Method for collection and determination of $^{14}CO_2$ for in vitro metabolic studies

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SUMMARY A metabolic flask is described which allows for the rapid and periodic collection of $^{14}CO_2$ in a liquid scintillation counting vial.

KEY WORDS $^{14}CO_2$ determination · liquid scintillation · cellular metabolism · in vitro

A NUMBER OF TECHNIQUES have been developed for the collection and subsequent assaying of $^{14}CO_2$ liberated during metabolic studies in vitro (1–6). Although these methods have produced accurate and significant data, they have been limited in respect to their versatility and applicability. The method here described allows for the direct trapping of $^{14}CO_2$ in scintillation counting vials without any subsequent transfers such as would be encountered in previously described methods (1, 2, 4–6). Snyder and Godfrey have pointed out the difficulty encountered in transferring $^{14}CO_2$ from a Warburg flask to a counting vial (1). Previous techniques do not permit repetitive determinations of $^{14}CO_2$ evolved by the same sample, because of the design of the assembly and the need for a prolonged $^{14}CO_2$ collection period (1, 2, 6). The method described allows for rapid and multiple collections of evolved $^{14}CO_2$.

The metabolic flask (Fig. 1) consists of the following: (*a*) a 50 ml Erlenmeyer flask onto which has been fused the threaded portion of a liquid scintillation vial (T. C. Wheaton Co., Millville, N.J.); (*b*) a Teflon adapter (1.8 cm I.D.), made to our design by Kontes Glass Co., Vineland, N.J., which can screw onto the metabolic flask and allows for the attachment of a scintillation vial which serves as the trapping chamber for $^{14}CO_2$; (*c*) a serum cap to facilitate the addition of various solutions to the metabolic flask; and (*d*) a slip of Whatman 40 filter paper (approximately 1.5 × 4.0 inches), soaked with 0.6 ml of 10% KOH, placed inside the collection vial.

The $^{14}CO_2$ trapping efficiency of the system was determined by adding a standard $NaH^{14}CO_3$ solution to Krebs-Ringer phosphate buffer medium (pH 7.4). One milliliter of a 62.5% citric acid solution (7) was then injected into the metabolic flask, and the evolved $^{14}CO_2$ was determined at intervals from 5 to 60 min thereafter. All experiments were conducted at 37°C and the metabolic flasks were shaken at 60 cycles/min.

After the collection of $^{14}CO_2$ in the scintillation counting vials, all samples were dried overnight in a vacuum desiccator over phosphorus pentoxide in an attempt to minimize the quenching found with aqueous systems (7). To each vial was added 20 ml. of a scintillation solution containing 0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis[2-(5-phenyloxazolyl)]-benzene in toluene, and the radioactivity was determined.

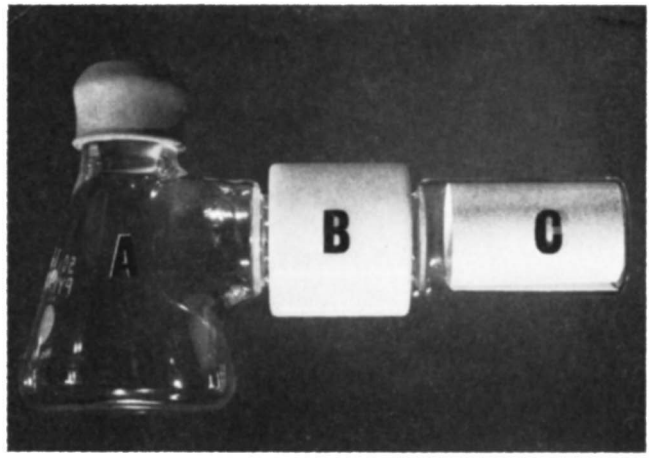

FIG. 1. Metabolic incubation flask for the collection of $^{14}CO_2$. *A*, 50 ml Erlenmeyer flask onto which is fused the threaded portion of liquid scintillation vial; *B*, Teflon adapter; *C*, scintillation vial (trapping chamber) containing filter paper soaked with 10% KOH.

Since the distance for the passage of the CO_2 from the gaseous phase of the metabolic flask to the trapping surface of the filter paper is short and the internal diameter of the adapter is large, essentially all the evolved $^{14}CO_2$ was trapped at 15 min (Table 1). The time necessary for quantitative recovery of $^{14}CO_2$ with this method is significantly less than has been previously reported (1, 6).

Because of the design of the flask, one can easily remove the scintillation vial containing the trapped $^{14}CO_2$ and immediately replace it with another scintillation vial at various time intervals during an experimental procedure. When this removal–replacement procedure was employed, the cumulative recovery in three separate

TABLE 1 RECOVERY OF $^{14}CO_2$ FROM A STANDARD SODIUM CARBONATE SOLUTION AS A FUNCTION OF TIME*

Time	Number of Samples	Recovery
min		%
5	3	39.2 ± 2.7
10	6	74.6 ± 2.3
15	3	93.5 ± 3.9
20	8	93.8 ± 2.7
30	5	95.4 ± 1.8
45	3	95.8 ± 2.4
60	3	96.6 ± 2.8

* Values are expressed as means ± SEM.

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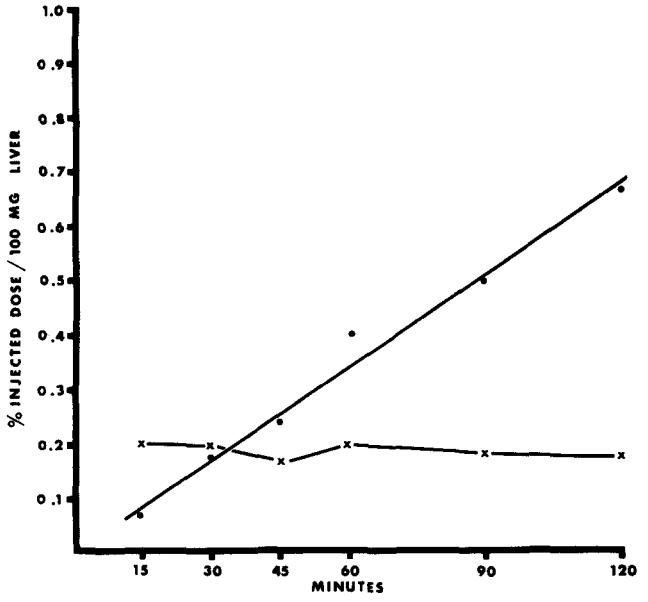

FIG. 2. Evolution of $^{14}CO_2$ by rat liver slices incubated with albumin-bound palmitic acid-1-^{14}C as a function of time (●), and the quantification of $^{14}CO_2$ evolved after the subsequent addition of citric acid solution to the incubation medium (x). Each point represents a mean of three determinations.

experiments at 10, 20, and 30 min was 70.6 ± 2.7, 91.3 ± 0.5, and 103 ± 1.2%, respectively. These values are similar to those observed when the vial was not replaced during the course of the experiment (Table 1). Thus, no detectable loss of activity was observed when the trapping chamber was replaced during the course of the experimental procedure.

The validity of employing the repetitive determination of $^{14}CO_2$ on a biological system was also evaluated. Eighteen rat liver slices obtained from three different rats were individually incubated in 5 ml of Krebs-Ringer phosphate buffer supplemented with 0.2 ml of normal rat plasma labeled with 1 μc of albumin-bound palmitic acid-1-^{14}C. The specific activity was 8.5 μc/μeq of free fatty acids. The albumin-bound palmitic acid was prepared with normal rat plasma according to the procedure of Borgström and Olivecrona (8).

At each time interval studied, i.e. 15, 30, 45, 60, and 120 min, the vial containing evolved $^{14}CO_2$ was removed and replaced with a new one. Citric acid solution was then injected, and the $^{14}CO_2$ previously trapped in solution was evolved and subsequently trapped in the new vial during an additional 15 min incubation. Since the incubation medium rapidly attained a constant radioactivity (Fig. 2), the evolved $^{14}CO_2$ is an excellent index of $^{14}CO_2$ production. The evolved $^{14}CO_2$ manifested a linear relationship with time during the 2 hr incubation period.

Repetitive determinations of $^{14}CO_2$ may be made on the same liver slice since under the conditions of the experiment the released $^{14}CO_2$ at the end of the experiment reflects the $^{14}CO_2$ activity of the medium throughout the experimental procedure, i.e., 15–120 minutes. This constant can be added to the evolved $^{14}CO_2$ trapped from the gas phase at each time interval and total $^{14}CO_2$ production can be quantitatively determined. Under different experimental conditions it would be necessary to ascertain the amount and constancy of $^{14}CO_2$ dissolved in the employed medium.

Since the time required to replace the scintillation vial is small and no subseqent transfers are involved in the determination of $^{14}CO_2$ activity, one can readily conduct simultaneous metabolic studies on relatively large numbers of samples.

An earlier version of this system has been employed in evaluating a variety of metabolic events relative to the metabolism of glyceryl tripalmitate-1-^{14}C by liver cells incubated in plasma or Krebs-Ringer phosphate buffer (9). In this study, the employment of a solution of ethanolamine in ethylene glycol monomethyl ether to trap the carbon dioxide (10) permitted the determination of evolved $^{14}CO_2$ production during the course of the experiment, i.e., without the necessity for overnight drying.

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