Measurement of Fecal C-14 Excretion

Kozhikot A. Kumaran, Stephen N. Wiener, and James B. Katz

The Mt. Sinai Medical Center, Cleveland, Ohio

Simultaneous measurements of fecal C-14 and expired 14CO2 in the breath are necessary to evaluate patients with various ileal abnormalities and bile salt malabsorption. Following the oral ingestion of the labeled bile acid, glycine-[1-14C]cholic acid, detection of increased fecal C-14 without abnormal expiration of 14CO2 identifies patients with ileal resection. This contrasts with the normal fecal C-14 content and abnormal expired 14CO2 found in patients with bacterial overgrowth. Fecal C-14 content was determined by utilizing Van Slyke combustion of the specimen and trapping the liberated 14CO2 with Scintisorb C. The method is simple, rapid, and accurate, and expands the diagnostic usefulness of the bile salt absorption test.


Measurement of the 14CO2 in the expired air following oral administration of the bile acid glycine-[1-14C]cholic acid is helpful in the diagnosis of altered bile salt metabolism (1,2). Improved detection of bile acid malabsorption can be achieved by the simultaneous determination of C-14 excretion in both expired air and in feces; the measurements allow bile acid malabsorption due to ileal disease to be differentiated from bacterial overgrowth due to small-intestinal stasis (1,3). While sampling of 14CO2 in expired breath has been shown to be simple and convenient (4), the determination of C-14 activity in the stool has been difficult, time-consuming, and expensive. The following method has been found to be accurate and rapid, and appears to solve problems inherent in previously described techniques.

MATERIALS

Potassium iodate (granular powder); chromic anhydride; ascarite; drierite or anhydrous magnesium perchlorate; gum cellulose (0.4% solution); fuming sulfuric acid (20% free SO3); phosphoric acid (85%); Scintisorb C*; glycine-[1-14C]cholic acid, 95% alcohol; anhydrous P2O5.

Preparations of the following reagents were carried out in a fume hood with appropriate caution.

A. Syrupy phosphoric acid. Measure 335 ml phosphoric acid (85%) into a 2-l heavy-duty Pyrex beaker placed on a magnetic stirrer. Using a spatula, weigh 100 g P2O5 (anhydrous) in a 500-ml Pyrex beaker using a triple-beam balance. Transfer the P2O5 in small portions into the phosphoric acid solution, with stirring. Caution must be observed because this reaction is exothermic, although the amount of heat generated during the reaction helps to dissolve the P2O5. When all the P2O5 is dissolved, the solution is cooled to room temperature and filtered through glass wool into a glass bottle for storage.

B. Van Slyke-Folch combustion fluid (5). Carefully weigh 50 g chromic anhydride in a 500-ml beaker using a triple-beam balance, and transfer into a one-liter Pyrex Erlenmeyer flask provided with a ground-glass stopper. Add 167 ml syrupy phosphoric acid to the flask, followed by 333 ml fuming sulfuric acid. Leaving the stopper off, heat the mixture on a hot plate to 140° to 150°C while gently rotating the flask at times to assist the solution of chromic anhydride. When 150° has been reached, stop heating, cool the solution to room temperature, and insert the glass stopper.

EQUIPMENT

The required equipment1 and schematic diagram of the assembly are shown in Fig. 1.

MEASUREMENTS

Informed consent was obtained from all patients before the test. After an overnight fast of ~8–10 hr, each patient ingested a gelatin capsule containing 5 μCi of glycine-[1-14C]cholic acid along with a glass of water. Twenty-four-hr stool was collected in a weighed gallon paint can as described by Gordon (6). (When a 72-hr-stool is collected for quantitative fecal fat determination, the glycine-[1-14C] cholic acid capsule is administered on the third day of stool collection for the breath test and subsequent 24-hr C-14 fecal excretion measurement.) Collection and measurement of the expired 14CO2 and the collection of breath were accomplished by the previously described method (2,4).

The stool collection was weighed and 100 ml 0.4% gum cellulose solution was added, along with sufficient water to make the mixture up to 1000 g. One liter of 95% alcohol was then added and the total weight of the mixture determined. The contents were homogenized by shaking for 20 min in a paint shaker. Immediately following that, about 2-ml portions of the homogenate were transferred in duplicates to weighed combustion tubes. The wet

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For reprints contact: K. A. Kumaran, GI Lab, The Mt. Sinai Medical Center, University Circle, Cleveland, OH 44106.
weight of the specimen in the tube was determined to the nearest 10 mg. The specimen was made alkaline by adding 1 ml 0.1 N NaOH, then evaporated to dryness in a water bath at 80°-90°C using a stream of air.

Combustion of the specimen as prepared above was performed by adding 300 mg potassium iodate to the specimen in the combustion tube (Fig. 1). The cleaned and dried joints as well as the stopcock in the condenser were well lubricated using a small amount of the previously described syrupy phosphoric acid delivered by a Pasteur pipet. No other type of lubricant is recommended for this purpose. The condenser was connected to the combustion tube. The stopcock was closed and ~10 to 15 ml of Van Slyke-Folch combustion fluid was poured into the funnel. Each of three bubblers, connected in series by silicon tubing, was filled with ~4-ml portions of Scintisorb C solution. The bubblers were attached to the drying tube. The combustion fluid was introduced into the specimen tube by opening the stopcock and applying a small amount of air pressure at the top of the funnel using a rubber bulb. The stopcock was closed and the solution was slowly brought to a boil, then boiled vigorously for one minute. (Cooling by circulating water through the condenser was not required.) The stopcock was then connected to the ascarite column and the system was aerated for 5 min. The valve was adjusted to provide for the passage of three to five bubbles per second.

The Scintisorb solution in the first bubbler was transferred into a scintillation vial. This bubbler was rinsed using the solutions in the second and third bubblers, which were again rinsed out with 3 ml fresh Scintisorb solution. The vials were counted in a scintillation counter. Fifteen ml of fresh Scintisorb solution was used for background counts. Percent excretion of C-14 in the stool was calculated as follows:

\[
\text{% excretion} = \frac{\text{dpm}}{\text{DPM}} \times \frac{W}{w} \times 100
\]

where dpm is the counts in the burned specimen after correcting for the background and counter efficiency; DPM is counts in the initial 5 μCi radioactivity; W is the total wet weight of the stool homogenate; and w is the wet weight of the stool specimen burned.

RESULTS

Data showing the accuracy and efficiency of the method are shown in Table 1. One capsule of 5 μCi glycine-[1-14C]cholic acid was homogenized with a 24-hr stool collection. The final weight of the homogenate was 1800 g. Approximately two-gram portions of the homogenate were used for the determination.

Previously described laboratory methods (7,8) for measuring C-14 in the stool are not easily applied to routine clinical testing since they require dried powdered stool to be burned in an oxygen atmosphere in a special chamber using electrical ignition. The 14CO2 evolved is absorbed in a measured amount of absorbant and the radioactivity counted in a scintillation counter. Though the method was found efficient, it is time-consuming and requires considerable expertise. As a result, most investigators have not measured C-14 in the stool.

The method described here is simple and rapid, and can be performed by a chemist in most hospital chemical laboratories. Excellent recovery is obtained, and the method is reproducible (Table 1) and linear. Total time required for combustion is about 15 min, and repeated determinations can be performed using the same apparatus by simply changing the bubblers and specimen tube. The optimum quantity of specimen for combustion appears to be about 2 g wet weight (150 mg dry weight). At amounts greater than approximately 3 g, the Scintisorb solution became supersaturated and formed crystals upon refrigerated storage at 5°C. Except for the weighing of the specimen, no other critical measurements are involved in this method. Use of an efficient 14CO2 absorbant such as Scintisorb C further simplifies the procedure. The 4-ml portion of Scintisorb solution in the first bubbler was found to absorb 90-95% of 14CO2 from the oxidation of the specimen. Little or no radioactivity was detected in the solution from the third bubbler. Thus, total recovery of absorbed 14CO2 was accomplished by rinsing the first bubbler with the solutions in the second and third.

The method described can also be used for determining C-14 in other body fluids. Carbon-14 activity has been measured in bile obtained from patients during a secretin test for pancreatic function. Altered bile acid metabolism due to bacterial overgrowth results in an increased 14CO2 specific activity in the breath, along with normal C-14 excretion in the stool. Patients with bile salt malabsorption may show only an increased fecal C-14 content. Simultaneous measurements of the C-14 in stool and in the breath are needed if one is to broaden the diagnostic usefulness of the test and to discriminate between these groups of patients.

![Figure 1: Equipment and schematic diagram of combustion apparatus.](image)

**TABLE 1. FECAL C-14 MEASUREMENT**

<table>
<thead>
<tr>
<th>Wet weight of specimen (g)</th>
<th>CPM-blank</th>
<th>DPM</th>
<th>Recovery (%)</th>
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<tr>
<td>1.85</td>
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<td>1.60</td>
<td>6,079</td>
<td>9,951</td>
<td>101.8</td>
</tr>
</tbody>
</table>

FOOTNOTE

* Isolab Inc., Akron, Ohio.
† Andrea Scientific Glass Works, Geneva, Ohio.

REFERENCES


Southwestern Chapter
Society of Nuclear Medicine
28th Annual Meeting

March 17–20, 1983
Lincoln Plaza
Oklahoma City, Oklahoma

Announcement and Call for Abstracts

The Scientific Program Committee of the Southwestern Chapter of the Society of Nuclear Medicine invites submitted abstracts of original work in Nuclear Medicine from members and nonmembers of the Society of Nuclear Medicine to be considered for the 28th Annual Meeting to be held March 17–20, 1983 at the Lincoln Plaza in Oklahoma City, Oklahoma.

The program will include submitted scientific papers, invited speakers, and teaching sessions covering areas of current interest in Nuclear Medicine. The program will be approved for credit toward the AMA Physicians Recognition Award under Continuing Medical Education Category 1 through the Society of Nuclear Medicine.

Scientific exhibits also are solicited for this meeting. Use the abstract submission guidelines listed below. Descriptions of the exhibits, including size, shape, and necessary lighting and support requirements should be listed on a separate sheet. Exhibits will be judged on scientific content in the technologist and professional level categories.

The Southwestern Chapter 5th Annual Nuclear Medicine refresher course will be held March 17, 18, 1983. The course will include reviews of basic science, instrumentation, radiopharmaceuticals and in vitro and diagnostic imaging techniques. Nuclear Medicine Scientists, Technologists and Physicians interested in a state of the art review are invited to attend.

Abstract forms may be obtained from:

Southwestern Chapter
1209 Lair Avenue
Metairie, LA 70003
Tel: (504) 733-0063

Abstracts must be received in Chapter Office by Dec. 1, 1982 (Postmark)

Additional information may be acquired from:

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