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Liquid Scintillation Vial for Cumulative and Continuous Radiometric Measurement of In Vitro Metabolism

EDWARD U. BUDDEMEYER

Division of Nuclear Medicine, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205

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A two-compartment vial is described in which suspensions of bacteria, cells, or tissues may be cultured and their growth and metabolism measured radiometrically by using a liquid scintillation counter. The device consists of a scintillation vial lined with a cylinder of scintillating paper into which is placed a sterilized inner culture vial containing a carbon-14 substrate. The assembled device can be carried by the sample transport systems of conventional liquid scintillation counters. Evolved $^{14}CO_2$ is collected and measured cumulatively and continuously. The device can be constructed simply and economically from readily available reagents and glassware. Data are given on relative sensitivity and on the effect of the color and transparency of the inner vial. A pilot experiment with bacteria (*Escherichia coli*) is described.

Radiometric methods are among the more sensitive of analytical procedures. The application of these methods to the in vitro measurement of metabolism can be accomplished by supplying ^{14}C-labeled substrates and collecting and measuring the radioactivity of the evolved $^{14}CO_2$.

Several devices have been produced for the purpose of measuring the $^{14}CO_2$ released by bacterial, viral, or tissue cultures. In one of these (1), the atmosphere above the culture is periodically sampled, drawn into an ionization chamber, counted, and then discharged into an absorber. The procedure has been fully automated, but sensitivity is limited since the method of sampling is nonconservative and an ionization chamber is not distinguished by an especially high intrinsic sensitivity for ^{14}C.

Efforts have been made to apply the highly sensitive technique of liquid scintillation counting to this problem. A typical device (2) consists of an incubation flask to which is attached, through a side arm, a removable scintillation vial. The vial is lined with a cylinder of paper which is wetted with potassium hydroxide solution to act as a CO_2 trap. After a suitable incubation period the vial is disconnected, charged with a scintillation "cocktail," capped, and counted. The method is sensitive but not susceptible to automation, since the assembled device cannot be carried by the sample transport systems of liquid scintillation counters. Furthermore, even if the device could be transported, it could not be counted in the assembled form, because scintillations do not occur unless the side vial contains a scintillation cocktail. Most such cocktails are toluene-based, and their presence in the side vial would produce intolerable concentrations of bacteriostatic toluene vapor in the incubation flask.

For the routine assay of large numbers of samples, it would be advantageous to have a device which is not only sensitive but also can be counted automatically. Such a device would have to be entirely contained within a scintillation vial.

MATERIALS AND METHODS

The basic aim was to design a sterilizable growth and metabolism chamber that could be placed within a scintillation vial and then surrounded by a nontoxic scintillator.

The growth chamber. The first requirement is that the inner vial fit through the neck of the scintillation vial. The scintillation vials at hand could accomodate commercially available 16-mm diameter vials and tubes. In initial trials, plastic (styrene) inner vials were used because they were easy to cut to the desired length. For a pilot experiment with bacteria, sterile glass vials were used.

Beta particles from ^{14}C-substrates in the inner vial must not reach the surrounding scintillator except by first being metabolized to $^{14}CO_2$. The wall thickness of commercial vials is more than sufficient to totally absorb the beta particles of ^{14}C.

In the first models, gas-phase communication between the inner and outer vial was facilitated by drilling a ring of holes near the top of the inner vial. This refinement was later found to be unnecessary, it being sufficient that the height of the inner vial (59

mm) be such as to leave some space between its top and the outer vial cap. In either case, maintenance of phase separation depends upon gravity, so that once the inner vial is charged with ^{14}C substrates the assembled units must thereafter be kept upright.

In a liquid scintillation counter the counting vials are viewed from opposite sides by two horizontally opposed photomultiplier tubes. The circuitry is such that, in order to be counted, a scintillation must be "seen" simultaneously by both photomultiplier tubes. This circumstance raises the possibility that an optical obstruction (i.e., an inner vial) in the middle of the scintillation vial might greatly reduce the detected counting rate. An experiment to examine this possibility is subsequently described.

The scintillator. Several scintillation mixtures that do not employ toxic solvents have been described in the literature. These include water slurries of anthracene crystals (4) or of beads of scintillating plastic (3). Beta particles emitted from samples (^3H_2O, $Na_2{}^{14}CO_3$) dissolved in the water interact with the adjacent scintillator by direct incidence. There is little chance for interference by chemical quenching since the method does not depend upon the efficient transfer of excitation energy between molecules of the solvent, as is the case in the usual dilute solutions of scintillators in toluene or dioxane. The principal determinant of counting efficiency is the ratio of the volumes of the scintillator and the aqueous sample solution. Excess water results in self-absorption of the beta particles before they can reach the scintillator. The mass of scintillator (approximately 1 g) required to support the needed volume of alkali solution (approximately ½ ml) is likely to be prohibitively expensive for large scale use.

It was suspected that the essence of the slurry method, that is, intimate contact between sample and scintillator, might be more economically accomplished if both were absorbed on some inert support within the scintillation vial. A cheap support medium, wettable by both organic and aqueous solutions, is filter paper. In an initial trial, strips of Whatman no. 40, of a size to fit within a scintillation vial, were dipped in a concentrated toluene solution of 2,5-diphenyloxazole–1,4-bis-(5-phenyloxazolyl)-benzene (PPO-POPOP) (New England Nuclear "Liquid-fluor" concentrate) and dried in a hood. The strips were found to have accumulated 45 ± 2 mg of the PPO-POPOP mixture, about the same quantity that is contained in the usual 15 ml of cocktail employed in routine liquid scintillation counting. Strips so treated were used in the experiments which follow.

The assembled unit. Strips of Whatman no. 40 filter paper, 8.8 cm long by 3.7 cm wide, dipped in PPO-POPOP and air-dried, were formed into cylinders and placed within a scintillation vial. The uncapped vials were left overnight in an evacuated desiccator to remove any traces of toluene. After removing the vials from the dessicator, the paper cylinders were wetted with 0.5 ml of 1 N NaOH, and the vial was immediately capped and left for several hours to permit the alkali solution to become evenly distributed on the paper. The inner metabolism vial was then inserted, and the device was assembled for use (Fig. 1).

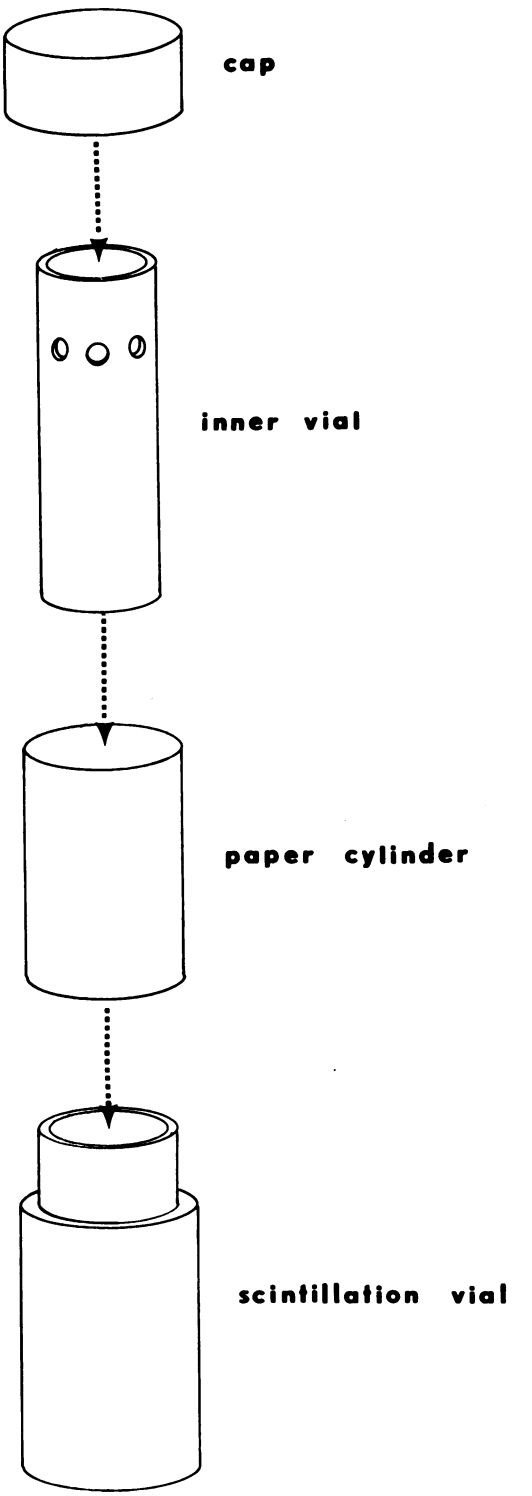

FIG. 1. *Components of the metabolism-detecting scintillation vial (exploded view).*

Sensitivity. Sensitivity was measured by placing 0.2 μCi of $NaH^{14}CO_3$ (New England Nuclear #NLS-086 S, 1 μCi/ml) in 0.2 ml of solution in the inner vial followed by 1 ml of 5% H_3PO_4 (to cause release of $^{12}CO_2$) and then capping the vials and counting the assembled units at several intervals. Five units so prepared were counted in a liquid scintillation counter (Packard model 3003) at 40% gain with a base level setting of 50 pulse height units and an upper level of 1,000. Detected counting rates were compared with those obtained when five samples of the same activity were counted in the same pulse height "window" in regular vials using a conventional scintillation cocktail (15 ml of Bray's mixture) at a gain setting (20%) previously determined to be optimum for that cocktail.

The effect of the inner vial. Nine units were prepared as in Sensitivity (above) except that a 24-h accumulation period was allowed for complete absorption of the evolved $^{14}CO_2$ on the paper cylinders. The effect of the hollow, transparent inner vial (plus contents) was determined by comparing the counting rates observed with the vials in place to that obtained after the inner vials were removed and the device was recounted. In addition, the effect of the color and transparency of hypothetical inner vials was examined by recounting the same units after the insertion of 16-mm diameter slugs of three different kinds: a solid, transparent Plexiglas rod; an opaque, white Teflon rod; and an opaque, black rubber rod.

Pilot experiment with bacteria. The results of the experiments previously described indicated that it was feasible to use commercially available (Rochester Scientific #7499 glass inserts, #7494 plastic vials) vials-within-a-vial for this trial. These consisted of a 20-mm diameter by 50-mm high glass inner vial and a plastic outer vial.

Sixteen of the glass inserts were plugged with gauze pledgets and sterilized in an autocalve. A like number of outer vials were lined with cylinders of scintillating paper as previously described.

Four of the sterilized vials were aseptically charged with 5 ml of glucose-free Trypticase soy broth (BBL) and 1 μCi of uniformly labeled [^{14}C]glucose (in 0.1 ml of solution) to act as blanks. The remaining six pairs of vials were similarly charged with 4 ml of Trypticase soy broth and 1 μCi of [U-^{14}C]glucose. The pairs were inoculated in duplicate with suspensions of *Escherichia coli* (Center for Disease Control, Atlanta, Ga.) in 1 ml of Trypticase soy broth. Six levels were used: 10^6, 10^5, 10^4, 10^3, 10^2, and 10^1 bacteria. All units were assembled as in Fig. 1, incubated at 37 C, and counted at various intervals. The units were placed in the refrigerated counting chamber only for the 1-min counting period and were then immediately returned to the incubator.

RESULTS

Sensitivity. Detection of evolved $^{14}CO_2$ was rapid (Table 1). The relative efficiency, including that of evolution, collection, and counting, reached 17% of that obtained by direct addition of $NaH^{14}CO_3$ to Bray's mixture.

Spectral analysis revealed that scintillations detected on the paper were not as intense as those in the cocktail, requiring higher gain settings for maximal efficiency. The lower intensity is probably due to the semiopacity of the paper and to some interference with complete light collection due to the presence of the inner vial.

TABLE 1. *Counting rate and relative efficiency at several intervals*

Elapsed time	Observed count rate (counts/min)	Relative[a] efficiency (%)
15 min	8,359 ± 785	13.42
2 h	10,627 ± 660	15.81
21 h	11,300 ± 988	17.26

[a] Relative to standard counted in Bray's mixture.

Effect of the inner vial. The results are presented in Table 2. Compared with the empty vial, the presence of a transparent, hollow cylindrical inner vial (plus contents) caused a 9% reduction in detected counting rate. A solid, transparent insert (Plexiglas rod) caused a slight increase in counting rate, perhaps by acting as a lenticular lens. An opaque, white insert (Teflon rod) caused an appreciable (40%) decrease in counting rate but not so severe as to preclude the use of this technique. This finding is of practical significance, since at a later stage of development it may prove desirable to use opaque, white inner vials to nullify the effect of highly colored specimens (e.g., blood) or media. Opaque black inserts (black rubber rod) had, as expected, a pronounced effect.

Detection of bacterial metabolism. The results of this experiment, plotted as a semilogarithmic graph, are presented in Fig. 2. The time required for detection of bacterial metabolism was inversely proportional to the number initially present. Detected activity in the two vials containing the fewest bacteria (a total of 10/vial) was distinguishable from blank activity within 8 h. After 24 h of incubation, all bacteria-containing vials reached an activity in excess of

TABLE 2. *Effect of various inserts*

Insert		Relative efficiency (mean ± SD)[a]
None	9	100.00[b] ± 6.45%
16 mm (outer diameter) hollow styrene vial	9[c]	91.10 ± 1.80%
16 mm Plexiglas rod	3[c]	102.20 ± 1.52%
15 mm Teflon rod	3[c]	60.54 ± 3.73%
16 mm black rubber rod	3[c]	18.48 ± 0.65%

[a] SD, standard deviation.
[b] Reference level.
[c] Number of pairs.

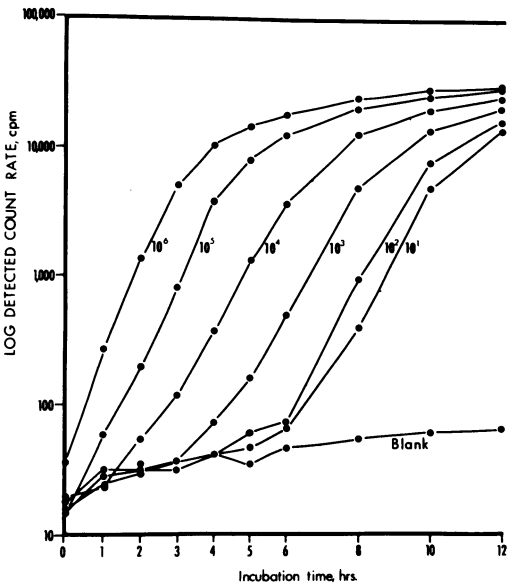

FIG. 2. *Detected activity as a function of incubation time and numbers of E. coli.*

30,000 counts/min (mean and standard deviation: 39,151 ± 4,946 counts/min, n = 12). Blank activity at that time was 97 ± 17 counts/min (n = 4). Over the period from day 1 to day 4 (not shown in Fig. 2), blank activity increased at the rate of 36 counts per min per day.

DISCUSSION

A simple, economical device is described that permits the detection of $^{14}CO_2$ evolved from in vitro cellular metabolism by a liquid scintillation counter. The method of sampling is cumulative and nondestructive so that growing specimens can be counted as frequently as desired without interrupting the experiment. The problem of chemical quenching does not arise, and color quenching can be dealt with by using opaque, white inner vials. The assembled units can be carried by the sample transport systems of conventional liquid scintillation counters. If the available liquid scintillation counter is a refrigerated model (as in these experiments), metabolism can be followed by placing the samples in the counting chamber only for the 1-min counting period. With some modifications, an ambient-temperature liquid scintillation counter may be used as a multisample, automatic detector and measurer of bacterial (or other) metabolism. This possibility is being explored.

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LITERATURE CITED

1. DeLand, F. H., and H. N. Wagner, Jr. 1970. Early detection of bacterial growth with carbon-14 labeled glucose. J. Lab. Clin. Med. **75**:529–534.
2. Saba, T. M., and N. R. DiLuzio. 1966. Method for collection and determination of $^{14}CO_2$ for *in vitro* metabolic studies. J. Lipid Res. **7**:566.
3. Steinberg, D. 1958. Radioassay of C^{14} in aqueous solutions using a liquid scintillation spectrometer. Nature (London) **182**:740.
4. Steinberg, D. 1960. A new approach to radioassay of aqueous solutions in the liquid scintillation spectrometer. Anal. Biochem. **1**:23.