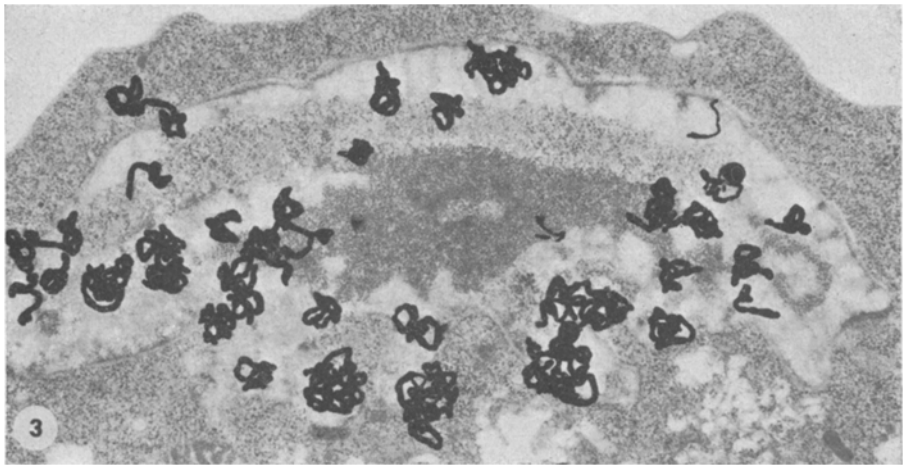

Fig. 3. Autoradiograph (^{3}H-thymidine) of a nucleus in a poliovirus infected cell, 6 h p.i. The silver grains are found over the destained, condensed chromatin. The RNA-containing structures are free of silver grains and retain their normal contrast. ×15,000.

and leaving all other parameters constant, because changing developing and fixation time or concentration might affect the ultrastructural preservation adversively.

In order to find the substance responsible for the destaining effect, we tested the developer (Microdol X), another developer (D 19), the fixer and the acetic acid alone or in different combinations. It was found that a destaining effect on sections not coated with emulsion only appeared if the sections were in contact with one of the developers. The fixer, the acetic acid alone, or both in combination, did not change the staining pattern.

Likewise, the high pH-values of the developers are not responsible for the destaining of DNA: distilled water, adjusted to the pH of the developers (7.9 for Microdol X and 10.7 for D 19) has no destaining effect. The two substances (Metol and sodium sulfite) common to both developers, were tested for their destaining action: only sodium sulfite (14%) showed the same Uac extracting property as the complete developer.

Thus, the method described can be used not only with autoradiographs but with every aldehyde-osmium fixed specimen by floating the block stained sections for 5 min empirically by changing the acetic acid treatment only on a 14% sodium sulfite solution and afterwards adjusting the degree of destaining by the acetic acid treatment prior to a lead citrate stain. This procedure might be useful if a distinction between RNA and DNA with already conventionally fixed and embedded material turns out to be necessary later in the course of an investigation.

Summary. A simple method for distinction between RNA- and DNA-containing structures in aldehyde- and osmiumtextroxide-fixed electron microscopic autoradiographs (or ordinary thin sections) is described: the developer and the acetic acid used for processing autoradiographs extract selectively uranium acetate from DNA containing-structures which, after staining with lead citrate, leads to a characteristically 'bleached' appearance of the DNA.

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Evaluation of a Conductometric Method to Determine the Volume Fraction of the Suspensions of Biomembrane-Bounded Particles

Although frequent need has been felt for a simple and rapid method to determine the volume concentration of cells or isolated organelles in suspension, there have so far been few methods applicable to routine biological work. In this communication we propose a new method which is based on simple conductometry and can be used as a substitute for the conventional microscopic and space-marker methods, provided the suspended particles are bounded by intact, limiting membranes and their electrical conductivity is several orders of magnitude smaller than that of the suspending medium.

Principle. According to theories[1,2], the relative conductivity of a suspension of non-conducting spheres is related to its volume fraction Φ as:

$$\varkappa/\varkappa_m = (1-\Phi)^{1.5} \quad (1)$$

where $\varkappa$ and $\varkappa_m$ are the conductivities of suspension and medium, respectively. Remarkable points of Eq. 1 are: that since derived originally in an effort to extend the MAXWELL-WAGNER theory[3] on the conductivity behavior of dilute dispersions of spherical particles to more concentrated ones, this relation would hold for any values of Φ, and that a variety of experiments including suspensions of glass beads[1] and oil-in-water type emulsions[4,5] have given strong evidence for the usefulness of Eq. 1 in dealing not only with monodisperse but with moderately polydisperse systems. In addition, that some anomaly in particle shape, unless extreme in its extent, is allowable, has also been confirmed[1] by experiment. Hence it is reasonable to expect that we can determine the electrically effective volume fraction Φ_E of a given suspension

[1] R. E. DE LA RUE and C. W. TOBIAS, J. electrochem. Soc. *106*, 827 (1959).
[2] T. HANAI, Kolloidzeitschrift *171*, 23 (1960).
[3] K. W. WAGNER, Arch. Electrotech. *2*, 371 (1914).
[4] T. HANAI, N. KOIZUMI, T. SUGANO and R. GOTOH, Kolloidzeitschrift *171*, 20 (1960).
[5] R. E. MEREDITH and C. W. TOBIAS, J. electrochem. Soc. *108*, 286 (1961).

Conductometric determination of mean cell volume of mouse lymphoma L5178Y cultured in Fischer's medium supplemented with 10% bovine serum

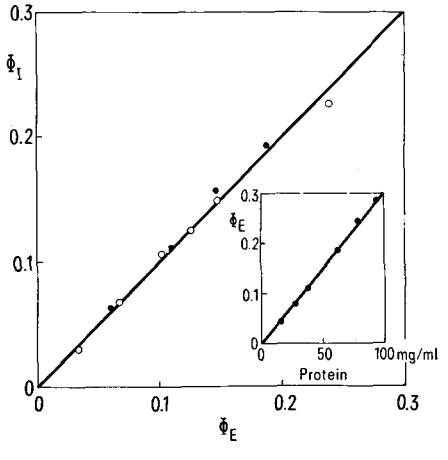
Suspension No.	Temperature (°C)	G[a] (μmhos)	G_m[a] (μmhos)	G/G_m	Φ_E	Cell No.[b] (ml^{-1})	Mean cell vol.[c] (pl)
1	25	5050	7992	0.633	0.263	2.42×10^8	1.09
2	30	8508	9097	0.935	0.0439	4.07×10^7	1.08

[a] Conductances were measured at a frequency 1 kHz. Accuracy, within 0.2%. [b] Counted with a hemocytometer after appropriate dilution. [c] Estimated from Φ_E divided by cell No. in ml^{-1}.

of biological particles only through measuring the ratio $\varkappa/\varkappa_m$ and using Eq. 1, when the constituent particles are assumed to behave as an insulator to externally applied electric field.

Procedure. 1. Equilibrate the particulate material with the suspending medium. 2. Transfer a small aliquot of suspension to a conductivity cell, and wait for thermal equilibration. 3. Read conductivity $\varkappa$ (or conductance G) with a Wheatstone bridge or a conductivity meter, both operated at sufficiently low frequency. 4. Read $\varkappa_m$ (or conductance G_m) for the particle-free medium. 5. Calculate Φ_E using Eq. 1.

Comments on practice. 1. When replacement of test media is required, washing by centrifugation and resuspension repeated 3 times is quite effective. We saved the final washings for the determination of $\varkappa_m$ or G_m. 2. The cell we used was of an all-glass, water-jacketed type with platinized Pt disc electrodes. The sample cavity was about 0.2 ml. Such a small volume is prerequisite for measuring samples of very low yield and also convenient for dealing with a single preparation under various conditions. 3. The measuring frequency should be chosen as low as possible; otherwise, the measurement will be seriously affected by a conductivity dispersion phenomenon[6] which leads to an erroneously low Φ_E. Temperature regulation (preferably, to within 0.05 °C) is another important factor for making reliable measurements. 4. If we read G_m under the same measurement condition as for G, the ratio G/G_m may immediately replace $\varkappa/\varkappa_m$ in Eq. 1. 5. The use of a nomogram representing Eq. 1 will facilitate the calculation of Φ_E.



Comparison of simultaneous determinations of volume fractions, Φ_I and Φ_E. The test suspensions of varied volume fractions were made of synaptosomes(●), prepared by the method of GRAY and WHITTAKER[7], or of liver mitochondria (○), prepared by the method of HOGEBOOM[8], both from Wistar rats. Composition of the suspending medium (mM): NaCl, 50; sucrose, 180; and *Tris*-HCl, 10, pH 7.3 at 25 °C. Osmolality, 0.28 Osm · kg^{-1}. Inset: A linear relationship between Φ_E and protein content (determined by a biuret method with bovine serum albumin as standard) for isolated liver mitochondria[8] in suspension. Medium as above. Specific volume of the specimen was readily calculated from slope of the line.

Comparison with the other methods. As a check for reliability of the method described, we first compared the determinations of mean cell volume by the conductometric and the microscopic techniques. The specimen used was a random population of a cultured cell line, mouse lymphoma L5178Y, with excellent sphericity in shape; its cell volume was calculated to be 1.05 pl by using a mean diameter of 12.6 μm estimated from phase microscopy. With the same population, the conductivity measurements were carried out and the result is shown in the Table. It is apparent that the final estimates for cell volume agreed very well with the microscopic one (1.05 pl).

Next we examined a possible equivalence of the conductometric and the space-marker determinations on the suspensions of subcellular organelles (mitochondria and synaptosomes). Using ^{14}C-inulin as the space-marker, we determined the volume fraction of inulin-inaccessible space Φ_I on 0.1 ml aliquots of the suspensions of known Φ_E by means of combined centrifugation and radiocounting. The results of such a simultaneous determination of Φ_E and Φ_I are plotted in the Figure. Within experimental errors, the observed points fell on a line of identity showing a satisfactory agreement between the two independent methods compared. In addition, subsidiary evidence was provided by demonstrating a linear relationship between Φ_E and the particulate concentration such as expressed on a protein basis (see Figure, inset).

Finally, it may be relevant to point out the osmotic behavior of these isolated organelles in suspension. When the effect of medium osmolality upon the specific volume of particles was examined using the conductometric method described above, we could confirm the Boyle-van't Hoff relation obtained previously with a light scattering technique[9]. Such a finding made it possible to estimate the osmotic dead space of synaptosomes to be 46% of the volume at 0.3 Osm/kg.

[6] H. P. SCHWAN, in *Advances in Biological and Medical Physics* (Eds. J. H. LAWRENCE and C. A. TOBIAS; Academic Press, Inc., New York 1957), vol. 5, p. 147.

[7] E. G. GRAY and V. P. WHITTAKER, J. Anat. *96*, 79 (1962).

[8] G. H. HOGEBOOM, in *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN; Academic Press, Inc., New York 1955), vol. 1, p. 16.

[9] K. KAMINO, K. INOUYE and A. INOUYE, Biochim. biophys. Acta *330*, 39 (1973).

Limitations. The use of a medium of much lowered conductivity or the case of very leaky membranes reduces accuracy of the measurement accordingly. Likewise, this method is difficult to apply to very dilute suspensions because the ratio $\varkappa/\varkappa_m$ approaches unity in this case. Shape complexity as found in red cells[10], for example, also impairs the straightforward application of Eq. 1, though not altogether inapplicable to spheroids or ellipsoids such as isolated mitochondria. With regard to other shape effects, a modification of the basic equation, e.g. by introducing an empirical parameter other than 1.5 into the exponent of Eq. 1, has been reported to be quite effective[1,11].

Summary. Supported by the fact of correspondence between the results of several independent techniques compared, we recommend here a conductometric method as a simple, nondestructive and reliable tool for determining the volume fraction of the suspensions of membrane-limited particles of biological relevance. It requires only conductivity measurements on a suspension and its medium[12].

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[10] H. Fricke, Phys. Rev. *24*, 575 (1924).

[11] R. E. Meredith and C. W. Tobias, in *Advances in Electrochemistry and Electrochemical Engineering* (Eds. P. Delahay and C. W. Tobias; John Wiley & Sons, Inc., New York 1962), vol. 2, p. 15.

[12] We wish to thank Dr. Y. Doida, Dept. of Experimental Radiology, Kyoto University, for his kind supply of L5178Y cells with the micrographic data. This research was supported in part by a grant from the Ministry of Education, Japan.

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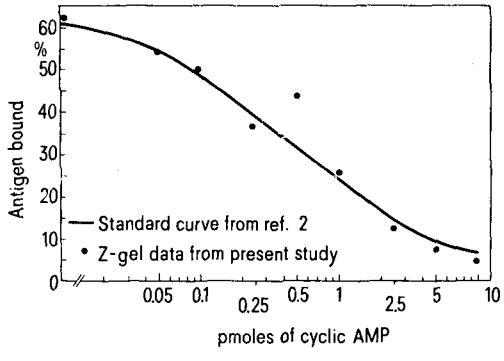
A Technical Improvement in the Radioimmunoassay of Cyclic-AMP

The radioimmunoassay of Steiner et al.[1] is a method which allows for the rapid measurement of cyclic-AMP in a large number of samples. The procedure involves the competition between isotopically labeled and unlabeled cyclic-AMP for specific antibody present in limiting amounts. As a result, the quantity of labeled antigen-antibody complex which is formed is inversely related to the amount of unlabeled antigen present. The quantity of unlabeled cyclic-AMP in a sample is then determined by comparison with a standard curve[2].

Separation of free from bound antigen has been achieved by precipitation of the complex with a 60% solution of saturated ammonium sulfate[2]. This suffers from the drawbacks that, owing to the small volume of the reaction mixture, the pellet obtained following centrifugation is very small and minute loses can cause significant variations. In addition, the ammonium sulfate precipitate is physically unstable. Thus, there tends to be a progressive redispersion of the pellet into the supernatant with standing, which can result in loss during the subsequent decantation. This potential source of error becomes especially important when a large number of samples are being processed, due to the longer time required.



Percent antigen bound as a function of the amount of unlabeled cyclic AMP present in the reaction mixture. Points: Z-gel precipitation of bound antigen-antibody complex, mean values of 2 experiments. Curve: standard curve using ammonium sulfate precipitation redrawn from reference[2] (with permission).

The present study was designed to test the feasibility of replacing the ammonium sulfate precipitation with zirconyl phosphate gel (Z-gel). The latter has been used for precipitation in the radioimmunoassay of carcinoembryonic antigen[3-5].

Materials and methods. The assay of cyclic-AMP followed the procedure outlined in the Schwarz-Mann technical bulletin[2] with the exception that Z-gel (Roche, Nutley, N.J.) was substituted for ammonium sulfate in the precipitation step. The labeled antigen was a cyclic-AMP derivative, succinyl cyclic-AMP tyrosine methyl ester [^{125}I], (Scamp-TME). The reaction was carried out as follows: 100 µl Scamp-TME was added to glass tubes containing 300 µl of sodium acetate buffer (pH 6.2). 100 µl of cyclic-AMP antiserum was then added to each tube. The reaction was allowed to proceed for 1 h at 0–4 °C. To determine the optimum quantity of Z-gel required to precipitate the cyclic-AMP-antibody complex, Z-gel additions, ranging from 10% to 200% of the total volume, were made. The tubes were then allowed to stand for 0.5 h on ice, centrifuged at $800 \times g$ for 10 min at 4 °C, decanted, swabbed, and the residual solids radioassayed in a crystal scintillation counter.

To determine if the Z-gel non-specifically precipitated unbound Scamp-TME, tubes containing only Scamp-TME in buffer were precipitated with varying amounts of Z-gel, centrifuged, decanted and counted. The pellet was then washed with 2.5 ml of sodium acetate buffer, centrifuged again and recounted. The second counting was then compared to the first count to ascertain the amount of Scamp-TME 'trapped' by the Z-gel.

To determine the stability of the antigen-antibody complex following Z-gel precipitation and washing, Scamp-TME + antisera were allowed to react and

[1] A. L. Steiner, D. M. Kipnis, R. Utiger and C. Parker, Proc. natn. Acad. Sci., USA *64*, 367 (1969).

[2] Schwarz/Mann Product Bulletin, Cyclic-AMP Radioimmunoassay Kit (Schwarz/Mann, Orangeburg, N.Y. 1972).

[3] H. J. Hansen, K. P. Lance and J. Krupey, J. clin. Res. *19*, 143 (1971).

[4] D. M. P. Thomson, J. Krupey, S. O. Freedman and P. Gold, Proc. natn. Acad. Sci., USA *64*, 161 (1969).

[5] T. M. Chu and G. Reynoso, Clin. Chem. *18*, 918 (1972).