Modifications in Biphasic Liquid-Scintillation Vial System for Radiometry

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Several modifications of the biphasic liquid-scintillation vial system for radiometry have been tried in order to improve the counting efficiency.

The biphasic system consisted of an inner sterile vial containing medium and substrate, and an outer liquid-scintillation vial lined on the inside with filter paper impregnated with scintillation fluors and alkali. The system gave an overall counting efficiency of 14.6%. Substitution of methanolic NaOH for impregnation of the paper raised the counting efficiency to 29.1%. This could be further enhanced to 33.8% by lining only half of the outer vial with filter paper, thereby allowing improved optical transmission of scintillation light. Increasing the amount of fluor did not change the efficiency significantly.

A complete interchange in the system, whereby half of the inner vial was lined with filter paper and was otherwise empty, while the outer vial contained the medium and substrate, gave the highest efficiency (36.9%). This also allowed the use of larger amounts of medium and the inoculum.

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Detection of microbial activity, by measuring $^{14}CO_2$ evolved from the utilization of a suitable C-14-labeled substrate in a nutrient medium, is a well-recognized procedure (*1*). In most cases the rate of $^{14}CO_2$ output is a good index of the rate of replication of microorganisms. Equipment using an ionization chamber for such measurements is commercially available and has been used in a variety of applications (*2,3*).

Buddemeyer has described a biphasic liquid-scintillation counter (LSC) vial lined on the inside by Whatman filter paper impregnated with fluor. A smaller sterile vial containing nutrient medium, C-14-labeled substrate, and the inoculum is placed within the LSC vial (*4*). Labeled CO_2, released from the substrate by the microorganism, is trapped on the scintillating paper lining the outer vial, and can be measured in a liquid-scintillation counter.

By converting a commercially available liquid-scintillation counter for operation at 37°C, Buddemeyer was able to measure precisely the generation time for many of the rapidly growing microorganisms in culture (*5,6*). However, the efficiency of counting $^{14}CO_2$ in his system was as low as 7%.

Singh et al. (*7*), studying the effects of thyroxine on bacterial metabolism, used modifications of the Buddemeyer method that doubled the counting efficiency.

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We report here on various other modifications of the biphasic-vial system intended to improve the counting efficiency for gaseous $^{14}CO_2$. Attempts were made to increase light production by loading the paper with larger amounts of fluor/absorber. Uniform and enhanced alkali deposition was obtained by using methanolic NaOH. Efforts to improve optical transmission were made by (a) reducing the size of the paper, and thus lessening the optical obstruction in the vial; and (b) placing the coated paper in the center of the vial rather than around its circumference.

METHODS

Preparation of basic vial. Singh's modification of the Buddemeyer vial was used as the basic system against which the effects of the additional modifications were compared. The basic method employed a narrow-necked, clear-glass (low-potassium), screw-cap, standard liquid-scintillation vial as the outer container. A fluor solution was prepared by dissolving 10 g PPO and 0.125 g POPOP in 100 ml of dioxane. Before impregnation of the paper, 14 ml of fluor solution was mixed with 1 ml of 2 *N* aqueous NaOH. A strip of Whatman No. 40 filter paper, 7.8 × 4 cm, was dipped in the mixture, dried, and promptly placed within the scintillation vial so as to form a closely fitting, hollow cylinder lining the inner circumference of the vial (Fig. 1A). Carbon-14-labeled test substrate solutions were contained within a smaller, sterile inner vial (metabolism vial) placed in the center of the larger scintillation vial. Labeled CO_2 evolved from the substrate diffused out of the open-topped inner vial and was trapped on the treated paper, where

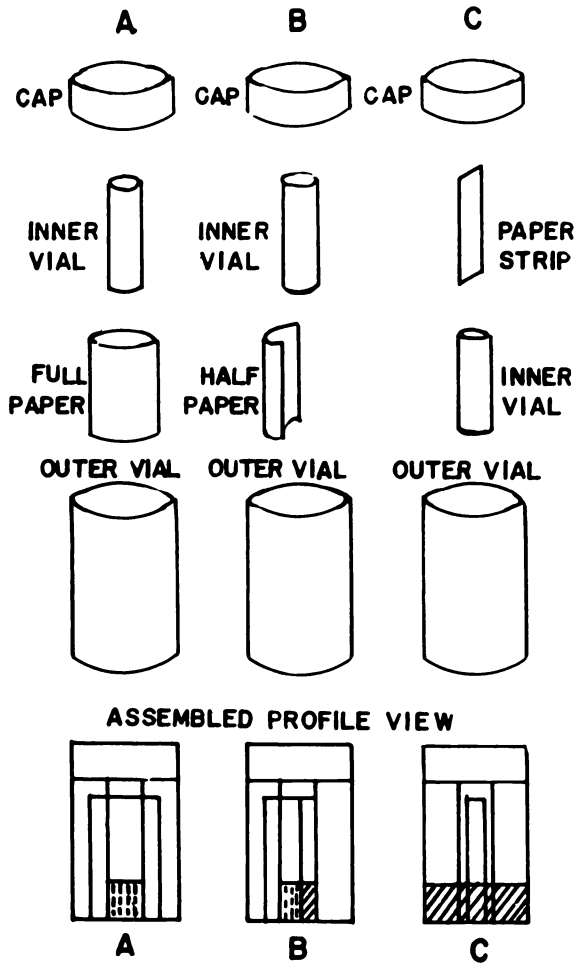

FIG. 1. (A) Full paper vial: Modified Singh vial (1977), inner sterile vial containing the nutrient medium and C-14 substrate; outer vial is lined with Whatman No. 40 paper (7.8 × 4 cm) impregnated with scintillation fluors containing alkali. (B) Half-paper vial: Same as in A, but size of paper lining outer vial is 4 × 4 cm. (C) Interchanged vial: Inner vial contains Whatman No. 40 paper (2 × 4 cm) impregnated with scintillation fluors and alkali, while outer sterile vial contains the nutrient medium and C-14 substrate.

it produced scintillations detectable by the liquid-scintillation counter.

Modifications. *The use of methanolic NaOH.* The mixture of fluor and aqueous alkali was not perfectly homogeneous, showing initial turbidity and evidence of phase separation on standing. A more nearly uniform coating was obtained by using alcoholic NaOH. A saturated solution was prepared by saturating anhydrous methanol with NaOH (~8 g NaOH/100 ml). A mixture of fluor solution and methanolic NaOH was prepared: 12 ml dioxane fluor solution to 3 ml methanolic NaOH. The fluor/absorber mixture obtained with methanolic NaOH was less turbid and more homogeneous.

The use of half-sized paper. On the hypothesis that a smaller paper would offer less optical obstruction, half-sized papers (4 × 4 cm) were prepared as described above. These papers formed a semicylinder covering only half the circumference of the scintillation vial, leaving the other half optically clear (Fig. 1B).

The use of increased amounts of fluor and alkali. Half-sized papers were prepared as above except that progressively increased amounts of fluor/alkali were obtained by dipping the papers once, twice, thrice, or four times in the fluor/alkali mixture, with drying between dippings.

Interchanged vial. In this modification the assembly was interchanged by placing a 2- by 4-cm piece of Whatman No. 40 filter paper (previously impregnated with scintillation fluor and methanolic NaOH) in the inner vial and the labeled substrate in the outer vial (Fig. 1C).

Counting efficiency measurements. The procedure used for determining the absolute efficiency of counting trapped $^{14}CO_2$ was essentially the same as that described earlier (*4*). In brief, the method consists of placing a known quantity of carbon-14-labeled sodium bicarbonate (1 μCi of $NaH^{14}CO_3$, sp.act. 50 μCi/millimol in 1 ml of 0.05 *N* NaOH in the metabolism vial. The $^{14}CO_2$ from the $NaHCO_3$ was then released by adding 1 ml of 8.8% H_3PO_4. The $^{14}CO_2$ trapped was counted after periods of 30 min, and 5 and 24 hr.

A liquid-scintillation counter was used for the counting. To allow the weak pulses to be counted the $^{14}CO_2$ produced was counted with "Data" set at 1750, "Gate" at 1900, and the upper discriminator at infinity.

Counting efficiency with microorganisms. To compare the three modifications shown in Fig. 1, the test inoculum consisted of 100,000 *Escherichia coli* organisms in 1 ml peptone water containing 1 μCi of [U-^{14}C] glucose. The $^{14}CO_2$ produced was counted at 10 min, 2, 4, and 24 hr in the newer model counter.

RESULTS

Counting efficiency with various modifications of basic biphasic system. The counting efficiencies obtained with each modification at 24 hr, the time at which the chemical processes in the vial would have reached equilibrium, are shown in Table 1.

The basic arrangement (Fig. 1A), with aqueous alkali and 360° paper, gave an efficiency of 14.66%. Substitution of methanolic NaOH doubled the efficiency (29.1%; Table 1, B1). Further improvement (to 33.79%) was achieved when the scintillating paper lined only half of the outer cylinder (Fig. 1B, Table 1, B2). The filter paper certainly is not transparent, and when it covers the entire glass vial, it can absorb the light signals. When half of the vial is uncovered, it is probable that more of the light signals are seen in coincidence by the photomultipliers.

It was estimated that about 220 μEq (8.8 mg) of alkali are spread on the full paper. The half paper thus contains about 110 μEq of alkali that appears adequate to trap the 20 μmoles of $^{14}CO_2$ that could be generated from 1 μCi of $NaH^{14}CO_3$ (sp. act. 50 μCi/millimole). The amount of $^{14}CO_2$ produced by microorganisms is likely to be much lower than 20 μmoles.

The efficiency of counting remained unaffected whether the paper was dipped once or twice in the mixture of scintillation fluor and alkali. Dipping the paper more than twice actually reduced the counting efficiency from 34.4 to 26.0% (Table 1, B3a-B3c). The lower counting efficiency could be due to the heavier deposits of solute on the paper with increased absorption.

The interchanged vial arrangement used only one quarter the area of paper lining the inner vial and thus contained one fourth the amount of alkali and fluor compared with the standard vial. Nevertheless the counting efficiency obtained by the inverse vial was 36.86% (Table 1, B4).

The orientation of the vials with respect to the photomultipliers did not seem to make a significant difference in the count rates.

Counting efficiency with microorganisms. Different modifications showed the same order of enhanced counting efficiency for $^{14}CO_2$ evolved from *E. coli* (Table 2). The interchanged vial was still the best, and half paper was better than full paper. Counting in this case was done in the newer counter to prove that the change in counting efficiency is primarily due to vial modifications.

TABLE 1. ABSOLUTE COUNTING RATES FOR $^{14}CO_2$ WITH VARIOUS MODIFICATIONS OF BIPHASIC-VIAL SYSTEM

	CPM*	Counting efficiency (%)	Ratio modification/ Singh
A. Basic biphasic vial system:			
Modified Buddemeyer vial (Singh et al. 1977) Whatman #40 paper (7.8 × 4 cm) impregnated with fluors and aqueous alkali.	325672 (305,146–344,181)	14.66	1.00
B. Modifications:			
1. Same as in A but with 2 *N* methanolic NaOH	646896 (614,418–694,950)	29.13	1.99
2. Same as in B1, except for half-cylinder paper, 4 × 4 cm	750179 (710,021–822,287)	33.79	2.30
3.			
a. Same as in B2 but paper dipped twice	762500 (742,378–755,546)	34.35	2.34
b. Same as in B2 but paper dipped 3 times	595407 (548,597–666,876)	26.82	1.83
c. Same as in B2 but paper dipped 4 times	579344 (458,159–654,769)	26.10	1.78
4. Interchanged vial	818406 (760,686–889,168)	36.87	2.52

* Each value is a mean of triplicates and range.

DISCUSSION

The biphasic double vial liquid-scintillation technique first described by Buddemeyer can be modified to achieve counting efficiencies as high as 37% for evolved $^{14}CO_2$. The modified versions are as economical as the original, using only a cheap, disposable filter-paper support, a small quantity of readily available scintillation fluor, and standard glass vials.

It was observed that slight modifications in the way the paper was impregnated produced better counting efficiency. Use of methanolic 2 *N* NaOH appreciably improved counting rates possibly by reducing turbidity besides improving trapping of $^{14}CO_2$ on the filter papers. A modest improvement in sensitivity is obtained when only part of the vial is lined with the specially impregnated paper. The improvement is attributed to reduced light obstruction and a correspondingly greater likelihood of simultaneous detection of the scintillations by both photomultiplier tubes. Curiously enough, the apparent asymmetrical arrangement of the paper does not show any dependence between detected counting rate and the orientation of the vials with respect to the photomultiplier tubes. In addition the culture in inner vial can be observed through the uncovered side—for example, to check the turbidity associated with rapidly growing organisms.

It is also possible to interchange the vial arrangement by placing the fluorescent paper in the inner vial and the medium, labeled substrate, and test organisms in the larger, outer vial. The counting efficiency obtained this way is slightly higher than that obtained with the usual arrangement. The outer vial is also more capacious so that a larger volume of medium or inoculum can be accommodated should that be desired.

The enhancement of counting efficiencies with half paper and interchanged vial appeared valid in the presence of microbial growth (Table 2) and even when a different model of counter was used.

TABLE 2. MEASUREMENT OF $^{14}CO_2$ EVOLVED FROM METABOLISM OF *E. COLI

	10 min (cpm)	2 hr (cpm)	4 hr (cpm)	24 hr (cpm)
Full paper	220† (113–292)	32,779 (31,025–34,142)	106598 (102,146–110,427)	144450 (142,015–149,005)
Half paper	152 (124–166)	62412 (62,066–62,653)	192210 (174,047–205,123)	248474 (236,522–258,558)
Interchanged vial	219 (186–267)	37,731 (35,100–38,220)	144877 (144,150–145,447)	305394 (291,312–318,593)

* 1 ml peptone water + 1 μCi [U-^{14}C]glucose + 100,000 *E. coli.*

† Each value is a mean of triplicates and range. A newer model liquid scintillation counter was used for this experiment.

The outstanding advantage of these biphasic-vial scintillation techniques—whether original or modified—is that the collection of labeled CO_2 is continuous and cumulative. When used for the intended purpose of measuring bacterial growth, the detected activity continuously increases with time in proportion to the metabolic activity of the sample until the labeled substrate is exhausted or growth ceases, whichever occurs first. The vials may be counted as often as desired without compromising the sensitivity of detection. The improved sensitivity of the modified versions may permit earlier detection of growth; moreover by choosing the option of equal sensitivity, one can realize up to a fivefold saving in the amount of labeled substrate.

Using these economical vials, any liquid-scintillation counter can serve as an automatic, highly sensitive, quantitative detector of bacterial metabolism. This radiorespirometric technique may find its most productive application in the study of infectious diseases in developing countries, where the use of these vials in a standard, multipurpose, liquid-scintillation counter obviates the need for special instrumentation.

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