatrie. Monatsschr Kinderheilkd 139, 434 – 441

Krumbiegel P (1980): Isotope Terminology – Interregional Training Course Materials. ZfI-Mitteilungen, Leipzig, 33

Krumbiegel P, Teichmann B, Faust H et al. (1988): [^{15}N]Methacetin Urine Test to Measure Liver Function. Methodology for Application in Pediatrics. J Pediatr Gastroenterol Nutr 7, 333 – 340

Krumbiegel P (1991): Stable Isotope Pharmaceuticals for Clinical Research and Diagnosis. Verlag Gustav Fischer, Jena, 57 – 72

Krumbiegel P (1991 a): Stable Isotope Pharmaceuticals for Clinical Research and Diagnosis. Verlag Gustav Fischer, Jena, 19 – 24

Labenz J, Gyenes E, Rhul GH et al. (1991): Amoxyllin-Omeprazole Treatment of Helicobacter pylori Infection. Eur J Gastroenterol Hepatol 3 (Suppl.1), 10

Lee A and Fox JG (1997): Animal Models for Vaccine Development. In: Ernst PB, Michetti P, Smith PD et al. (eds.). The Immunobiology of H. pylori: From Pathogenesis to Prevention. Philadelphia, Lippincot-Raven, 55 – 72

Lee A, O'Rourke J, De Ungria MC et al. (1997): A Standardised Mouse Model of Helicobacter pylori Infection: Introducing the Sidney Strain. Gastroenterol 112, 1386 – 1397

Lembcke B (1997): Atemtests bei Darmerkrankungen und in der gastroenterologischen Funktionsdiagnostik. Schweiz Rundsch Med Prax 86, 1060 – 1067

Leodolter A (1997): ^{13}C -Harnstoff-Atemtest zur Diagnose der Helicobacter pylori-Infektion – Validierung und Optimierung des Testverfahrens. Dissertation bei der Otto von Guericke Universität Magdeburg, 27 – 30

Lifschitz CA, Boutton TW, Carazza TF et al. (1988): A Carbon-13 Breath Test to Characterise Glucose Absorption and Utilisation in Children. J Ped Gastroenterol Nutr 7, 842 – 847

- Lim AG, Wagner DA, Tosces PP et al. (1993): ^{13}C -Xylose Breath Test for Bacterial Overgrowth. *Gastroenterol* 104, A 259
- Lind T, Megraud F, Unge P et al. (1999): The MACH2 Study: Role of Omeprazole in Eradication of *Helicobacter pylori* with 1-Week Triple Therapies [See Comments]. *Gastroenterol* 116, 248 – 253
- Lionetti P, Amarri S, Silenzi F et al. (1999): Prevalence of *Helicobacter pylori* Infection Detected by Serology and ^{13}C -Urea Breath Test in Perinatally Infected Children. *J Pediatr Gastroenterol Nutr* 28, 1 – 6
- Logan RPH, Dill S, Bauer FE et al. (1991): The European ^{13}C -Urea Breath Test for the Detection of *Helicobacter pylori*. *Eur J Gastroenterol Hepatol* 3, 915 – 921
- Logan RPH, Polsen RJ, Misiewicz JJ et al. (1991): Simplified Single Sample ^{13}C Carbon Urea Breath Test for *Helicobacter pylori*: Comparison with Histology, Culture and ELISA Serology. *Gut* 32, 1461 – 1464
- Logan RPH, Gummet PH, Schaufelberger HD et al. (1994): Eradication of *Helicobacter pylori* with Clarithromycin and Omeprazole. *Gut* 35, 323 – 326
- Lotterer E, Ramaker J, Lüdtker FE et al. (1991): The Simplified ^{13}C -Urea Breath Test – One Point Analysis for Detection of *Helicobacter pylori* Infection. *Z Gastroenterol* 29, 590 – 594
- Ma JL, Jou WC, Gail MH et al. (1998): *Helicobacter pylori* Infection and Mode of Transmission in a Population at High Risk of Stomach Cancer. *Int J Epidemiol* 27, 570 – 573
- Maes BD, Ghoo YF, Geypens BJ et al. (1995): Influence of Octreotide on the Gastric Emptying of Solids and Liquids in Normal Healthy Subjects. *Aliment Pharmacol Ther* 9, 11 – 18
- Malaty HM, el-Zimaity HM, Genta RM et al. (1996): Twenty-Minute Fasting Version of the US ^{13}C -Breath Test for the Diagnosis of *Helicobacter pylori* Infection. *Helicobacter* 1, 165 – 167
- Mannesmann AG, Düsseldorf, Bundesrepublik Deutschland (1993): Nichtdispersives Infrarotspektrometer. EP 0584 897
- Marshall BJ and Warren JR (1984): Unidentified Curved Bacilli in the Stomach of Patients with Gastritis and Peptic Ulceration. *Lancet* 1984, 1311 – 1315
- Martin JG and Redgrave TG (1998): A ^{13}C CO₂-Breath Test to Assess the Metabolism of Triglyceride-rich Lipoprotein Remnants in Mice. *J Lipid Research* 39, 691 – 698
- Mathews DF (1986): A Critical Assessment of Methods of Measuring Metabolic Concentrations by NMR Spectroscopy. *NMR in Biomedicine* 1, 1 – 10
- Mendall MA, Goggin PM, Molineaux N et al. (1994): Relation of *Helicobacter pylori* Infection and Coronary Heart Disease. *British Health Journal* 71, 437 – 439
- Mendall MA, Patel B, Ballam B et al. (1996): C-reactive Protein and Its Relation to Cardiovascular Risk Factors: A Population Based Cross-Sectional Study. *British Medical*

Journal 312, 1061 – 1065

MIC Medical Instrument Corporation, Solothurn, Schweiz (1994): Vorrichtung zur Bestimmung des $^{13}\text{CO}_2/^{12}\text{CO}_2$ -Konzentrationsverhältnisses in einer Gasprobe. EP 0634 644

Mion F, Rousseau M, Brazier J-L et al. (1995): Human Hepatic Macrovesicular Steatosis: A Non-invasive Study of Mitochondrial Ketoisocaproic Acid Decarboxylation. *Metabolism* 44, 699 – 700

Miwa H, Murai T, Ohkura R et al. (1997): Effect of Fasting Subjects' Posture on ^{13}C -Urea Breath Test for Detection of *Helicobacter pylori* Infection. *Helicobacter* 2, 82 – 85

Murnick PE and Peer BJ (1994): Laser-Based Analysis of Carbon Isotope Ratios. *Science* 263, 945 – 947

Nedrud JG and Czinn SJ (1997): Oral Immunisation for the Prevention and Treatment of Infection with *Helicobacter pylori*. In: Ernst PB, Michetti P, Smith PD et al. (eds.): *The Immunobiology of H. pylori: From Pathogenesis to Prevention*. Philadelphia, Lippincot – Raven, 273 – 286

Nomura A, Stemmermann GN, Chiou PH et al. (1991): *Helicobacter pylori* Infection and Gastric Carcinoma among Japanese Americans in Hawaii. *N Engl J Med* 325, 1132 – 1136

Nomura A and Stemmermann GN (1993): *Helicobacter pylori* and Gastric Cancer. *J Gastroenterol Hepatol* 8, 294 – 303

Ohara S, Kato M, Asaka M et al. (1989): The UbiT 100 $^{13}\text{CO}_2$ Analyser: Comparison between Infrared Spectrometric Analysis and Mass Spectrometric Analysis. *Helicobacter* 3, 49 – 53

Opekun AR Jr, Klein PD and Graham DY (1995): [^{13}C]Aminopyrine Breath Test Detects Altered Liver Metabolism Caused by Low-Dose Oral Contraceptives. *Dig Dis Sci* 40, 2417 – 2422

Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan (1995): Method for Spectroscopically Measuring Isotopic Gas and Apparatus thereof. WO 97/140 29

Palocz-Andresen M (1995): Gerät für Infrarotanalysen von insbesondere gasförmigen Stoffen. DE 44 13 670

Parsonnet J, Friedman G, Vandersteen DP et al. (1991): *Helicobacter pylori* Infection and the Risk of Carcinoma. *N Engl J Med* 325, 1127 – 1131

Patel P, Mentall MA, Carrington D et al. (1995): Association of *Helicobacter pylori* and *Chlamydia pneumoniae* Infection with Coronary Heart Disease and Cardiovascular Risk Factors. *British Medical Journal* 311, 711 – 714

Perry F, Clemente R, Pastore M et al. (1998): The ^{13}C -Urea Breath Test as a Predictor of Intra-gastric Bacterial Load and Severity of *Helicobacter pylori* Gastritis. *Scand J Clin Lab Invest* 58, 19 – 28

Perry F, Clemente R, Pastore M et al. (1998a): *Helicobacter pylori* Infection May Undergo Spontaneous Eradication in Children. *J Pediatr Gastroenterol Nutr* 27, 181 – 183

- Pfaffenbach B, Wegener M, Adamek RJ et al. (1995): Nicht-invasiver ^{13}C -Octansäure-Atemtest zur Messung der Magenentleerung einer festen Testmahlzeit – Korrelation mit der Szintigraphie bei Diabetikern und Reproduzierbarkeit bei gesunden Probanden. *Z Gastroenterol* 33, 141 – 145
- Pfaffenbach B, Schaffstein J, Adamek RJ et al. (1996): ^{13}C -Acetat-Atemtest zur nichtinvasiven Beurteilung der Magenentleerung einer flüssig-festen Testmahlzeit bei Diabetikern. *Dtsch. Med. Wschr.* 121, 713 – 718
- Pfaffenbach B, Gotze O, Szymanski C et al. (1998): The [^{13}C]Methacetin-Breath Test for Quantitative Liver Function Analysis with an Isotope-Specific Nondispersive Infrared Spectrometer in Liver Cirrhosis. *Dtsch Med Wochenschr* 123, 1467 – 1471
- Pfeifer S, Pfliegel P and Borchert HH (1984): Pharmakokinetik, Bioverfügbarkeit und Biotransformation. Verlag Volk und Gesundheit, Berlin
- Rost KL and Roots I (1994): Accelerated Caffeine Metabolism after Omeprazole Treatment is Indicated by Urinary Metabolic Ratios. Coincidence with Plasma Clearance and Breath Test. *Clin Pharmacol Ther* 55, No. 4, 402 – 411
- Rothenbacher D, Bode G, Adler G et al. (1997): Use of Commonly Prescribed Antibiotics is Not Associated with Prevalence of *Helicobacter pylori* Infection in Adults. *J Gastroenterol* 32, 1096 – 1099
- Rothenbacher D, Bode G, Berg G et al. (1998): Prevalence and Determinants of *Helicobacter pylori* Infection in Pre-School Children: A Population Based Study from Germany. *Int J Epidemiol* 27, 135 – 141
- Rothenbacher D, Bode G, Berg G et al. (1999): *Helicobacter pylori* among Pre-School Children and Their Parents: Evidence of Parent-Child Transmission. *J Infect Dis* 179, 398 – 402
- Sauer PJJ, Lafeber HN and Sulkers EJ (1988): Measurement of Energy Metabolism by Stable Isotopes and Indirect Calorimetry. *Klin Ern* 34. Use of Stable Isotopes in Clinical Research and Practice. Int. Workshop Berlin, Zuckerschwerdt, München, 62 – 69
- Schoeller EA and Klein PD (1979): A Microprocessor Controlled Mass Spectrometer for the Fully Automated Purification and Isotope Analysis of Breath Carbon Dioxide. *Biomed Mass Spectrom* 6, 355 – 359
- Schofield WN (1985): Predicting Basal Metabolic Rates: New Standards and Review of Previous Work. *Hum Nutr* 34 C (Suppl. 1), 5 – 41
- Schröder E (1991): Massenspektrometrie. Springer Verlag, Berlin et al.
- Shreeve WW, Cerasi E and Luft R (1970): Metabolism of [$2-^{14}\text{C}$]Pyruvate in Normal, Acromegalic and HGH-treated Human Subjects. *Acta Endocrinol* 65, 55 – 69
- Shuishi Miyakawa MD, Makoto Hayakawa MD, Abihiko Horiguchi MD et al. (1996): Estimation of Fat Absorption with the ^{13}C -Trioctanoin Breath Test after Pancreato-duodenectomy or Pancreatic Head Resection. *World J Surg* 20, 1024 – 1029

- Siemens AG (1997): Infrared Gas Analyser. DE 196 47 632
- Siemens AG (1997a): Nondispersive Gas Analyser. DE 196 08 907
- Sipponen P, Kosunen TU, Valle S et al. (1992): Helicobacter pylori Infection and Chronic Gastritis in Gastric Cancer. J Clin Pathol 45, 319 – 323
- Sipponen P (1994): Gastric Cancer – A Long Term Consequence of Helicobacter pylori Infection. Scand J Gastroenterol 29 (Suppl. 201), 24 – 27
- Sipponen P, Riihelä M, Hyvarinen H et al. (1994): Chronic Non-atrophic (“Superficial”) Gastritis Increases the Risk of Gastric Carcinoma – A Case-Control Study. Scand J Gastroenterol 29, 336 – 340
- Sprinson DB and Rittenberg D (1949): The Rate of Interaction of the Amino Acids of the Diet with Tissue Proteins. J Biol Chem 180, 715 – 726
- Suehiro M, Kuroda A, Maeda M et al. (1987): Automated ^{13}C CO₂ Analysing System for the ^{13}C Breath Test. Radioisotopes 36, 7 – 13
- Taniguchi Y, Kimura K, Sato K et al. (1995): Substitute, Inexpensive Diagnostic Analysis of Helicobacter pylori Infection by Infrared (IR) Spectrophotometer for ^{13}C -Urea Breath Test. Gastroenterol 109, A 953
- Tanis AA, van den Berg JW, Kroneman R et al. (1998): Human Liver Glycogen Metabolism Assessed with a ^{13}C -Enriched Diet and a ^{13}C -Breath Test. Eur J Clin Invest 28, 466 – 474
- Tofts PS and Wray S (1988): Amino Acid and Substrate Turnover in Man Using GC-MS. In: Dietze G et al. (eds.): Clinical Nutrition and Metabolic Research. Proc. 7, Congress ESPEN, München 1985, Karger, Basel, 208 – 222
- VEB Junkalor Dessau, Bundesrepublik Deutschland (1988): Einrichtung zum Nullabgleich des Meßsignals in einem nichtdispersiven Infrarot-Gasanalyser. DD 269 444
- Wegener M, Schaffstein J and Börsch G (1988): Physiologie und Pathologie der Magenentleerung. Grundlagen, Untersuchungsmethoden und Therapie. Med Klin 10, 331 – 335
- Wetzel K, Hübner G and Gebhardt O (1961): Zur Auswertung von Markierungsversuchen in dynamischen Systemen. Abh dtsh Akad Wiss Berlin, Klasse Chemie, Geologie, Biologie, H. 7, 613 – 619
- Wetzel K, Hübner G and Gebhardt O (1963): Mathematische Analyse der Verwertung von zugefüttertem Ammoniumbicarbonat für die Synthese von Milch- und Körpereiweiß. Arch Tierernährg 13, 424 – 442
- Wetzel K, Gebhardt O and Hübner G (1966): Untersuchungen zum N-Stoffwechsel beim laktierenden Rind unter Verwendung von oral verabreichtem Harnstoff-(^{15}N). 12. Mitt.: Verwertung von zugefüttertem Harnstoff für die Synthese von Milch- und Körpereiweiß bei Milchkühen. Arch Tierernährg 16, 425 – 435

Wetzel K, Gebhardt O and Hübner G (1966 a): Über die Verwertung von NPN-Verbindungen für die Synthese von Milch- und Körpereweiß beim laktierenden Rind. Z Naturforsch 21 b, 183 –187

Wetzel K, Winkler E, Faust H et al. (1968): Über die Auswertung von Markierungsexperimenten zum Stickstoff-Stoffwechsel von Pflanzen mittels Modellbetrachtungen. studia biophysica Berlin 11, 27 – 39

Wetzel K and Gerstenberger H (1978): Verfahren zur Bestimmung des relativen ^{13}C -Gehalts der Atemluft. DD 137 323

Wetzel K, Junghans P, Faust H et al. (1979): New Results in the Use of ^{15}N in Medical Research. Proceedings of the III. International Conference on Stable Isotopes, Oak Brook, Illinois, 23. –26. May 1978. Academic Press New York et al. 591 – 609

Wetzel K and Fischer H (1998): Verfahren zur Herstellung ^{13}C -markierter Verbindungen. DE 198 20 078.1

Wetzel K and Fischer H (1998a): Über die Interpretation bei ^{13}C -Atemtests gewonnener Meßergebnisse. Unveröffentlichte Arbeit

Winkler E, Faust H, Wetzel K et al. (1978): Theoretische Aspekte der Untersuchung des Stickstoffmetabolismus mit ^{15}N beim Menschen. II. Probleme der Auswertung und Interpretation klinischer Untersuchungen. Isotopenpraxis 14, 367 – 372