of the processes underlying the reported relaxation effect is not yet clear. The effect is similar to the relaxation process reported previously in muscle tissue (*5*), except for a time constant which is about one-twentieth as large as that for blood and about 0.1 msec. The effect may be indicative of a double-layer structure of the ghost envelope due to two different molecular components of different dielectric losses (*6*).

Even more likely is the existence of a mechanism which restricts ion exchange across the membrane and which necessitates about 0.1 msec to become effective. The fact that similar relaxation effects could not be observed in normal cell suspensions (*1*) could be related to an increase in permeability in the case of hemolyzed cells and consequent shift of the relaxation spectrum from frequencies too small to be investigated up to the range demonstrated. The simple frequency dependence of the membrane properties of lysed cells reported here and of muscle cells reported previously (*5*) makes it necessary to differentiate between "static" and "dynamic" permeability values, the former applying to long-time, and the latter to short-time, stimuli, and with the transition characterized by a single rate constant in the case reported in this article.

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Suspension Counting of Carbon-14 in Scintillating Gels

Several techniques have been described for the incorporation of weak alpha and beta emitters into a scintillation counting medium in order to achieve the high detection efficiency, short resolving time, and energy-discrimination facilities which are inherent in scintillation counting methods. These techniques have included direct solution

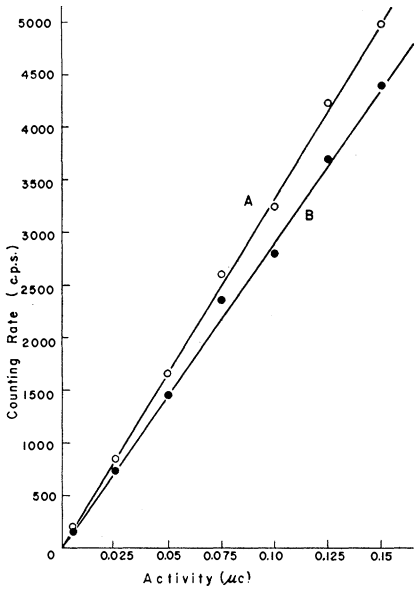

Fig. 1. Linear dependence of counting rate on activity with fixed weight (0.1770 g) of suspended $BaCO_3$ in 10 ml of gel. Curve *A* was obtained with 1.5 kv on the photomultiplier tube, curve *B* with 1.4 kv.

in an organic medium (*1*), the use of inorganic salts in water-dioxane solutions (*2*), the syntheses of nonquenching solvents (*3*), and the use of quaternary ammonium salts (*4*). Hayes (*5*) described the counting of tagged materials in suspension in liquid scintillators. The disadvantage of rapid settling of the suspended material inherent in this method was overcome by the use of scintillating gels as described by Funt (*6*) and more recently by White and Helf (*7*). In this article we report an investigation of the counting efficiencies of scintillating gels in the estimation of C^{14} and their dependence on the concentration and specific activity of the suspended material.

Samples containing $BaC^{14}O_3$ of known specific activity were prepared from active Na_2CO_3, obtained from Atomic Energy of Canada Limited. Scintillating gels were formed from a liquid scintillator solution containing 4.0 g/lit of *p*-terphenyl and 0.1 g/lit of 1,4-bis 2,5-phenyloxazolyl benzene (POPOP) (*8*). To this solution, 70 g/lit of aluminum stearate was added for the formation of rigid gels, and the desired weight of $BaC^{14}O_3$ was then incorporated. A 10-ml scintillator volume was used, and the precipitate was dispersed uniformly throughout the colloidal solution by vigorous shaking. Gelation was produced by inserting the glass vial containing the suspension into water at 80°C. Vials 22 by 10 cm were used in the preparation and measurement of the samples. The vials were surrounded by MgO reflectors, and the corks were lined with aluminum foil for the counting experiments. They were bonded with silicone fluid to Dumont K1295 or K1190 photomultiplier tubes. The counting equipment consisted of a standard scintillation counting assembly, including cathode follower, Atomic 204 linear amplifier, constant voltage supply, Dynatron N/101 discriminator, and Tracerlab 105 scaler.

The linear dependence of counting rate on specific activity is illustrated in Fig. 1. For this experiment, a constant weight of $BaCO_3$ (0.1700 g) was suspended in 10 ml of the gel. In the range of specific activities from 0.005 to 0.150 μc, the counting rate was found to be linearly dependent on the activity. For the preparation of these samples, a constant volume of Na_2CO_3 solution, prepared from varying ratios of active and inactive Na_2CO_3 of the same molarity, was used. The tests were conducted with samples of high activity, and it was thus possible to set the discriminator to count all pulses greater than 4 v without unduly increasing the background and noise counting rate contribution. In Fig. 1, curve *A* was taken with a potential of 1.5 kv on a 1-inch K1190 photomultiplier tube mounted in a massive lead shield. Under these conditions, an average background counting rate of approximately 50 count/sec was obtained in a counting rate (corrected for background) of between 200 and 5000 count/sec. For samples of lower activity, it would be desirable to reduce the background count. A lower potential on the photomultiplier tube (1.4 kv) produced curve *B* with the same discriminator settings. In this instance, the background was reduced to approximately 18 count/sec. However, the over-all counting efficiency was reduced as a result of this drop in amplification, and a greater

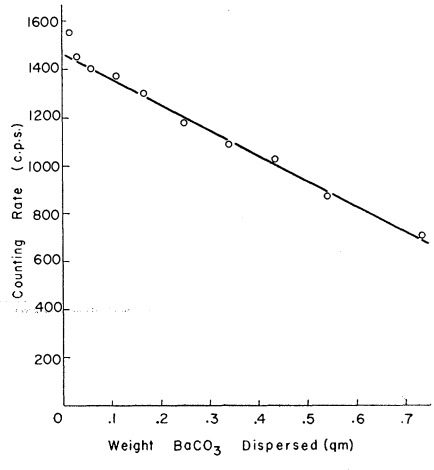

Fig. 2. Variation of counting rate with weight of suspended material at constant activity (0.05 μc).

Table 1. Optical transmittance of scintillating gels of aluminum stearate. I, transmitted radiant power; I_o, incident radiant power. The absorbance $A = abc$, where a is the absorptivity, b is the thickness of the cell, and c is the concentration in grams per liter.

Al stearate concn. (g/lit)	5000 A		4500 A	
	I_o/I	log I_o/I	I_o/I	log I_o/I
10	1.015	0.0064	1.029	0.0123
30	1.068	0.0286	1.092	0.0382
50	1.098	0.0407	1.128	0.0522
70	1.138	0.0561	1.178	0.0712

decrease would be inevitable if the discriminator bias were set higher in order to further reduce the background contribution for less active samples.

The variation of counting efficiency with total weight of precipitate at a constant specific activity is shown in Fig. 2. The samples were prepared from a fixed volume of active Na_2CO_3, mixed with varying volumes of inactive Na_2CO_3 of the same molarity. For this experiment a total activity of 0.05 μc was maintained in each sample. The counting data were obtained on a Dumont K1295 2-inch photomultiplier tube. The counting efficiencies dropped from 75.6 percent for 1 percent by weight of precipitate to 37.2 percent for 7.5 percent by weight of precipitate in these tests. The absolute counting efficiencies quoted are based on the stated activity of the Na_2CO_3 solution used, but the indicated accuracy of this source was ± 10 percent, and the calculated efficiencies may be in error by this amount. The effects of particle size and density were not investigated, but it seems obvious that settling and self-absorption effects would be reduced if a material of lower density than $BaCO_3$ (density 4.4) were used.

Some gels were formed in evacuated cells in order to eliminate the effects of oxygen quenching (*9*). An increase in pulse height and in counting efficiency was observed under these conditions. For example, the count of a sample containing 0.1665 g of $BaCO_3$ and 0.05 μc activity increased from 1360 to 1585 count/sec.

The optical transmission of the gels was also investigated. The thickened systems used by White and Helf (*7*) appeared to have poor transmittance as judged from the published photographs of their samples. The transmission characteristics of aluminum stearate gels were measured with a Beckman DK-1 recording spectrophotometer using 1-cm quartz cells. The data at 5000 and 4500 are shown in Table 1.

The data of Table 1 yielded a linear Beer's law plot whose slope gave a value for the absorptivity a of 0.00082 lit/gm-cm at 5000 A and 0.00104 at 4500 A.

Settling effects were negligible after the first day. A sample containing 0.05 g of $BaCO_3$ gave identical results within experimental error after 3 weeks' standing.

These counting experiments indicate that the scintillating gel technique provides a simple and effective means of assessing the activity of beta and alpha emitters. High sensitivity, good reproducibility, and linearity of response with activity are observed over a useful range of suspended material. The method compares favorably with the new technique recently described by Passman, Radin, and Cooper (*4*). In the same scintillator volume, about 7 times as much carbon can be incorporated as by their CO_2 method. The counting efficiencies appear better than those recently reported by White and Helf (*7*) for scintillating gels. This may be due to the superior optical properties of the gels reported here, but much of the difference is undoubtedly due to the wider gate settings used in our discriminator. In comparison with other techniques for assaying C^{14}, (*10*), scintillating gels offer many advantages. The samples are readily stored, vacuum manifolds are not needed, and compounds can be measured directly without the necessity of combusting or destroying the sample. The over-all sensitivity is superior to that obtainable with most gas counting techniques, and pulse height discrimination can be employed when one is counting radiation of differing energy (*11*).

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Potentially Simple Technique for Rearing "Germ-Free" Fish

"Germ-free" animals, those deprived of detectable microorganisms, are highly useful for studies in nutrition, disease, infection, and immunity. Investigators who have felt the need for this type of animal for the past 50 years have encountered many difficulties because rearing germ-free animals is a laborious and complicated procedure. Many complex techniques are involved, the necessary equipment is elaborate and costly, and the animals require careful and constant attention (*1*).

In the course of some experiments on the role of oral incubation in the cichlid fish, *Tilapia macrocephala*, L. R. Aronson and I (*2*) discovered that dipping fish eggs into a 0.04-percent formaldehyde-aquarium water solution for 10 minutes resulted in germ-free eggs which, when transferred aseptically to sterile water, grew into germ-free fry. Of 25 trials (consisting of 10 eggs per trial) treated in this manner (*3*), eight trials remained germ-free died during the experiment, Those embryos which did not remain germ-free died during the experiment, since certain of the bacteria proved to be lethal contaminants (*2*). The germ-free embryos, however, survived for 2 to 3 weeks after hatching, using the large quantity of yolk as their source of food. When these fry eventually died of starvation after the yolk was absorbed, they did not disintegrate. Some of the dead fish were kept as long as 4 months, when they were discarded. No microorganisms could be cultured from a number of the dead fry when culture was attempted in Difco nutrient broth, Difco nutrient agar, and thioglycollate media. Likewise, no mold and fungal growths were detected.

Since this problem is not related to the work of the department of animal behavior, it was not pursued, but the observations suggest that *Tilapia macrocephala* could be fed aseptically and brought to germ-free maturity in an appropriately equipped bacteriological laboratory with fewer of the complications that are encountered in other forms. For example, Baker and Ferguson (*4*) found