\( ^{14}\text{CO}_2 \) in Breath*

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Abstract. The diagnosis of metabolic disorders can be made by detecting \( ^{14}\text{CO}_2 \) in the breath. This is possible because \( ^{14}\text{CO}_2 \) can label any organic compound without any deteriorations in the nature of the compound. This type of analysis is dependable, noninvasive and simple to perform with a scintillation counter.

Introduction

The nearly universal fate of ingested carbon atoms is oxidation to \( \text{CO}_2 \) and excretion in the breath. In 1784, Lavoisier when analyzing the breath of animals, showed this qualitatively by observing the parallel between metabolism and combustion. Although Dodds [29–31] used analysis of \( \text{CO}_2 \) pressure in breath as a diagnostic tool for various gastrointestinal disorders at an early stage, major progress awaited the advent of radioisotope technology, especially the availability of \( ^{14}\text{C} \)-labeled compounds. A typical experiment involves administration, usually oral, of a labeled compound, followed by analysis of radioactivity, as \( ^{14}\text{CO}_2 \), in the breath.

Labeling with \( ^{14}\text{CO}_2 \) enjoys several advantages over the use of other isotopes, e.g., \( ^{131}\text{I} \), \( ^{99}\text{Te} \), which have been widely used for labeling:

1. All organic compounds can be labeled, usually specifically without seriously affecting their molecular weight.
2. The labeled material is a natural product with unchanged biochemical, pharmacological, and other properties, rather than an artificial derivative.
3. Oxidation to \( \text{CO}_2 \) is a highly predictable metabolic pathway.

4. The rate of \( \text{CO}_2 \) released in breath is within narrow parameters.
5. Analysis is noninvasive and, with the assistance of a liquid scintillation counter, simple and straightforward.
6. Despite the very long physical half-life (5730 years) of \( ^{14}\text{C} \), the biological half-life of \( ^{14}\text{C} \)-labeled substrates is usually short, often in the order of hours or minutes.

Methods

Originally \( ^{14}\text{CO}_2 \) was determined in precipitated \( \text{Ba}^{14}\text{CO}_3 \) [38] or by a cumbersome total collection method [35, 40, 81, 83, 97]. In 1966, Abt and von Schuching [1] described a modification of the breath analysis technique that proved to be simpler, faster, and more accurate. In view of the fact that in the fasting and resting person \( \text{CO}_2 \) production remains approximately constant, these investigators employed periodic rather than continuous sampling. In this technique, which has subsequently become widely used [4, 8, 22, 32, 33, 43, 53, 65, 94], subjects exhale directly through a tube into a liquid scintillation vial containing a known quantity of hyamine hydroxide, which reacts with \( \text{CO}_2 \) on an equimolar basis. The tube is provided with a water-removing device (calcium chloride pellets) and a butterfly valve to avoid reverse flow. When color change of an acid-base indicator (phenolphthalein) present in the hyamine hydroxide indicates that a known amount of \( \text{CO}_2 \) has been trapped, exhalation is stopped, scintillation liquid is added, and a radioassay for \( ^{14}\text{C} \) is performed in a liquid scintillation counter. Since total amounts of both \( \text{CO}_2 \) and radioactivity are known, specific activity may be readily calculated.

For in vitro or bacterial studies of \( ^{14}\text{CO}_2 \) production the trapping solution may be placed in a closed flask containing the culture medium and \( ^{14}\text{C} \)-labeled compound. Alternatively, an automated system has been developed [28] which features direct counting of radioactive gas in an ionization chamber. Vials containing culture media are passed below the ionization chamber and are sampled periodically by a descending needle through which gas is removed. For bacterial or viral studies the needle is sterilized by heating between samplings. \( ^{14}\text{CO}_2 \) monitoring has found wide use in the detection of infection [5, 16, 25, 27, 77] since \( ^{14}\text{CO}_2 \) will be produced from \( ^{14}\text{C} \)-glucose if microorganisms are present.

Most \( ^{14}\text{CO}_2 \) breath tests use 2–5 \( \mu \text{Ci} \) of \( ^{14}\text{C} \). Available data suggests that 5–10 \( \mu \text{Ci} \) is a relatively safe amount of radioactivity.
for the benefit obtained. The total integrated body irradiation dose following administration of 10 μCi of 2-14C-glycine is about 1% of that from natural and cosmic sources [7]. After administration of cholyt-14C-glycine, patients eliminated 60% of the dose within one week [44] and more than 98% within 2 1/2 months [32].

Nonradioactive 13C-labeled materials, completely safe for use even in children and during pregnancy, offer promise for future use, although analysis of 13CO2, requiring mass spectrometry, is considerably more difficult and costly [39, 82, 87, 90].

Fat Absorption

The gastrointestinal digestion and absorption of fat is a complex process which depends on a number of factors for its integrity and normal function. Furthermore, significant differences in the digestion and absorption of various ingested lipids are due to differences in the solubilities of the liberated fatty acids. Fatty acids may be roughly divided into three groups: (1) short chain (six or fewer carbons), which are water soluble; (2) medium chain (8–12 carbons), sparingly soluble in both water and organic solvents; (3) long chain (16–24 carbons), soluble only in organic solvents. The third category includes palmitic, oleic, and stearic acids, which esterify with glycerol to form triacylglycerols (triglycerides), make up a vast majority of the fat in the average American diet.

The first phase of fat absorption occurs in the lumen of the intestine. Poorly soluble dietary lipids, emulsified in mixed micelles with the amphipathic components of the bile (conjugated bile acids, phospholipids, cholesterol) undergo lipolysis into fatty acids and monoacylglycerols. Pancreatic lipase catalyzes this partial hydrolysis. When a mixed micelle, now composed of fatty acids and mono- and dicylglycerols in addition to bile constituents, reaches the microvillus of the intestinal epithelial cell, it is dissolved into its components. Fatty acids, mono- and dicylglycerols pass across the membrane of the microvillus into the mucosal cell, while the bile salts are recirculated.

Improper operation of the intraluminal digestive phase may be due to inadequate quantities of bile in the intestine, as in extrahepatic biliary obstruction or biliary cirrhosis, or in resection, bypass, or disease of the terminal ileum, preventing reabsorption of bile salts. Alternatively, bile salts may be deconjugated and rendered ineffective in micelle formation. Deconjugation, which is caused by bacterial enzyme action, can result either from bacterial overgrowth of the normally sterile small intestine or from malabsorption in the terminal ileum and passage into the colon, where deconjugation is produced by action of the bacterial flora. The former cause is most frequently seen in the stagnant loop syndromes, especially that following Billroth II gastrectomy.

The second phase of fat absorption, occurring within the mucosal cells, is essentially a reversal of the first. The fatty acids and acylglycerols are reassembled into glycerols, a process which requires energy in the form of ATP and involves the action of acyltransferases on the fatty acyl CoA’s. A host of mucosal cell disorders, including gluten enteropathy, tropical sprue, regional enteritis, lymphoma, and amyloidosis, may impair this phase.

Finally, triglycerols and other lipids coated with proteins in the form of chylomicrons are secreted from the basal or lateral wall of the mucosal cell into the lymph. Submucosal disorders such as Whipple’s disease, scleroderma, or intestinal lymphangectasia may interfere with this phase of fat absorption.

Early applications of radioisotopes in the study of fat absorption centered around the use of 131I-triolein [12, 21, 48, 59, 76, 86, 92]. Poor accuracy was soon reported in many cases [6, 64, 75]. Although accuracy was somewhat improved by use of radiochemically pure 131I-triolein [56, 96], the low reliability of this method and its difficulty of administration have removed it as a diagnostic tool.

14C-labeled fats were used to study the absorption of fats in animals as early as 1948 [37, 57], although extensive attention to this technique occurred only with the disillusionment over 131I-triolein. In an early paper, Van Handel and Zilversmit [98] demonstrated that in animals 14C-labeled fat gave data of greater reliability than the corresponding 131I-labeled materials. Rothfeld and Rabinowitz [74] found a similar superiority in evaluating neomycin-induced steatorrhea in humans. Schwabe and co-workers [83] found reduced exhalation of 14CO2-specific activity in patients with malabsorption following oral administration of trioctanoylglycerol, but considerable overlap existed with controls and the test was unreliable in individual instances. Abt and von Schuching [1], using discontinuous sampling with 12C-tripalmitin and 14C-triolein, observed a similar decrease in 14CO2 breath activity among malabsorbers. Kaihara and Wagner [53], using a 14C-tripalmitin test in patients with fat malabsorption and neomycin-induced steatorrhea, reported good correlation between 14CO2-specific activity in expired air and other measurement of fat absorption. They pointed out that the test could not be used in patients with grossly disturbed metabolism (fever, thyrotoxicosis, diabetes) or abnormal fat clearance from the blood (some forms of hyperlipidemia). Antar et al. [4] confirmed the usefulness of the 14C-tripalmitin test but reported normal absorption of 14C-trioctanoylglycerol even in the presence of advanced bowel wall dysfunction and grossly impaired 14C-tripalmitin absorption. Similar positive data led Bhatia et al. [8] and Tompkin et al. [94] to suggest