Comp. Biochem. Physiol., Vol. 63A, pp. 483 to 484
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0300-9629/79/0801-0483$02.00/0

AN ACCURATE MICROASSAY FOR MEASURING FILTRATION RATES OF SMALL INVERTEBRATES USING LATEX BEADS*

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(*Received* 16 *October* 1978)

Abstract—1. An indirect approach of measuring filtration rates by the clearance of uniform latex (polyvinyltoluene) beads from suspension is described.
2. Aliquots of suspensions are dried at 95°C. These dried samples are stable indefinitely.
3. Dried samples are eventually extracted with dioxane and read spectrophotometrically at 267 mμ.
4. Described approach lends itself to accurate measurements on small suspension feeders in small volumes and to the simultaneous running of multiple experimental chambers.

INTRODUCTION

The indirect approach of measuring filtration rates by the clearance of particles from suspension by aquatic organisms is well known (Coughlan, 1969; Jørgensen, 1975), where particle suspensions such as colloidal graphite or clay and cultures of algae have been used. The concentrations of particles have been commonly assessed photometrically or by the use of radioactively labelled algae cultures. The use of electronic counters have expanded the application of indirect methods and helped refine the interpretation of results due to the accurate determination of particle concentrations. Recently a variety of uniform particles have become available for assessing filtration. These include Sephadex beads and particles made from semipermeable polymer membranes surrounding solutions of proteins or cellular homogenates (Poulet & Marsot, 1978). Uniform latex beads are available which can be used as pure suspensions or with proteins adsorbed to the surface (Bangs & Kenny, 1976). The technique reported here provides an accurate microassessment of filtration using small volumes of suspended latex beads and tiny freshwater clams. The concentration of beads is assessed spectrophotometrically by reading the absorption of latex dissolved in dioxane. This approach facilitates the simultaneous running of large numbers of filtration experiments.

MATERIALS AND METHODS

Uniform suspensions of latex beads were purchased from Dow Diagnostics, P.O. Box 65811, Indianapolis, Indiana, 46268, USA. Beads composed of various latexes (Polystyrene, Styrene-Butadiene, Vinyltoluene/t-butylstyrene, Polyvinyltoluene, and Styrene-Divinylbenzene) and of diameters between 0.085 and 90.7 μ are available. All of these latexes have neutral or near neutral buoyancy in water with specific gravities of one or nearly one at 25°C (Bangs & Kenny, 1976). The assay has been developed using suspension of polyvinyltoluene (PVT) beads with a mean diameter of 2.020 μ (S.D. = 0.0135 μ) and a specific gravity of 1.027 at 25°C. These PVT beads are supplied at 10 *weight percent* aqueous suspension. Periodic agitation of this stock suspension is necessary to ensure uniform dispersion since prolonged standing results in settling of some beads. For experiments with freshwater systems, dilutions of PVT beads remain in suspension long beyond the necessary few hr. We have not been successful in making dilute suspensions in filtered sea water (natural or synthetic) since the beads immediately clump. However, others have been regularly using suspensions of the same size PVT beads in conjunction with an electronic counter for filtration experiments with marine organisms (personal communication, Leslie G. Williams, August, 1978). Apparently the use of sulfonate surfactants during the process of making these beads and the subsequent washing leaves varying trace levels of surfactant in each batch. According to Leigh Bangs (personal communication, August, 1978) the variation in surfactant level explains why some stock suspensions of latex beads cannot be suspended in sea water. One can wash these beads with commercially available surfactants to insure reliable suspensions in sea water.

This assay was developed using the tiny sphaeriid clam, *Musculium partumeium*. These clams range in size from about 1.4 mm to about 9.0 mm shell length corresponding to tissue dry weights of about 0.05 and 5.0 mg respectively. Most adults grow to shell lengths of between 5.0 and 7.0 mm with tissue dry weights between 1.0 and 2.0 mg. Measurements of filtration can be made on a single adult whereas approximately 15 of the smallest clams must be placed in a chamber in order to obtain reliable results. Immediately prior to each experiment a dilute suspension of 10% PVT is prepared by mixing three drops in 250 ml of filtered pond water. Each animal or group of clams was placed in a 50 ml Erlenmeyer flask with 10 ml of filtered pond water and allowed to equilibrate for about 30 min at constant temperature. Ten ml of the dilute PVT suspension was added to each chamber (10 ml filtered pond water + 10 ml of dilute PVT suspension = 20 ml total volume of experimental chamber) with clams and initially stirred. Clams were allowed to filter undisturbed for four hr in the experimental chambers. Their respiratory-feeding current provides adequate mixing of the suspension during experiments. Control chambers without clams for initial PVT concentrations and blank chambers with only filtered pond water (no PVT or clams) were also established. At the end of each experimental run, a 10 ml aliquot of each experimental (*s*), control (*c*), and blank (*b*) chamber is dried at 95°C for 24 hr in separate foil covered 100 ml beakers. It is critical that experimental aliquots are not drawn from

* Supported by grants from The University of Dayton Research Council.

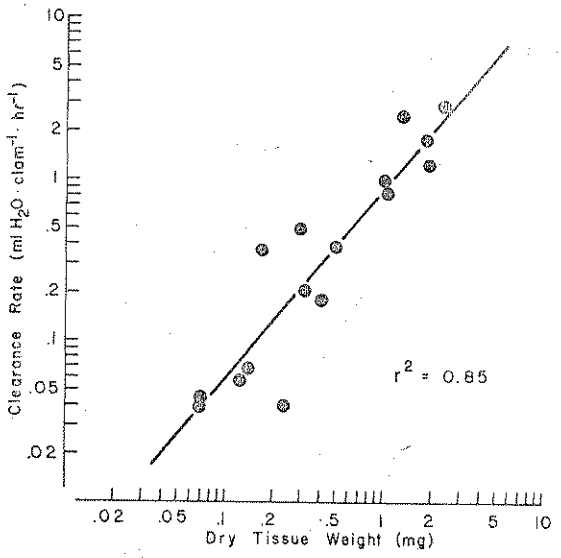

Fig. 1. Filtration rates in relation to body mass for *Musculium partumeium* at 22°C.

the bottom of the chamber in order to avoid PVT laden feces and pseudofeces. Dried samples are stable indefinitely. Each dried sample was eventually extracted with 2.0 ml of dioxane and read against pure dioxane at optimum absorption for PVT at 267 mμ (Weisman & Korn, 1967) with a Beckman DU spectrophotometer. Dissolved material in filtered pond water makes a small but significant contribution to the readings at 267 mμ. This can be corrected for from readings on dioxane extracts of dried filtered pond water (b). Values for control concentrations of PVT (c) and corrections for pond water (b) represent the average absorption of at least three values. By comparing the final concentrations ($s - b$) of the experimental chambers with the initial concentration ($c - b$) it is possible to calculate the clearance rates from an equation which is equivalent to those used by many others (Coughlan, 1969).

$$m = \frac{M \cdot \log_{10}(E_c^- - E_b^-) - \log_{10}(E_s^- - E_b^-)}{\log_{10} e \cdot t}$$

where m = clearance rate, M = volume of suspension, E_c^- = average absorption of control: equivalent to initial concentration of PVT plus absorption due to dissolved material in filtered pond water. E_s^- = absorption of experimental: equivalent to final concentration of PVT plus absorption due to dissolved material in filtered pond water. E_b^- = absorption of blank: value represents contribution of dissolved material in filtered pond water. t = experimental time period, e = the natural base of logarithms.

RESULTS AND DISCUSSION

Figure 1 gives a set of typical results obtained on *Musculium partumeium* using this technique.

The use of latex beads is convenient since stocks can be maintained on the laboratory shelf. Moreover, the use of these beads for measurements of filtration lends itself to experiments with accurate controls on particle size and concentration. However, these beads are not a natural food material and may influence the filtration rates of experimental suspension feeders due to "unpalatability" or lack of "palatability". The assay outlined here lends itself to the simultaneous running of multiple experimental chambers on the same day and the reading of results at convenience when the dried samples are extracted with dioxane. Since latex beads are not a natural food source, the results of Fig. 1 cannot be claimed to represent "absolute" or "natural" rates. However, this type of data is excellent for examining certain adaptational patterns and can be corrected by comparison to rates determined on suspensions of more natural food sources. We are using this technique to collect the large amount of data necessary to properly assess seasonal changes in the filter feeding strategy of populations of clams adaptated to natural environmental conditions.

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