An isotope technique for measuring lactose absorption

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SUMMARY Expired radiocarbon dioxide has been collected by a simple autotitration method following the ingestion of lactose-1-¹⁴C. With this method, which is suitable for clinical use, 12 subjects with alactasia have been readily separated from 24 normals, both groups being defined by strict criteria.

This test, which may be used to measure the absorption of other sugars, is especially suitable for population surveys and may be used to investigate the distribution of disaccharidase deficiency.

A further advantage is that false low readings resulting from rapid plasma clearance of absorbed sugar do not occur with this method although they may do so in up to one in three lactose tolerance tests, thereby overestimating the prevalence of alactasia.

Studies of radiocarbon breath excretion have been used to investigate intermediary metabolism in animals (Godfrey and Snyder, 1962; Tolbert, Kirk, and Baker, 1956) and in man (LeRoy, Okita, Tocus, and Charleston, 1960; Thompson, Buskirk, and Whedon, 1961; Kinney, Morgan, Domingues, and Gildner, 1964). Using the same principle, fat absorption in man (Schwabe, Cozzetto, Bennett, and Mellinkoff, 1962; Cozzetto, 1964; Abt and von Schuching, 1966; Kaihara, and Wagner, 1968) and monosaccharide absorption (Holt and Somersalo, 1966) have also been measured.

In the past the complex equipment required to collect and measure expired radiocarbon-dioxide has not enabled the method to become available for routine clinical use. A simple apparatus has therefore been constructed, based on designs published by Abt and von Schuching (1966), and has been used to measure lactose absorption. By using this method good separation of normal from lactase-deficient subjects is achieved by analysis of the cumulative record of radiocarbon-dioxide.

MATERIALS AND METHODS

The apparatus is shown in Figure 1. It consists of a glass canister, 20 × 7 cm (OD) containing 500 g of self-indicating silica gel. A short length of flexible rubber hose is attached to the upper end via a Pyrex grip-seal joint (B24) and a Ruben non-return valve is interposed between this and a further length of tubing ending in a mouthpiece. A similar joint is present at the lower end of the equipment but has been modified so that expired air passes through a glass inner tube projecting 3.5 cm below the lower flange. A liquid scintillation vial cap

set into an araldite sleeve is attached to the lower flange (3 cm ID). The top of the cap is bored out so that the inner glass tube can pass through and excess air can pass up around it. A liquid scintillation vial containing the reagents is screwed into the cap. The glass tube should then be about 1.5 cm from the bottom of the vial. Expired air will now pass into the vial and escape up around the glass tube and out via the side tube as shown. Radiocarbon-dioxide is trapped by a known amount of accu-

FIG. 1. Apparatus for CO₂ collection in vivo.

FIG. 2. Lactose utilization test: Two curves from the same subject after the ingestion of 25 g and 50 g lactose (+5 μ C lactose-1- 14 C).

rately titrated hyamine hydroxide, using phenolphthalein as indicator. The specific activity of the trapped carbon dioxide is then measured at 5°C in a liquid scintillation spectrophotometer (Nuclear Chicago 720 series). Lactose-1- 14 C (7.5 mc/mM) was obtained from the Radiochemical Centre, Amersham. Patients with lactase deficiency were selected according to the following criteria: (1) history of intolerance to milk; (2) symptoms occurring after ingesting 50 g lactose (any two of diarrhoea, cramping, bloating, borborygmi, or gas flatulence); (3) maximal rise in plasma reducing substances of less than 21 mg per 100 ml after the ingestion of 50 g of lactose; (4) normal monosaccharide absorption (glucose/galactose tolerance test showing a rise of plasma reducing substances of 30 mg per 100 ml or more, after the ingestion of 25 g each of glucose and galactose); (5) jejunal lactase level of less than 2.5 units/g wet wt mucosa.

Normal subjects were selected on the basis of absence of the above criteria, together with a normal follow-through barium meal.

Total plasma reducing substances were measured on a Technicon AutoAnalyzer using a method based on the reduction of potassium ferrioxalate (Hoffman, 1937). Postabsorption blood glucose levels were checked using the glucose oxidase method of Marks (1959). Jejunal disaccharidase estimations were carried out according to the method of Burgess, Levin, Mahalanabis, and Tonge (1964).

CLINICAL METHOD Patients were studied after an overnight fast, at rest, and were not allowed to smoke. After ingestion of 500 ml of 10% lactose (50 g) containing 5 μ C of lactose-1- 14 C the subject breathed into the mouth-piece of the equipment at timed intervals. Before each collection exactly 1 ml of titrated hyamine hydroxide, 1 ml of ethanol, and 3 drops of phenolphthalein (1%) are gently mixed in a liquid scintillation vial which is then attached to the apparatus as shown. The dried expired air

is blown on to the reagents and at the end-point saturation of the hyamine with CO₂ is shown by decolorization of the indicator. After this when collection is terminated it is not critical since further CO₂ trapping does not occur. Each collection takes about three minutes and requires very little 'work' by the patient since the internal resistance of the apparatus is low.

Then 15 ml of scintillant (4 g, 2,5-diphenyloxazole, 0.3 g p-bis 2(5-phenyloxazolyl)-benzene POPOP to each litre of toluene) is added to the vial and the specific activity of the trapped CO₂ measured. All samples were counted in triplicate and readings within 3% of each other were accepted for analysis. Quench corrections were carried out using the pulse height shift method (Baillie, 1960).

EXPRESSION OF RESULTS Expired radiocarbon dioxide may be plotted as a cumulative record but is more conveniently recorded as a curve based on the percentage of the ingested dose expired as radiocarbon dioxide per 800 mM of CO₂. This latter figure represents the average hourly excretion of CO₂ over 24 hours by an adult. This percentage is obtained from the relationship:

$$x = \frac{c \cdot (800/M) \cdot 100}{S}$$
 where c = cpm of sample, M = molarity of hyamine, S = cpm of ingested lactose- 14 C. Figure 2 shows two curves obtained in the manner described from the same patient. On one occasion, however, the subject ingested only 25 g of lactose. Both graphs are similar and have approximately the same area indicating that the subject was able to hydrolyze and absorb 50 g of lactose as well as half this amount. A more objective measurement of the percentage of the oral dose absorbed in normal subjects can be obtained from the following expressions which allow an approximation of the degree of absorption from three measurements only, namely, the two- three- and five-hour figures. For most

FIG. 3. Radiocarbon breath excretion curves (1 to 4 = lactose-deficient subjects; 5 to 9 = normal subjects).

purposes only these three measurements need be made.

$$\sum_0^2 = a \cdot y/S \cdot 100$$

$$\sum_0^3 = \frac{y \cdot (3a + b)/2 \cdot 100}{S}$$

$$\sum_0^5 = \frac{y \cdot (3a + 3b + 2c)/2 \cdot 100}{S}$$

a = cpm/mM CO_2 (2 hr)

b = cpm/mM CO_2 (3 hr)

c = cpm/mM CO_2 (5 hr)

S = cpm/lactose-1- ^{14}C

y = 800 mM

It is apparent that the five-hour figure obtained from these three measurements may overestimate absorption if peak excretion of radiocarbon-dioxide occurs between three and five hours and is therefore not measured. In most cases the peak excretion of radiocarbon dioxide is found to occur between one and three hours (see Fig. 4).

RESULTS

Figure 3 shows the radiocarbon dioxide excretion curves from five normal subjects and four cases of lactase deficiency. The prompt excretion of carbon dioxide reaching a peak at two to three hours is noted. Lactase-deficient subjects are characterized by a low two- to three-hour excretion with a five-hour figure that approaches that found in normal subjects. It may be assumed that this latter value is related to lactose fermentation in the colon with subsequent absorption of small ^{14}C -metabolites, although the possibility that reserve ileal lactase may be con-

tributing is being studied further. Figure 4 shows pooled data from six cases of lactase deficiency and nine normal subjects. The marked difference between the two-hour excretion figures ($p < 0.01$) and the five-hour overlap are again seen.

These data can also be used to separate normal from lactase-deficient subjects by employing the 2:5 hour percentage ratio (Sammons, Morgan, Frazer, Montgomery, Philip, and Phillips, 1967). In order to measure the peak activity in normals the three-hour radiocarbon-dioxide value is also recorded and the three values are then related according to the equations given. Figure 5 shows that this method separates lactase-deficient subjects from (p normals < 0.001).

FIG. 4. Lactose utilization test in normal subjects and in patients with hypolactasia.

FIG. 5. Radiocarbon breath excretion after 50 g lactose + 5 μ c lactose-1- 14 C (mean $\pm$ 1 SD).

DISCUSSION

Lactose tolerance tests have been widely used to diagnose lactase deficiency. The peak rise in blood glucose, however, does not always correlate with jejunal lactase activity. For example, Newcomer and McGill (1966) showed a maximal rise in blood sugar of less than 20 mg per 100 ml in 10 of 36 tests (28%) in 18 healthy subjects with normal jejunal lactase, and a later study (Newcomer and McGill, 1967) in eight out of 33 normal subjects. Capillary blood glucose levels were found to give higher values but were normal in four out of 10 cases of lactase deficiency (McGill and Newcomer, 1967). These

discrepancies are not fully explained but it is clear that many factors apart from jejunal lactase influence the maximum blood sugar obtained. Sugar loading tests are influenced by the rate of hydrolysis, rate of absorption, and rate of clearance from the blood, whereas the lactose utilization test described is not influenced to the same extent by the rate of disappearance of absorbed glucose from the blood since it gives a cumulative record of the absorbed isotope by expressing the expired radiocarbon dioxide as a percentage of the oral dose. Holt and Somersalo (1966) demonstrated almost complete absorption of monosaccharides in children using a similar procedure, even when a flat sugar tolerance curve was obtained. In earlier studies continuous breath excretion was measured but the equipment necessary for this procedure was not generally available. Direct titration of expired CO_2 at timed intervals is a much simpler procedure. The method described may also be used for other sugars. Figure 6 shows the time course for radiocarbon dioxide breath excretion after the ingestion of D-glucose- 14 C (U) and compares it with blood glucose levels. Counts significantly above background may be obtained within 15 minutes and these continue to rise well after the blood glucose has fallen to its fasting value. Factors that may alter the excretion of carbon dioxide in the breath include the patient's nutritional state, metabolic activity, and the total CO_2 pool. In order to standardize conditions the test was performed after a 12-hour fast with the patient resting throughout. Normal subjects in this study were included only if their nutritional status was judged to be normal and in the absence of fever or evidence of respiratory disease or metabolic acidosis. Recycling of 14 C residues may also affect the rate of radiocarbon dioxide excretion. The extent to which this occurs in any one subject is not known. As seen from Table I less than half of the ingested

FIG. 6. Glucose utilization test: the time course for radiocarbon breath excretion after the ingestion of 50 g glucose + 5 μ c D-glucose- 14 C compared with blood glucose levels.

lactose is accounted for by CO_2 breath excretion in the first five hours, even though absorption is almost

of active patient participation. This means that three or four patients may be studied at the same time using only one piece of equipment.

TABLE I

RADIOCARBON BREATH EXCRETION IN NORMAL AND LACTOSE-DEFICIENT SUBJECTS

Mean Age (Range) (yr)	No. of Patients	Radiocarbon Breath Excretion (% Ingested Dose Excreted per 0.8 M CO ₂) (Mean ± 1 SD)				
		M	F	2 Hours	5 Hours	2 to 5 Hours
Normal subjects (31-76)	56.1	8	16	8.22 ± 1.99	35.39 ± 6.29	23.01 ± 2.65
Lactase-deficient subjects (30-75)	55.7	5	7	2.41 ± 1.16	17.65 ± 6.25	13.26 ± 4.1

certainly complete by this time. An extended time course study has confirmed that more than 98% of the ingested isotope (lactose- $1\text{-}^{14}\text{C}$) could be accounted for in terms of $^{14}\text{CO}_2$ excretion by 48 hours. This delay is not due to galactose breakdown since the isotope is tagged to the glucose moiety of lactose.

It is not envisaged that this test will play much part in the diagnosis of sporadic cases of disaccharidase deficiency, except where enzyme assays are not available, or sugar loading tests give equivocal results. The chief advantages are that large numbers of patients can be screened in a relatively short time. Because of this it should be a useful technique for studying the distribution of disaccharidases in large populations. In addition our experience with this test in over 100 patients with a variety of conditions has not produced a single false positive result when compared with the results of the lactose tolerance test. Results from a representative normal subject illustrate this point.

Mrs J. R., aged 36, was investigated for diarrhoea and 'milk intolerance', and the following results were obtained. Lactose tolerance test, peak rise of glucose 10 mg%; glucose/galactose tolerance test, peak rise of glucose 22 mg%; lactose utilization test, 2 hours = 10.46% (normal 8.22 $\pm$ 1.99); jejunal disaccharidases lactase = 7.88 U per g wet weight (normal > 2.5 U); and sucrase/lactase ratio = 1.56 (normal 0.3 to 3.7). The possible reasons for a flat sugar loading test in a normal subject have already been discussed.

Because of the nature of this test it is acceptable to most patients and involves less than 15 minutes

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