Reports

Dielectric Properties of the Membrane of Lysed Erythrocytes

The dielectric properties of the (plasma) membrane of normal erythrocytes are frequency independent at frequencies below 10 kcy/sec. (*1*). On the other hand, the properties of the membrane of hemolyzed erythrocytes have been reported to change with frequency in the range from 500 cy to 100 kcy/sec (*2*). Data available at the beginning of the present investigation (*3*) were not adequate to determine whether this frequency dependence extended over many octaves, as anticipated from a study of a (plasma) membrane whose dielectric properties change as a power function of frequency (*4*), or whether it is limited to a smaller frequency spectrum similar to that reported by one of us in the case of muscle cells (*5*).

For these reasons, it was decided to measure the capacitance C (in micromicrofarads) and resistance R (in ohms) of suspensions of hemolyzed erythrocytes, extending the range of observation to frequencies as low as possible. As a first step, cells hemolyzed with distilled water were investigated. Heparinized fresh beef blood was washed with Ringer Tyrode at *p*H 7.6 and centrifuged to high cellular volume concentration. The erythrocytes were resuspended in triple their volume of distilled water. Measurements were carried out at 25°C and evaluated under precautions previously summarized (*1*). Figure 1 shows the results obtained from one typical experiment. The points represent individual measurements.

If the dispersion arises from a relaxation process with a single relaxation time $T = 1/2\pi f_0$, it must be possible to represent the data by the following relationships:

$$C = C_\infty + \frac{C_0 - C_\infty}{1 + (f/f_0)^2};$$

$$R = R_0 - (R_0 - R_\infty)\frac{(f/f_0)^2}{1 + (f/f_0)^2}$$

where C_0, C_∞ and R_0, R_∞ are the low- and high-frequency limits of the capacitance and resistance, and f_0 is the relaxation frequency determined by the magnitude of the dispersion of resistance and capacitance as follows:

$$R_0 - R_\infty = 2\pi f_0 (C_0 - C_\infty) R_0 R_\infty$$

The derivation of these equations is based only on the assumptions of linearity and exponential response of polarization to a step-function potential. Introducing the measured values of $R_0 - R_\infty = 1.18$ ohm and $C_0 - C_\infty = 34$ μμf, we determine $f_0 = 1.9$ kcy/sec. The curves corresponding to these parameters have been drawn as solid lines in Fig. 1. Agreement between measurement and theory is seen to be as exact as measurements could be obtained: resistance, ±0.001 percent; capacitance, ±[1/*f* (kcy/sec) ±0.5] percent).

The results obtained do not depend on current strength, and thus they characterize the passive behavior of the lysed erythrocyte suspension. The capacitance of the (plasma) membrane, as calculated from the present data above 10 kcy/sec, is about 0.9 μf/cm² of surface. The dielectric constant of the solution is almost completely controlled by the contributions of the (plasma) membrane. Hence, the dispersion in the capacitance reflects proportionally a dispersion in the capacitance of the membrane itself.

The magnitude of the resistance of the suspension, on the other hand, is controlled largely by the suspending fluid, and the dispersion caused by the presence of the membranes is a very small fraction of the total resistance. Hence, it is impossible to compute the resistance of the membrane in the same manner as is possible to compute the capacitance. However, the low frequency dispersion reported here establishes an upper limit of the membrane resistance above 10 kcy/sec. If the membrane were a perfect insulator at low frequencies (below 100 cy/sec), it follows from membrane capacitance and ratio of total conductance and capacitance change that the resistance of the membrane above 10 kcy/sec is 100 ohm-cm².

The phenomenon of frequency-dependent dielectric properties of the (plasma) membrane of hemolyzed erythrocytes, as demonstrated, proves that biological interfaces do not necessarily behave in the manner predicted by polarization theory (*4*). The exact nature

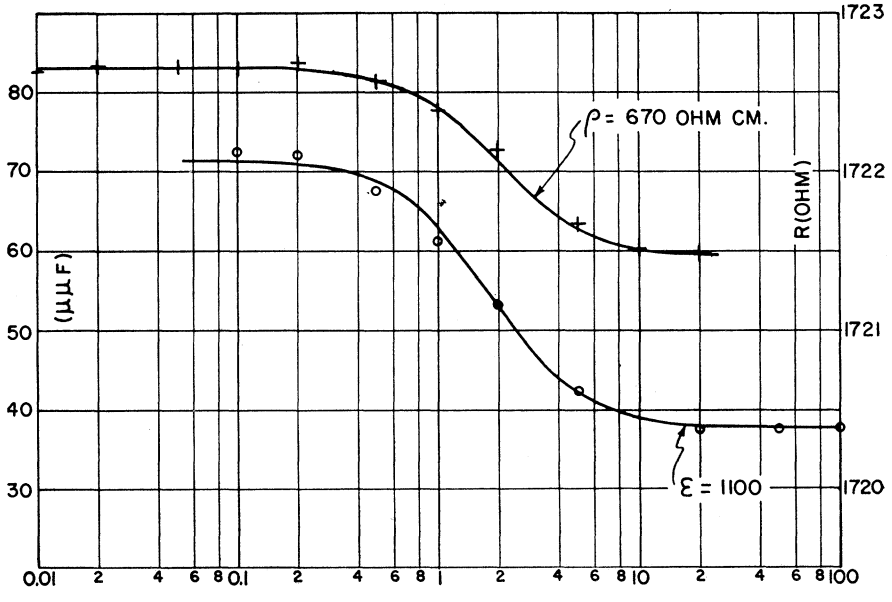

Fig. 1. Frequency dependence of capacity in micromicrofarad and resistance in ohm of lysed erythrocyte suspension. Frequency in kilocycles per second; dielectric constant ε = 1100 above 10 kcy/sec; specific resistance ρ = 670 ohm-cm.

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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.

H. P. Schwan
E. L. Carstensen
Electromedical Division, Moore School of Electrical Engineering, and School of Medicine, University of Pennsylvania, Philadelphia

References and Notes

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18 January 1957.

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

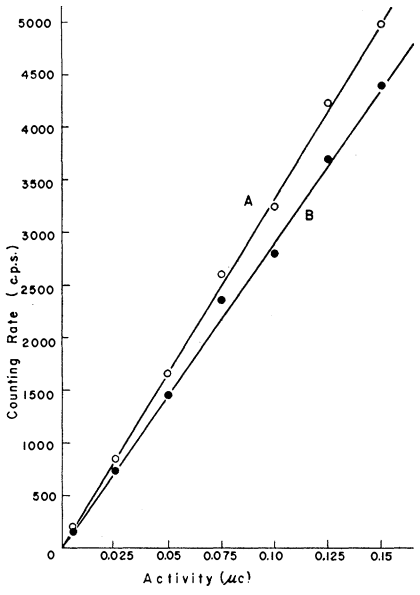

Fig. 1. Linear dependence of counting rate on activity with fixed weight (0.1770 g) of suspended $BaCO_3$ in 10 ml of gel. Curve *A* was obtained with 1.5 kv on the photomultiplier tube, curve *B* with 1.4 kv.

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

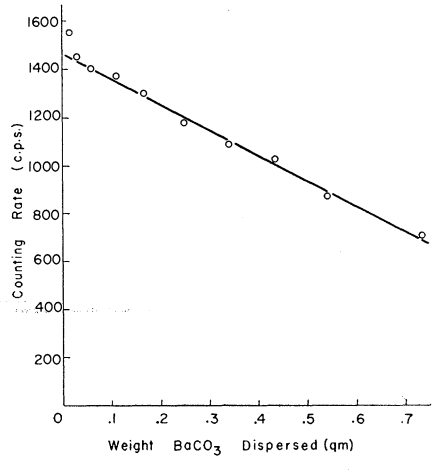

Fig. 2. Variation of counting rate with weight of suspended material at constant activity (0.05 μc).