Collecting $C^{14}O_2$ in a warburg flask for subsequent scintillation counting

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» This report describes an improved technique for the collection of $C^{14}O_2$ in a Warburg flask and for the subsequent transfer of the radioactive material for liquid scintillation assay. The CO_2 absorbent used in this work was Hyamine®,[1] which is a high-molecular-weight quaternary amine. Passmann *et al.* were the first to use this substance in collecting $C^{14}O_2$ for liquid scintillation counting, and their article describes some of the important characteristics of Hyamine, for example, its CO_2 absorbing capacity and its effect on counting efficiency (1).

Before we arrived at the method which is subsequently described in detail, we made two unsuccessful attempts to transfer the $C^{14}O_2$ from a Warburg flask to a counting vial. The use of a connecting diffusion tube (9 or 7 mm i.d.) gave poor recoveries (approximately 80% in 3 hours and 90% in 14 hours, after shaking at 37°), and a second procedure, in which the Hyamine was injected into the center well or side arm of the Warburg flask and an aliquot of the solution transferred directly to the vial, was not quantitative because of volume changes during the diffusion period. However, the use of a removable vessel in collecting the CO_2 gave very good recovery and minimized the equipment needed and the possible errors inherent with the other methods. We have used this technique successfully in a preliminary study of the oxidation of C^{14}-labeled fatty acids.

Figure 1 illustrates the arrangement of a Warburg flask for collecting the labeled CO_2. The incubation mixture in the main compartment consists of 5 ml Krebs-Ringer bicarbonate buffer gassed with 95% O_2, 5% CO_2, C^{14}-labeled substrate, and the enzyme system. The side arm contains 0.3 ml 6 N H_2SO_4. The vessel positioned on top of the center well is empty during the enzymatic incubation but, at the termination of the incubation, the vessel serves as a receptacle for 0.5 ml of the hydroxide of Hyamine which is injected from a tuberculin syringe through the rubber cap covering the flask. Once the Hyamine has been injected, the sulfuric acid is poured into the main compartment to release the carbon dioxide from the solution. After allowing the flask to shake in a Dubnoff shaker for 3 hours at 37°, the vessel containing the Hyamine (and carbon-14) is transferred *in toto* to a glass counting vial that contains 5 ml of 0.4% PPO (2,5-diphenyloxazole) and 0.01% POPOP (1,4-bis-2-[5-phenyloxazolyl]-benzene) in toluene. The counting vial is capped, the contents are mixed, and the radioactive content of the solution is measured with a liquid scintillation spectrometer. The presence of any volatile compounds in enzymatic studies might invalidate interpretations based on $C^{14}O_2$ collected in this manner.

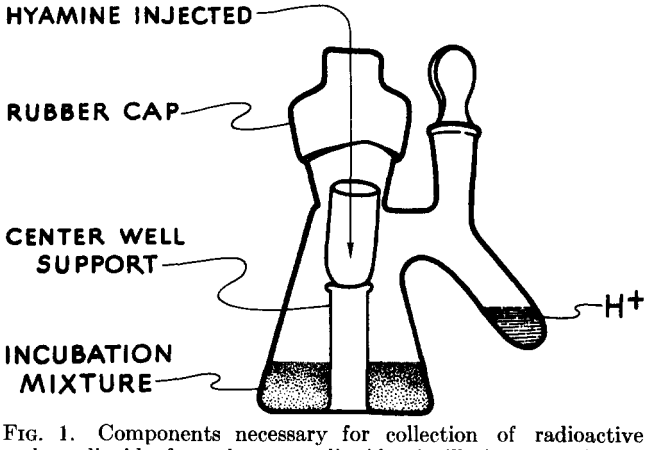

FIG. 1. Components necessary for collection of radioactive carbon dioxide for subsequent liquid scintillation counting.

The recovery of $C^{14}O_2$ from a standard sodium bicarbonate solution was 94.5%, 97.1%, and 98.1%, respectively, at 1, 2, and 3 hours after addition of the acid to the main compartment. The reproducibility of this method can be seen from results obtained with four samples treated in the manner described: 4485, 4441, 4443, and 4439 cpm. The standard deviation is 0.49% of the mean value. The glass vessel, prepared from 10 mm (i.d.) pyrex tubing, did not affect the counting characteristics of carbon-14 in this system. Although Primene (2) was not used in these experiments, it presumably could be used as a less expensive substitute for Hyamine in collecting the carbon dioxide.

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[1] Trade name for 1 M *p*-diisobutyl-cresoxyethoxyethyl dimethylbenzylammonium hydroxide in methanol, obtained from Rohm & Hass Co., 712 Locust Street, Philadelphia 5, Pa.

REFERENCES

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