Procedural Notes on an Intestinal Fat Absorption Test as Determined by the Excretion of $^{14}CO_2$

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An innovative method for the measurement of intestinal fat absorption with ^{14}C-labeled tripalmitate is being investigated. Five microcuries of glyceryl ^{14}C-tripalmitate are administered orally. The maximum specific activity of the $^{14}CO_2$ contained in the patient's expiration at 3–5 hr is directly proportional to the intestinal absorption of fat. It is hoped that this test will provide an inexpensive, technically simple, yet sensitive, alternative to present 24- and 48-hr collected fecal fat determinations. Since nuclear medicine departments are being asked to make this test available on a routine clinical basis, this paper is intended to aid the technologist in establishing such a procedure.

In 1966 Abt and von Schuching *(1)* developed a clinical test that gave information about a patient's ability to absorb fats. Rather than administering radioactive long-chain fatty acids intravenously in order to study the kinetics of fat metabolism, they administered the fat orally. Their intent was to monitor the $^{14}CO_2$ excreted due to the metabolic breakdown of the radioactive fat. They showed that the specific activity of carbon dioxide in expired air is a valid measure of the adequacy of intestinal absorption of fat, specifically, glyceryl ^{14}C-tripalmitate.

In 1967 Kaihara and Wagner *(2)* measured the maximum specific activity of $^{14}CO_2$ in the expired air 3–5 hr after the administration of 5 μCi of the tripalmitin to a patient who had fasted for 12 hr. Following their method, the patient blows through a tube containing calcium sulfate into a collection vial. The $CaSO_4$ absorbs the moisture in the patient's breath. The collection vial contains hyamine hydroxide, a monoalkalide, which absorbs carbon dioxide. The carbon dioxide neutralizes the base as indicated by the decoloration of phenolphthalein. The trapped $^{14}CO_2$ is then counted in a liquid scintillation counter and compared to the amount of activity ingested. Kaihara and Wagner correlated their findings with the chemical determination of fat in feces and blood, showing that the results of this test are useful in corroborating the diagnosis of a patient with either intestinal malabsorption or chronic pancreatitis.

Materials

Glyceryl ^{14}C-tripalmitate is commercially available from Amersham/Searle, Tracerlab, or New England Nuclear. It can be prepared by means of the esterification of ^{14}C-palmitic acid with glycerol *(3)*. The Amersham/Searle Co. produces 100-μCi quantities dissolved in arachis oil to a concentration of approximately 100 μCi/ml. Thin-layer chromatography in a system of cylcohexane/ethyl acetate (4:1) shows the radiochemical purity of the compound to be 98%. To provide for greater accuracy in preparing the administered doses, the tripalmitate is diluted with 6–10 ml of Dunwody's USP mineral oil. This allows for an administered volume of 0.3–0.5 ml of fat for each of 20 doses. The doses are put into gelatin capsules and refrigerated.

A 1-*M* hyamine hydroxide in methanol solution was obtained from Amersham/Searle. It is kept tightly sealed and refrigerated. The stock solution is standardized by titration with 0.1 *M* HCl. One milliliter of the hyamine is diluted to 10 ml with methanol. A drop of 1% by weight of phenolphthalein in ethanol is added. (Molarity of the hyamine hydroxide) = (milliliters HCl used in titration × 0.1 *M* HCl)/(1.00 ml of hyamine used.) Titration with the HCl usually yields 0.90–0.95 *M*.

Scintillation cocktail was made with 1 liter Scintillar grade toluene, 4 gm 2,5-diphenyl oxazole (PPO), and 100 mg of 1,4-bis [2-(5-phenyl oxazole)] benzene (POPOP). Fifteen milliliters were used per vial.

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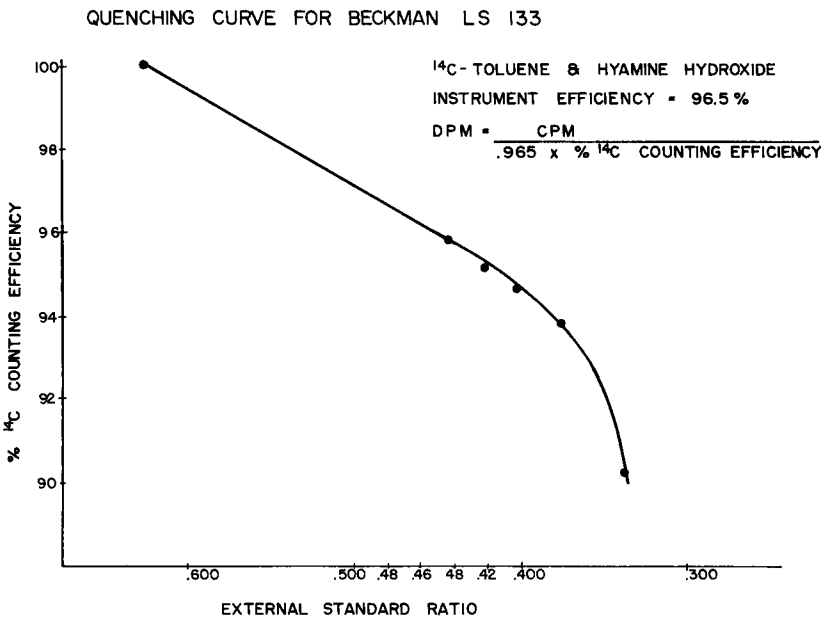

FIG. 1. Quenching curve prepared for use with hyamine-methanol carbon dioxide collection system.

Low-potassium disposable scintillation vials were obtained from Kimble.

A Beckman LS-133 liquid scintillation counter with an efficiency of 96.5 ± 0.3 (1 s.d.)% was used to count the samples. A sealed, argon-purged ^{14}C standard, provided by Beckman, was used with each counting, as was a duplicate of the neutralized system used to collect the patient's CO_2. The latter served as a background count. The Beckman LS-133 has an external standard ratio that is indicative of the degree of quenching. A quenching curve was prepared (Fig. 1) for use with the hyamine-methanol carbon dioxide collection system. A quantity of the ^{14}C-tripalmitate (dilute enough so as to have negligible chemical quenching) was counted. To this, increments of hyamine hydroxide were added. The external standard ratio was plotted as a function of the counting efficiency. A smaller total volume of chloroform or water can be used so that the geometry remains more nearly constant.

The liquid scintillation counter was set to a limit of 50 min or to 0.5% error-equivalent to 140,000 counts. All of the vials, including those with very low activity, were counted to within a 3% error.

Calculations

The results are expressed as a percent of the administered dose per millimole of carbon dioxide as described by Kaihara and Wagner *(2)*.

$$\%\ \text{dose/mmole}\ CO_2 = \frac{\text{dpm of sample}}{\text{dpm administered}} \times \frac{1}{\text{molarity of hyamine}} \times 100.$$

This assumes nothing about the rate of expiration of individual patients. The maximum value of the three samples (3,4, and 5 hr after administration of the fat) was used as an indicator of intestinal absorption of fat.

Procedural Notes

Because of the viscous nature of the fat, it is difficult to deliver an accurate, reproducible aliquot of the tripalmitate. Table 1 provides a comparison of the delivery of mineral oil by an automatic pipette and by a 1.0-ml volumetric glass pipette. It is clear that no method of pipetting a viscous fat will deliver 100% accuracy unless the pipette is washed with an organic solvent. The percent error in a given dose would range from 1.2% (0.5 ml by glass pipette) to 9.0% (0.5 ml by automatic pipette). This takes into account the error in a given dose plus the error in a standard dose to be counted at the time of the test.

The traditional method of counting a similarly prepared standard dose and equating it with the

Table 1. Comparison of the Delivery of Mineral Oil

Method	Volume desired (ml)	Volume delivered* (ml)	s.d. (n=6)	Accuracy (%)+ (n=6)	Precision (%)† (n=6)
Automatic pipette (Becton-Dickinson)	0.3	0.266	0.0041	88.7	3.1
	0.5	0.442	0.0100	88.4	4.5
Volumetric glass 1.00-ml pipette	0.3	0.264	0.0023	88.0	1.7
	0.5	0.452	0.0014	90.4	0.6

* *Volume by weight as determined by the density @ 20° C of the mineral oil, (0.886 gm/ml).*

\+ *Accuracy is defined as volume delivered over volume desired expressed in percent.*

† *Precision is defined as 2 s.d. over the volume delivered expressed in percent.*

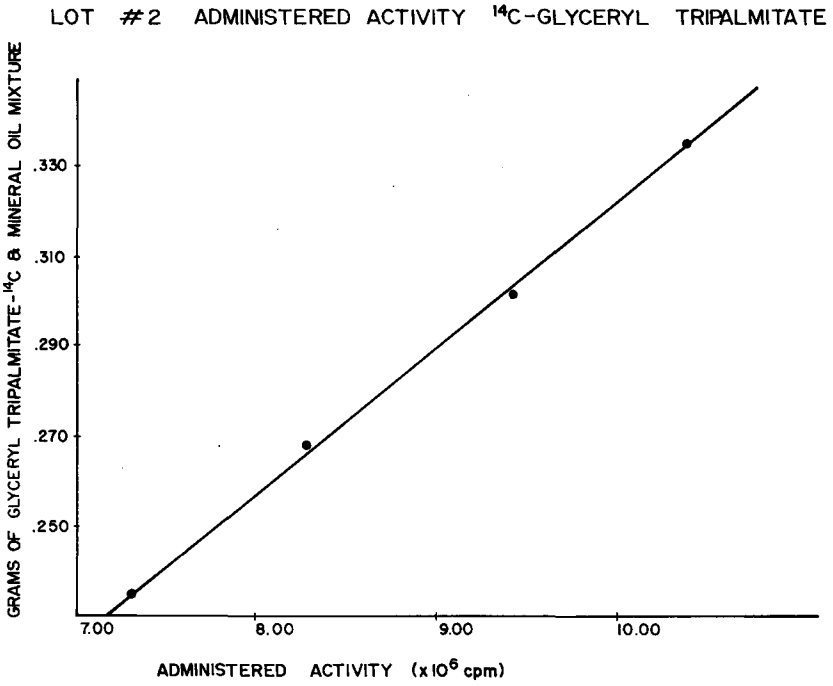

FIG. 2. Graph used to determine administered dose after activity per milligram is converted to activity per gram.

patient's dose has several disadvantages. In addition to the error in delivery, a standard dose of fat of between 400 and 500 mg dissolved in 15 ml of scintillation cocktail will cause significant chemical quenching. Furthermore, 5 μCi of ^{14}C is a greater activity than most liquid scintillation counters can count. Considerable risk of counter contamination exists when that active a standard is used.

Clearly an indirect method for determining the administered dose is preferred. The activity in the administered dose can be calculated by accurately measuring the weight of the dose and the specific activity of the fat. Empty gelatin capsules are numbered and weighed to the fourth place. The glyceryl tripalmitate, diluted with mineral oil, is dispensed in about 5-μCi doses into these capsules, which are then reweighed. The error in the weight per dose is less than one-tenth of 1%. The specific activity of the fat can be determined by weighing a 50-ml volumetric flask, adding a known amount of tripalmitate-mineral oil mixture (preferably 50 mg), and then diluting with scintillation-grade toluene. The desired concentration is about 1 mg/ml since this provides sufficient activity for accurate statistical counting in a short period of time (less than 10 min per vial) in a volume of fat so small as to minimize chemical quenching. Using a volumetric glass pipette to obtain various volumes and knowing the concentration of the solution one can obtain the specific activity of the glyceryl tripalmitate-mineral oil mixture. After converting activity per milligram to activity per gram, a graph (Fig. 2) can be used to determine the administered dose. If the slope of this graph is 1 (the activity per gram is constant regardless of the amount of tripalmitate), there is no quenching and the administered dose need only be corrected for machine efficiency.

The actual apparatus for collecting the expired CO_2, as noted in Fig. 3, is very simple. Although there are many more elaborate systems, a drying tube with indicator $CaSO_4$ and two straws was

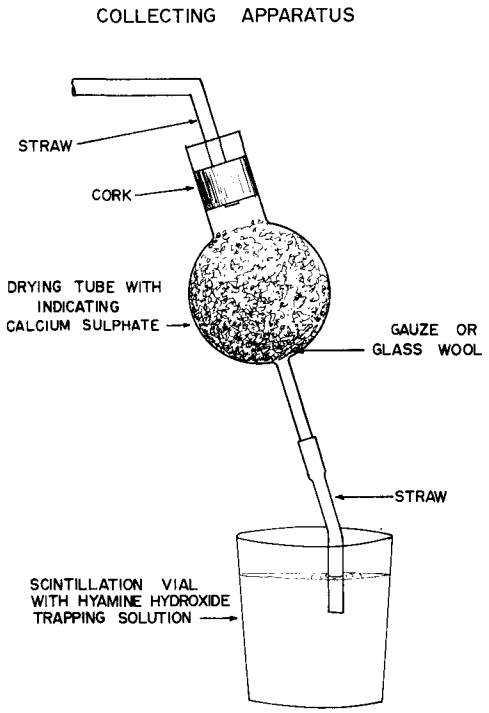

FIG. 3. Apparatus for collecting expired CO_2.

found to work more than adequately. If plastic straws are used and can be disposed of after each of the three uses, there is little risk of cross-contamination of the vials. Collection of the $^{14}CO_2$ by breathing over the solution and by inserting the straw into the solution yields similar results. The advantage to immersing the straw into the hyamine solution is that decoloration of the indicator occurs in most patients in 1–1.5 min compared with 4 or 5 min.

Kaihara and Wagner *(2)* showed that decoloration of the phenolphthalein indicator marks the maximum activity that the solution will collect. Further breathing into the collection system will have no effect.

The limiting factor in achieving accuracy and reproducibility of the collection system is the delivery of the hyamine hydroxide. Because it is a monobasic alkali, 1 ml of 1 *M* hyamine hydroxide (or 1 mmole) will be neutralized by exactly 1 mmole of CO_2.

Figure 4 shows the linear relationship between the error in the delivery of the hyamine and amount of radioactive carbon dioxide collected. A comparison of the delivery of hyamine hydroxide by automatic pipette and a volumetric pipette shows that they behave similarly (with an error in precision of about 3.5%). It is suggested that, regardless of which instrument is used, the same one be used when standardizing the stock solution.

The patient should abstain from eating for 12 hr before the test. Abt and von Schuching *(1)* administered the tripalmitin with either butter on a slice of bread or with cottonseed oil and milk. Kaihara and Wagner *(2)* administered it with 1 gm of corn oil per kilogram of body weight. The effect of a test meal in addition to the corn oil was insignificant. An alternative is to administer the tripalmitin in a capsule and allow the patient to eat a normal breakfast immediately thereafter. This is a matter of concern as studies with rats have shown that carbohydrates given with fat have inhibited the conversion of fat to CO_2 *(4,5)*. The best method is to administer the tracer dose of fat with a fat carrier.

Results and Conclusions

Kaihara and Wagner *(2)* found that 42 normal patients with fecal fat of less than 6 gm/day had an average value of 39.5 ± 12.0 (1 s.d.) × 10^{-4}%/mmole CO_2. Twenty patients with malabsorption or neomycin-induced steatorrhea had an average of 14.7 ± 5.8 (1 s.d.) × 10^{-4}%/mmole CO_2.

Preliminary results of this lab show six control subjects without gastrointestinal disorders to have an average value of 67.1 ± 7.9 × 10^{-4}%/mmole CO_2. Four patients with a malabsorption syndrome have averaged 25.8 ± 4.3 × 10^{-4}%/mmole CO_2. Fecal fat studies correlated well with our controls. Increased fecal fat was noted in those cases with a malabsorption syndrome, although it was not quantitated.

Our excretion values were higher than the values of Kaihara and Wagner, probably reflecting separate standardization. Until a radiopharmaceutical company standardizes this study, each new laboratory should run a series of normal controls. As with any experimental radioactive drug, approval must be obtained from the hospital committee that deals with drugs for human use and from the local radiation safety officer.

The technologist should ensure that each consul-

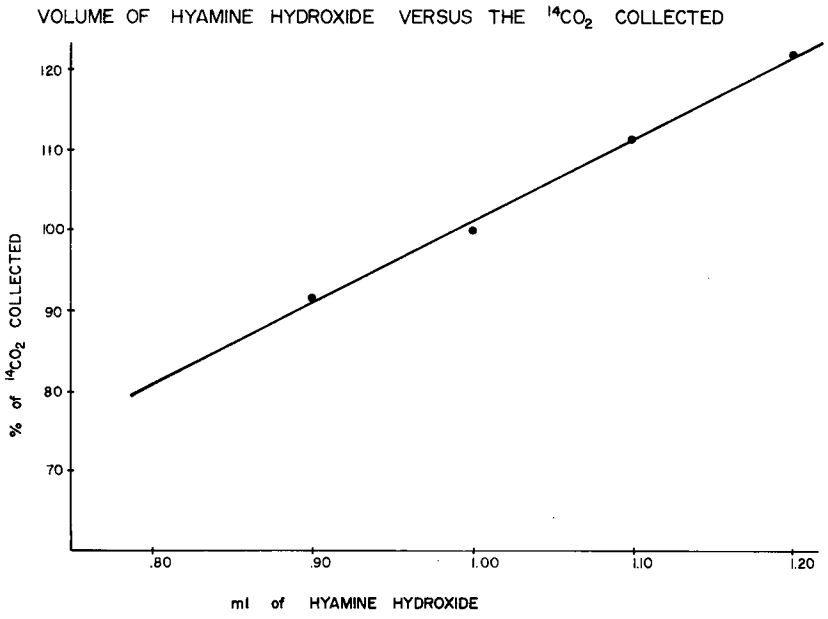

FIG. 4. Graph shows linear relationship between error in delivery of hyamine and amount of radioactive carbon dioxide collected.

tation request has a full history because the interpreting physician cannot make a decision without this information. In our series, normal CO_2 excretion values were obtained in a patient noted to have high stool fat. This case had an ileal resection with consequent bile salt loss. However, the amount of bile salts present was adequate to absorb the test dose of radioactive fat (without a fat carrier) but was not adequate to absorb the 100 gm of fat given in the diet to test for steatorrhea. Another patient had normal values of tripalmitate excretion at 3 and 4 hr, and then the value decreased to an abnormally low level at the 5-hr sample. This man had received 40 units of NPH insulin for his diabetes just before the study which may have altered absorption and metabolism of the radioactive triglyceride.

Acknowledgments

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