CONTINUOUS MEASUREMENT OF $^{14}CO_2$ BY LIQUID SCINTILLATION COUNTING

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I. INTRODUCTION

Radioactivity of $^{14}CO_2$ breathed out by an animal may be measured by liquid scintillation, after absorption of CO_2 in a basic liquid such as 10N KOH (Bruce, Newman, Pinchbeck, 1968), barium hydroxide (Major, Burczak, Kellogg 1979; Turner 1969), Hyamine-10X (Passman, Radin, Cooper, 1956; Godfrey, Snyder 1962) phenylethylamine (Woeller, 1961) or by low-temperature condensation (Horrocks 1968, Barendsen 1957).

These techniques involve the radioactivity measurement of samples obtained. For kinetic studies, which give many samples, it is easier to have a continuous radioactivity measuring system such as an ionization chamber (Chevallier 1962) through which $^{14}CO_2$ passes. The gas may also be trapped in a hyamine scintillation fluid and the increasing radioactivity of this mixture measured continuously by circulation through a coiled tube placed between the two-photomultiplier tubes (Suzuki, *et al.*, 1973).

The system presented here, allows continuous measurement of the radioactivity of $^{14}CO_2$; the gas is bubbled through a liquid scintillation cocktail enclosed in a special vial, which is placed directly in the detection chamber of a liquid scintillation counter.

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ISBN 0-12-549902-7

II. EXPERIMENTAL

A. Apparatus

A complete diagram of the experimental system is shown in Figure 1.

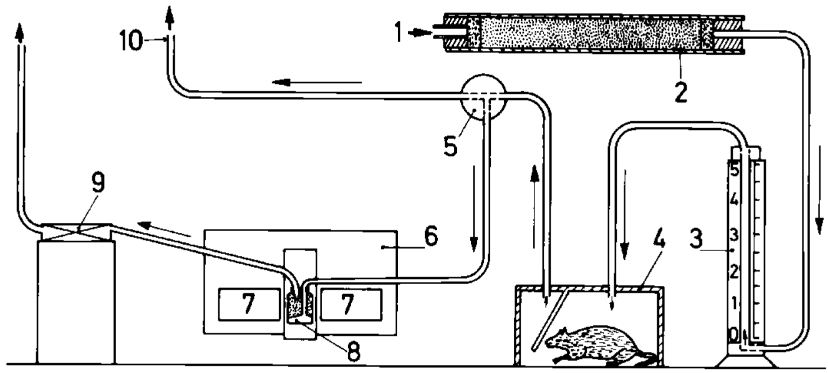

FIGURE 1. Complete diagram of experimental system. 1. Air input. 2. Soda lime for absorption of carbon dioxide. 3. Flow-meter. 4. Metabolism cage. 5. Three-way glass stop-cock. 6. Liquid scintillation counter. 7. Photomultipliers. 8. Counting vial. 9. Peristaltic pump. 10. Towards barium hydroxide for $^{14}CO_2$ absorption.

The apparatus consists of: a metabolism cage in which air enters from a flow-meter after elimination of CO_2. Expired $^{14}CO_2$ is transferred to the counting vial which is placed between counter. Air is circulated by a peristaltic pump and a filter pump. Part of the $^{14}CO_2$ may pass through an infra-red cell for measurement of the CO_2 concentration (Beckman Medical Gas Analyzer LB-2). The counting result may be recorded either directly with a linear or logarithmic recorder or after division by the CO_2 concentration value to record the specific activity (cpm/µmoles).

B. Counting Vial

The 25 ml counting vial is made of pyrex glass and is cylindrical with a 28 mm diameter and 47 mm height (Fig. 2). The glass stopper is crossed by two tubes, a gas outlet and inlet, the latter leading to the bottom of the vial and ending in a fritted disc. The $^{14}CO_2$ gas enters the vial through this disc (Simonnet 1974; Simonnet, Oria 1976). The vial is

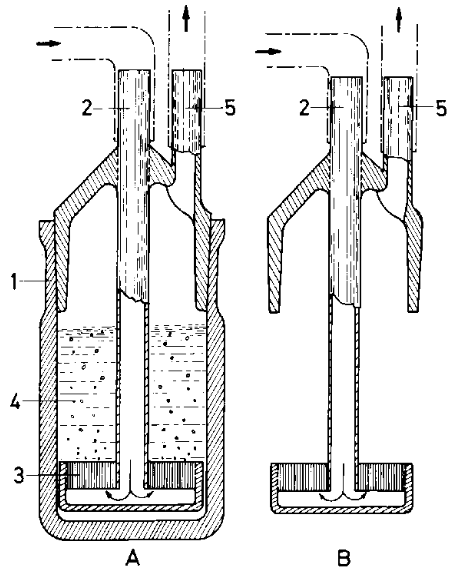

FIGURE 2. Cross-section of the counting vial.
A. Vial with stopper. B. Glass stopper.
1. Vial. 2. Gas input tube. 3. Fritted disc. 4. Scintillation cocktail. 5. Gas output tube.

filled with 13 ml liquid scintillation mixture (toluene or xylene-PPO-dMPOPOP : 1l/4g/0.1g). Beta particles emitted by $^{14}CO_2$ are detected as the gas passes through the liquid scintillation (Simonnet 1978). Connexions of the counting vial is provided by a black plastic tube resistant to the organic solvent.

C. Determination of the Counting Efficiency

In an Erlenmeyer, 50 µl $Na_2{}^{14}CO_3$ (0.5 µCi) are decomposed with HCl and the gas is sent through the counting vial by a peristaltic pump in order to count the radioactivity of the $^{14}CO_2$ formed. The counting efficiency is calculated according to the gas volume which passed through the detector during the measurement. In this detector system the counting efficiency for $^{14}CO_2$ is $88 \pm 2\%$.

III. RESULTS AND DISCUSSIONS

In order to compare results obtained with this technique and with other technique described by Pascaud (1963) and

Chevallier (1972) we determined the Acetyl-Coenzyme-A turnover in the rat.

A 350 g animal is intraperitoneally injected with 20 μCi [1,^{14}C]-sodium acetate. The animal is immediately placed in the metabolism cage in which the air flow is 900 ml/min. Part of the air outflow is transferred through the counting vial (35 ml/min) and another part through a gas washing bottle containing barium hydroxyde-satured solution; these are replaced every 10 min. Part of the $Ba^{14}CO_3$ is collected by filtration, washed with decarbonated water, ethanol then acetone and finally dried by heating with infra-red radiation. The radioactivity of the $Ba^{14}CO_3$ precipitate is measured by a low-background Tracer Lab. Geiger-Muller flow counter with a 150μg/cm^2 window. The results are corrected for self-absorption and expressed as the specific activity of $^{14}CO_2$ breathed out by the animal versus time (Fig. 3).

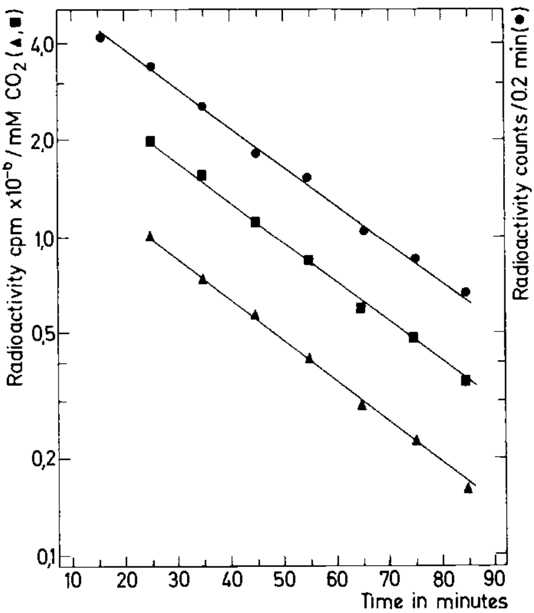

FIGURE 3. The specific activity of $^{14}CO_2$ breathed out by an animal is determined.
1. By continuous gas flow liquid scintillation counting (● ●).
2. After transformation of $^{14}CO_2$ into $Ba^{14}CO_3$ and measurement with a Geiger-Muller counter (▲ ▲) or by liquid scintillation counting (■ ■).

The radioactivity of $Ba^{14}CO_3$ samples is also measured by liquid scintillation counting (Fig. 3); for this purpose the same samples as before are introduced into a scintillation vial with

10 ml of a toluene-PPO and dMPOPOP mixture (1l/4g/0.1g). With this technique the activity measured is directly proportional to the quantity of $Ba^{14}CO_3$ (Fig. 4); this shows that the self absorption of the β^- particle is constant.

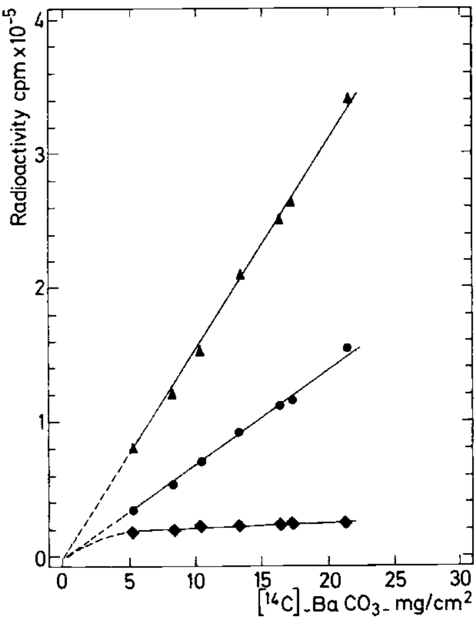

FIGURE 4. Radioactivity of $Ba^{14}Co_3$ layers of different thickness.
Radioactivity measured by: liquid scintillation counting with toluene-PPO-dMPOPOP (▲ ▲), and with a Geiger-Muller counter; values: before (◆◆) A_o and after (• •) A_x self-absorption correction

$$A_x = A_o(1-e^{-2.9x})/0.29x$$

x = thickness layer in mg/cm²

Moreover the activity is not influenced by the particle size of the samples. For these measurements the channel counter must be calibrated with a sample in which the ^{14}C is not in solution but in the form of $Ba^{14}CO_3$.

Finally, $^{14}CO_2$ exhaled by the animal has been measured both directly, by bubbling through the liquid scintillation cocktail and after transformation of $^{14}CO_2$ into $Ba^{14}CO_3$ in which case the activity is measured with Geiger-Muller and liquid scintillation counters. Results of these measurements expressed as counts/0.2 min or specific activity *versus* time (Fig. 3) show that the slope of the curves is the same with the three techniques.

The half-live is 25 minutes in agreement with the value found previously by Pascaud and Chevallier. Figure 5 shows results obtained in an experiment where a gas flow liquid scintillation counter is coupled with an infra-red cell for measurement of the CO_2 concentration and the logarithimic ratio (counts/concentration) is recorded directly as a function of time.

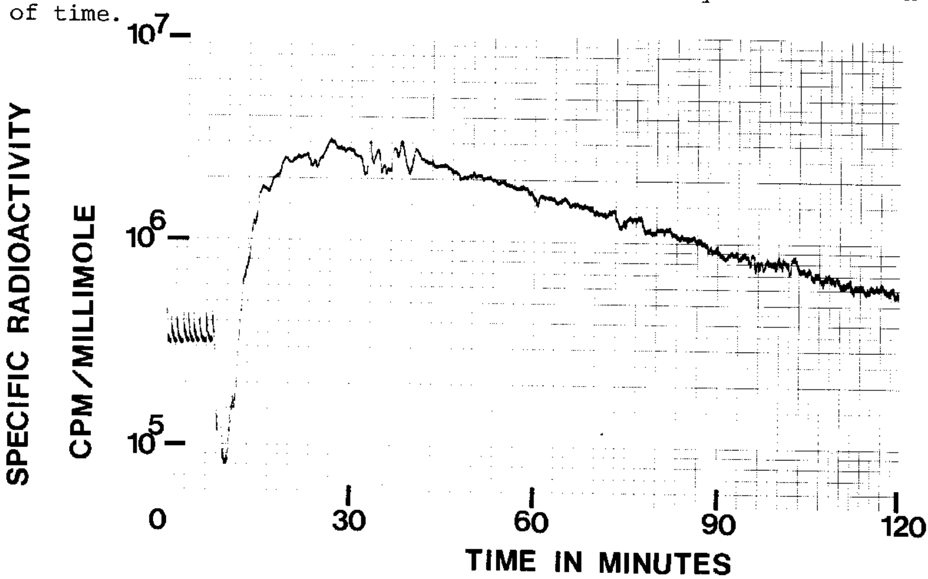

FIGURE 5. Variation in the specific activity of $^{14}CO_2$ breathed out by a rat.
Radioactivity is measured by continuous gas flow liquid scintillation. To obtain specific activity the radioactivity is divided by the CO_2 concentration measured with an infra-red cell; results are recorded as the logarithm of specific activity versus time.

A. Response Time

The response time of this gas-flow liquid scintillation was determined by two experiments. First the background is measured every 0.2 min for 2 minutes; then $^{14}CO_2$ is introduced into the metabolism cage; 0.5 min. later the radioactivity record increases (Fig. 6.1). Secondly during a $^{14}CO_2$ measurement the input is stopped, which allows measurement of the decontamination kinetics (Fig. 6.D).

The activity measured decreases quickly and a few minutes later the radioactivity is at normal background level. This shows that $^{14}CO_2$ causes contamination of the scintillation cocktail.

B. Advantages of This Apparatus

Dead volume, especially that of the probe, is smaller than in an ionization chamber and the time response is faster. The counting efficiency is high: 88% for the ^{14}C and the apparatus is convenient to use, for the detection and recording are automatic.

This apparatus may be used for detection of any radioactive gas insoluble in toluene.

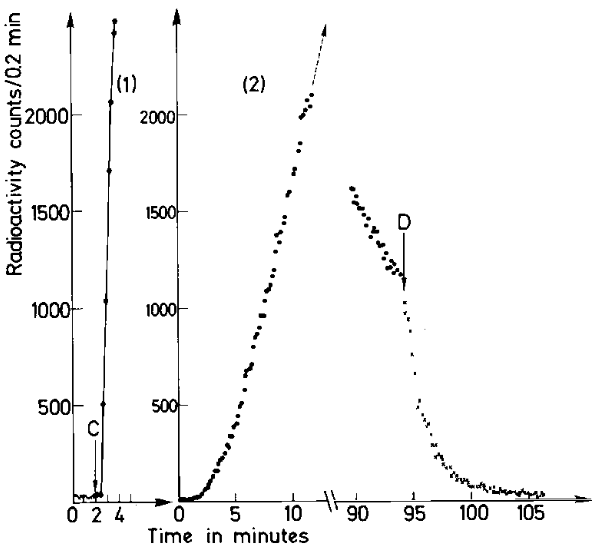

FIGURE 6. Duration of a measurement.
1) A 2 minute background measurement is followed by introduction of $^{14}CO_2$ (C↓). Radioactivity is detected 30 sec. later.
2) Detection kinetics of $^{14}CO_2$ after [^{14}C]-sodium acetate injection to the animal at t = zero.
3) Decrease in the radioactivity breathed out by the animal (●●●●); at 95 minutes (D↓) the input of $^{14}CO_2$ is stopped, allowing measurement of the decontamination kinetics (XXXX).

ACKNOWLEDGMENTS

The authors wish to thank Mr. A. Bissonnet and R. Ripon for the adaptation of the logarithmic converter and the construction of the electronic device for the specific gravity calculation.

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