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LIQUID SCINTILLATION COUNTING OF ^{14}C-DIATOM MATERIAL ON FILTER PAPERS FOR USE IN PRODUCTIVITY STUDIES

ABSTRACT

An improved method for the liquid scintillation counting of ^{14}C-phytoplankton material on membrane filter papers is described. The method involves the incorporation of standard ^{14}C-sucrose into the filter, and it clearly demonstrates that the counting efficiency of the sample is affected by the amount of material present. It is concluded that the cells and the membrane filter have a self-absorptive effect that previous methods of counting, using internal and external standardization, cannot have fully detected.

Liquid scintillation counting for the determination of ^{14}C-activity in filtered phytoplankton has come into increasing use since the publication of the paper by Wolfe and Schelske (1967), and there are several references to its use in the literature. Recently, Lind and Campbell (1969) have added information on the technique. The method appears to promise high counting efficiencies, without the problems of backscattering and self-absorption that greatly limit the Geiger-Müller counting method.

However, during the course of my studies of the excretion of metabolites by marine algae, certain inadequacies in the procedures have become apparent. Wolfe and Schelske (1967) determined the counting efficiency of their ^{14}C-phytoplankton samples on filter papers by adding internal standard ^{14}C-toluene to the fluor solution. This method estimates the counting efficiency of any dispersed material in solution, but does not take fully into account the self-absorption of the cells and the filter, which was assumed to be negligible—an assumption not supported by my results.

Wallen and Geen (1968) also concluded that self-absorption had no significant effect on counting efficiency with liquid scintillation counting. However, they used two counting methods: the first, like that of Wolfe and Schelske, with dry membrane filters (+ cells) that were simply placed in the fluor solution, and a second where the wet membrane filter was dissolved in a fluor solution containing ethylene glycol monomethyl ether. Although it is not clearly stated which of these two methods they used when they reached their conclusion regarding the absence of any self-absorption effect, by inference it appears to be the latter and so does not contradict my results.

Schindler (1966), similarly, used a fluor solution containing naphthalene to dissolve the membrane filters, leaving the cells or cell fragments to settle to the bottom of the scintillation vial. Both Schindler and Wallen and Geen then determined the counting efficiencies of their samples by adding a ^{14}C-internal standard (^{14}C-toluene in the former case; not stated in the latter). The cells are dispersed in the fluor solution (assuming frequent agitation, which is rather impracticable for automated counting of large numbers of samples), so the radioactivity within them will only be separated from the fluor solution by the cell walls and not by several layers of cells as is the case on a filter. The degree of self-absorption may thus be expected to be less, and the samples may behave more like heavily chemically and color-quenched homogeneous solutions. I have not yet tested these methods, but I suspect that since the solution is still heterogeneous, the use of internal standardization methods, using ^{14}C-toluene or the like, should be avoided, although the percentage error may not be high. However, these methods are not the main concern here.

Lind and Campbell (1969), however, applied an external standardization technique for the estimation of the counting efficiency of their scintillation vials containing cells on filter papers. The external standard method can only properly be used to determine the degree of quenching within a homogeneous fluor solution and should not be applied to heterogeneous ones.

The current work was undertaken to investigate the internal and external standardization methods, together with a third that I developed in connection with the counting of ^{14}C-material on filter papers using liquid scintillation counting techniques. My results clearly demonstrate that the only reliable method for determining the counting efficiencies of this material is by a channels ratio method, although the curve relating channels ratio to counting efficiency is markedly different from that for homogeneous solutions.

The following procedure was developed to simulate the counting of ^{14}C-material on filter papers and so enable the construction of a "quench" curve relating channels ratio to counting efficiency. Various amounts of a culture of the diatom *Phaeodactylum tricornutum* were filtered onto tared 50-mm membrane filters, washed with isotonic ammonium formate, dried, and weighed. Samples (0.1 ml) of a ^{14}C-sucrose solution in distilled water (sp activ ca. 36×10^3 dpm/0.1 ml) were microburetted onto the surfaces of the dried filters and allowed to soak in and dry. Sucrose was used because it is virtually insoluble in toluene and so will remain on the filter and not be eluted into solution. The dry membrane filters were placed in 20-ml scintillation vials, completely covered with fluor solution (10 ml of 0.5% Butyl-PBD in AR toluene), and counted. Two scintillation counters were used (a Packard Tri-Carb Model 3314 and a Philips Scintillation Analyser). Counts were recorded in two channels, set for optimum counting of a homogeneous solution, and from these counts the channels ratios were calculated. The specific activity of the original ^{14}C-sucrose solution was determined by counting 0.1-ml samples in homogeneous solution in a toluene : ethanol (3 : 1) fluor; from the channels ratios obtained, the specific activity could be calculated from the standard curve for the scintillation counter. With a knowledge of the specific activity of the sucrose solution, the counting efficiencies of the vials containing the filters could then be calculated. The counting efficiencies are plotted against their respective channels ratios (for the Philips counter) in Fig. 1 (curve a). Also plotted in Fig. 1 is the standard curve for counting a homogeneous solution (curve b).

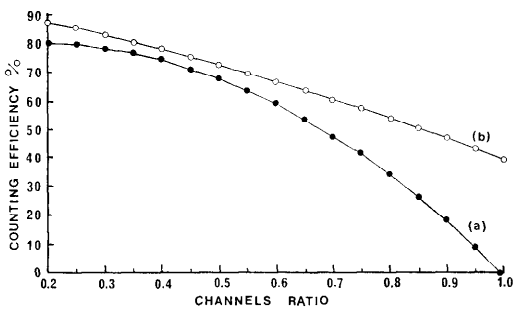

FIG. 1. Channels ratio calibration curves. Filter standardization curve (a) calculated as a second order polynomial from 220 points ($r = 0.970$). Channels ratio curve (b) is the standard curve for counting a homogeneous solution.

To measure the loss of radioactivity from the filters during counting, the fluor solution was decanted off into clean 20-ml vials and counted. The results indicated an average loss of less than 1%; other experiments, using cells previously incubated with ^{14}C-carbonate, indicated only a slightly higher loss (2–3%). If the exposure time to the fluor solution is kept to a minimum, the cells apparently neither dislodge from the filter nor lose much material into solution.

The Philips counter also had the facility for automatic external standardization and so the apparent counting efficiencies of some of the vials containing filters (+ cells) were determined by this method. Standard ^{14}C-toluene was then added to the vials, which were recounted so that the apparent counting efficiencies using the internal standardization method could also

TABLE 1. *Liquid scintillation counting of ^{14}C-sucrose on membrane filters coated with different weights of the diatom* Phaeodactylum tricornutum. *Counting efficiencies calculated from the known dpm (filter standardization, F.S.), from internal standardization (I.S.), and from external standardization (E.S.)*

Dry wt cells (mg)	Counting efficiency			
	F.S.	I.S.	E.S.	Solvent*
†	73.1	83.5	83.9	85.8
†	71.9	83.5	83.3	85.6
0.28	65.0	77.5	78.3	85.2
0.50	64.5	78.7	79.9	85.8
0.94	59.6	76.8	76.4	85.2
1.15	56.6	74.6	74.3	85.1
1.35	54.9	73.7	71.1	84.7
1.65	46.1	76.0	71.4	84.6
2.67	41.1	73.0	69.2	85.0
3.98	33.3	72.0	66.1	86.3
6.56	22.4	68.6	63.5	84.9
7.97	14.8	63.1	58.5	80.0
9.43	9.3	60.8	56.0	79.0
10.84	4.3	57.1	51.3	79.0

* The membrane filters (+ cells) were removed from the pots and the counting efficiency of the solution determined by external standardization.
† Filter alone.

be calculated. These results are shown for different weights of material on the filters and are compared with the results for the filter standardization method in Table 1.

The calculated counting efficiencies using internal and external standards are seen to be similar, and the efficiency determinations using an internal standard were in excellent agreement with those determined by reference to the channels ratio curve for homogeneous samples. Both these standardization methods, however, differ markedly from the filter standardization results; Fig. 1 clearly shows that the use of the quench curve for homogeneous solutions (curve b) for efficiency corrections on the filter samples would lead to serious errors, especially in the higher weight ranges of cellular material (i.e., at higher values for the channels ratio). For example, for 1 mg of dry cellular material on the filter, the filter standardization method gives a counting efficiency of about 50–60%, depending on the channels ratio, which is some 15–20% less than that derived from the internal standard or homogeneous channels ratio. For about 10 mg of dry material, the difference in counting efficiencies is almost an order of magnitude.

Both the apparent internal and external counting efficiencies decrease with greater weights of cellular material. This might be attributed to an increase in the chemical or color quenching of the fluor solution due to the elution of quenching agents (chlorophylls, etc.) from the cells. But if the filters are removed from the vials and the solutions recounted, there is seen to be only a slight drop in the counting efficiency (6.8%) as calculated by external standardization (Table 1, col 5). Thus the presence of the filter and the weight of cellular material on it have some effect on the counting efficiency as determined by internal and external standardization, but this change cannot be predicted by the channels ratio method for the counting of homogeneous solutions.

It seems, therefore, that the standardization methods used by Wolfe and Schelske (1967) and Lind and Campbell (1969) produce results of limited accuracy and should not be used to standardize the method of counting ^{14}C-cellular material on filter papers. Instead, the filter standardization method outlined here should be adopted. This method apparently accounts for any self-absorption of the cells and the filter paper as well as any color or chemical quenching in solution, but these latter forms of quenching are small as there is only a very slight leakage of material from the cells on the filter.

A method similar to that outlined here has been used by Davies and Hall (1969) for counting tritiated macromolecules on filter papers. In a few cases they also used ^{14}C-macromolecules and found that for milligram levels of these macromolecules, counting efficiencies of 80–90% were obtainable. Their situation is slightly different from mine where the radioactivity is contained within cells, which often have very thick cell walls, but it does exemplify the fact that this channels ratio method, although obviously the most accurate, prob-

ably should be standardized for each type of material to be counted.

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DISTRIBUTION AND SIGNIFICANCE OF VITAMIN B_{12} AND THIAMINE IN THE SUBARCTIC PACIFIC OCEAN[1]

ABSTRACT

The distribution of vitamin B_{12} and thiamine was investigated in the Subarctic Pacific Ocean extending from Kodiak Island to the Pribilof Islands. Vitamin B_{12} concentration was found to range from undetectable levels up to 3.39 ng/liter and thiamine from undetectable levels up to 445 ng/liter. Vitamin B_{12} was present in 86% of the samples and thiamine in 74%. High concentrations, especially of thiamine, were found in low nutrient areas. Thiamine showed a fair negative correlation with PO_4-P, NO_3-N, and SiO_3-Si concentrations whereas vitamin B_{12} had no significant correlation with anything else measured. Enrichment experiments showed that thiamine increased the relative uptake of ^{14}C whereas vitamin B_{12} had no significant effects.

INTRODUCTION

It is known that vitamins, especially B_{12}, thiamine, and biotin, may play an important role in influencing the productivity of the marine environment. Laboratory studies of pure cultures of marine microorganisms have shown that one or more vitamins may be required to maintain growth, which has led to the assumption that these vitamins are present in the natural environment. Several attempts have been made to measure the distribution of vitamins in the marine environment using bioassay methods (Provasoli 1963; Riley 1965). Although most work has involved the distribution of single vitamins, recent attempts have emphasized the combined bioassay for more than one vitamin (Vishniac and Riley 1961; Natarajan 1968; Carlucci and Silbernagel 1967). This investigation presents data on the distribution of vitamin B_{12} and thiamine in an area of the Subarctic Pacific Ocean extending from Kodiak Island to the Pribilof Islands in the Bering Sea.

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MATERIALS AND METHODS

Thiamine was assayed with the marine yeast *Cryptococcus albidus* (Saito) Skinner which is sensitive in the range of 10–300 pg thiamine/ml (Natarajan and Dugdale 1966). Vitamin B_{12} was assayed with the marine diatom *Cyclotella nana* Hustedt Strain 3H which is sensitive in the range of 0–2 pg B_{12}/ml (Ryther and Guillard 1962).

Samples were collected during cruise 28 (August 1966) of the RV *Acona* (Fig. 1).

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