CONDUCTIVITY AS A MEASURE OF THE PERMEABILITY OF SUSPENDED CELLS.

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(Accepted for publication, November 10, 1924.)

The electrolytic conductance of living cells has been used extensively as a measure of their permeability to electrolytes, and more particularly of the permeability of an hypothetical semipermeable plasma membrane supposed to delimit the protoplasm from the surrounding medium. There are, however, many possible sources of error in making such an interpretation, especially when suspensions of cells are studied. This paper is a critical consideration of this phase of the subject, and exposition of the precautions and corrections suggested by rather extended intensive study of the conductance of bacteria, red blood cells, unicellular algae, and yeast.

I.

The Influence of Cell Volume on the Conductance of Suspensions.

When studying the conductance of cell suspensions it becomes necessary to take many precautions in order to arrive at a fair estimate of the conductance of the cells themselves. The importance of concurrent determinations of the conductance of the suspending fluid and suspension has been emphasized by Green and Larson\textsuperscript{1,2} and by the writer in previous papers\textsuperscript{3,4} in which it was shown that the net conductance as therein defined is a fair measure of the relative conductance of normal cells. But under abnormal conditions the volume of

\textsuperscript{1} Green, R. G., Papers from The Mayo Foundation for Med. Education and Research and the Med. School, 1921, i, 577.
the suspended cells may change, and the net conductance (which is in reality a measure of resistance) must be corrected for the volume of the cells if their true relative conductance is to be determined. How is this volume to be estimated? This can be answered, at least in part, for red blood cells and bacteria, which will be considered separately. Adequate study has not yet been given to the cases of yeast or the green alga Chlorella.

a. Red Blood Cells.—The true volume of red blood cells has been sought by many methods which fall under about five heads: quantitative determination of certain ingredients in defibrinated blood, serum, and serum-free cells; estimation of certain substances in cells and surrounding fluid before and after dilution; estimation of the concentration in the surrounding fluid of a substance added to a suspension of blood cells; relative conductance of suspension and suspending fluid; and centrifugation of a cell suspension in a graduated tube until the mass of cells becomes translucent (hematocrit). In the hematocrit method the cell mass is supposed to become translucent only when all differences in refractive index at the fluid-cell surfaces, are eliminated by the elimination of all the suspending fluid. In the conductivity method the cells are assumed to be non-conductive, the observed slight conductivity of the densest obtainable masses of cells being attributed to the presence of residual traces of suspending fluid. The first three methods depend upon the assumption that the substance estimated is not taken up or given off by the cells.

We propose to show that of these five methods the hematocrit is the most nearly correct. The cell volume estimated by this method is consistently somewhat above that calculated from the other methods, and it has been with some reason that the hematocrit method has been distrusted. The possible errors in any of the other methods will be shown to be consistently in favor of minimizing the volume of the cells, and evidence will be presented to show that the conductivity data have not been correctly interpreted.

The first method was used by Bunge who determined the sodium content of defibrinated blood and of its serum; this method is available

only for blood whose cells contain no sodium, as is assumed to be the case for the pig, horse, or rabbit. Hoppe-Seyler's method can best be illustrated by data taken from an experiment by Stewart. The protein and hemoglobin content of 100 gm. of the defibrinated blood was 17.360 gm.; of 100 gm. of washed corpuscles, 14.254 gm.; the protein content of 100 gm. of serum was 5.424 gm. The serum in 100 gm. of defibrinated blood is given by the calculation

\[
\frac{17.360 - 14.254}{5.424} \times 100 = 57.26 \text{ gm.}
\]

But if 2.2 per cent of the protein and hemoglobin of the cells had been lost during washing, the figure 14.254 should have been 14.575, and the relative amount of serum should have been more than 10 per cent less, i.e. 51.35. A very small loss of protein and hemoglobin during washing, even one so small as to pass unnoticed, will greatly decrease the apparent volume of cells in a given sample of blood.

The second method was suggested by Bleibtreu who calculated the original volume of serum in defibrinated blood by estimating the protein nitrogen before and after dilution with 0.6 per cent NaCl. This presupposes that no change of cell volume occurs, and that no protein nitrogen escapes from the cells under the new conditions set up by dilution. The method has been severely criticized on account of both of these errors by Hamburger, Biernacki, Eykman, and Hedin and has been shown not to give self-consistent results when different degrees of dilution are compared. Scott has shown that both protein and non-protein nitrogen are lost by red blood cells to Ringer solution in which they are being washed. The change of cell volume might be made relatively negligible by using a strictly isotonic diluent, but if protein nitrogen diffused from the cells the decrease in

12 Hedin, S. G., Arch. ges. Physiol., 1895, lx, 360.
13 Scott, F. H., J. Physiol., 1915–16, i, 128.
concentration of protein nitrogen as a result of dilution would be too small. This would in turn lead to the conclusion that the original volume of serum was greater than it actually was, and in consequence that the cell volume was less than it really was. The liberation from the cells of a very minute amount of protein nitrogen would account for the difference between this and the hematocrit method. Similar comment may be made on other dilution methods and hence absolute certainty as to cell volume cannot be attained by these methods.

The addition of substances to serum is also unreliable. When introduced by Stewart, who added hemoglobin to defibrinated blood, there seemed to be every reason for supposing the erythrocytes to be impermeable to hemoglobin and hence to be incapable of taking any hemoglobin out of the solution. But the recent work of Brinkman and von Szent-Györgyi on the reversion of hemolysis, as well as some previous work mentioned by them make it entirely probable that some of the hemoglobin which Stewart introduced into the defibrinated blood was taken up in some way by the cells.

The work of Bayliss, while casting considerable doubt on the main conclusion of Brinkman and von Szent-Györgyi, does not disprove the supposition that at least some of the hemoglobin is absorbed by red blood cells under the conditions described. Such an absorption of hemoglobin, occurring in Stewart's experiments, would reduce the observed concentration of hemoglobin in the serum, and lead to an overestimate of the volume of serum, and to the calculated volume of erythrocytes being again too low. All three analytical methods probably lead to an underestimate of the volume of cells in a given suspension of erythrocytes.

The conductivity method also was introduced by Stewart as a method of determining the volume of erythrocytes in defibrinated blood. Formulæ developed empirically by Stewart, Röth, and Oker-Blom are theoretically unsound, since they give impossible values for the conductance of cells alone or suspending fluid alone. Formulæ were developed by the writer which gave the theoretical limits between

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which the conductance of a suspension should lie, but did not lead to a method of calculating the resistance exactly. 18

Formulae recently published by Fricke 19,20 are much more satisfactory. In these formulae there appears the constant β, a function of the shape and conductance of the internal phase, here the suspended cells, and of a quantity λ whose significance is not given. In Fig. 1 of the latter paper, 19 β appears as a function of \( \frac{a}{b} \) (a and b being the axes of spheroidal particles), and of \( \frac{k_2}{k_1} \) in which \( k_2 \) and \( k_1 \) are the conductivities of the suspended and suspending media, respectively. In applying the formulae to the conductivity of suspensions of erythrocytes as observed by Stewart 7 Fricke obtains good agreement between observed and calculated volumes by assuming the cells to be non-conductive and the ratio of diameter to thickness to be as 4:1. This gives \( β = -1.91 \). However, the red blood cells are not exactly spheroidal, but rather biconcave; and furthermore the ratio of their axes is still very much in doubt; and, as Ponder 21 so ably points out, they will be in doubt until methods are available much more precise than any we now possess. Apparently the ratio is more than 4:1

18 Paper presented at the meetings of the American Physiological Society, Toronto, Dec. 27, 1922. Since these formulæ have not heretofore been presented in print they are given here as a matter of record. If \( G \) is the specific conductance of the suspension as a whole, \( g \) that of the outer phase, e.g. suspending fluid, \( kg \) that of the inner phase, e.g. cells, and \( n \) the relative volume of cells in parts per mille of the suspension, then \( G \) lies between

\[
g \left[ 1 - \frac{n (1 - k)}{(n - 10n^{2/3}) (1 - k) + 1,000} \right]
\]

and

\[
g \left[ 1 - \frac{n (1 - k)}{100n^{2/3} (1 - k) + 1,000 \varepsilon} \right]
\]

In some cases \( k \) is so small that the first equation gives impossible values, showing that the current paths assumed in developing this equation are not those actually followed in these cases.

21 Ponder, E., Quart. J. Exp. Physiol., 1924, xiv, 37.
The rather doubtful value of this ratio gives to the constant $\beta$ quite a range of possible values at any given value of $\frac{k_2}{k_1}$.

Therefore when Fricke assumes that red blood cells act as non-conductors $\left(\frac{k_2}{k_1} = 0\right)$, and applies his formulae to the data given by Stewart and obtains good agreement, it does not follow that the premises are correct. If Stewart’s data on the cell volume, obtained by the analytical method, are too low, then better agreement may be obtained by assuming $\frac{k_2}{k_1} > 0$ and $\beta$ correspondingly smaller. Such a calculation has been carried out for $\frac{k_2}{k_1} = 0.05$ and $\beta = -1.74$; this value of $\beta$ corresponds to a ratio of diameter to thickness of the assumed spheroids equal to 4.2 : 1 approximately.

The figures given by different writers vary widely: Starling (Starling, E. H., Principles of human physiology, Philadelphia, 2nd edition, 1915, p. 811) gives the diameter of human erythrocytes as 7.1–7.8$\mu$, thickness at edge 2.5$\mu$, at centre 1–1.5$\mu$; this gives a mean ratio very nearly 4:1. Ponder (Ponder, E., Proc. Roy. Soc. London, Series B, 1922-23, xiv, 102) gives the corresponding dimensions as 7.8, 2, and 1.5$\mu$, respectively; ratio 4.5:1; but as a result of recalculation from the data of Gulliver (Gulliver, G., Proc. Zool. Soc. London, 1875, 474). Comes to the conclusion that the ratio for mammalian cells is about 3:1 at the edge and 4:1 at the center. Since Gulliver’s data were obtained by measurements of cells in dried smears (Gulliver, G., Phil. Mag., 1840, xvi, series 3, 23) it seems to the writer to be rather dangerous to transfer these data to cells lying in defibrinated blood. Schenck and Gürber (Schenck, F., and Gürber, A., Outlines of human physiology, translation, W. Zoethout, New York, 1900, p. 53) give the average diameter and thickness of human red blood cells as 7–8$\mu$ and 1.6$\mu$, respectively; the ratio is 4.4–5.0:1.0. These, which are fairly representative figures, suggest a figure somewhat above 4:1 as the most probable value of the mean ratio of diameter to thickness in the case of the cells under consideration.

This value seems to be about that to be expected by extrapolation along the $\frac{a}{b}$ axis for $\frac{k_2}{k_1} = 0.05$ in Fricke, Phys. Rev., 1924, xxiv, 575) make possible the exact calculation of $\beta$, but this would not materially affect the result of my recalculation.
The agreement obtained in this way is better than that given by Fricke, as may be seen in Table I.

**TABLE I.**

Comparison of the volume of erythrocytes in 100 volumes of suspension as observed and as calculated by the use of the formula

\[
\frac{k - k_1}{k - k_2} \left(1 - \frac{k_2}{k_1}\right) = \beta \cdot \frac{\rho}{1 - \rho}
\]

in which \(\rho\) = concentration per cent, \(k, k_1,\) and \(k_2\) the conductivities of the suspension, suspending fluid, and cells, respectively, and \(\beta\) a constant. In the recalculation \(\rho\) is corrected by increasing \(\rho\) observed by a uniform proportion.

<table>
<thead>
<tr>
<th>Calculation by Fricke.</th>
<th>Recalculation.</th>
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<tbody>
<tr>
<td>(\rho) observed</td>
<td>(\rho) calculated</td>
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<tr>
<td>90.7</td>
<td>88.4</td>
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<tr>
<td>82.1</td>
<td>81.9</td>
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<tr>
<td>74.5</td>
<td>74.4</td>
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<td>67.8</td>
<td>68.0</td>
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<td>61.6</td>
<td>62.1</td>
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<tr>
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<tr>
<td>11.4</td>
<td>10.8</td>
</tr>
<tr>
<td>Totals</td>
<td>-</td>
</tr>
<tr>
<td>Mean error</td>
<td>1.37</td>
</tr>
<tr>
<td>&quot; root square error</td>
<td>1.81</td>
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</table>
The average per cent discrepancies by Fricke's and the writer's calculations are 1.37 and 1.25, and the root mean square discrepancies, 1.81 and 1.79, respectively. Either standard of comparison favors the theory that the cells are slightly conductive and that Stewart's method of estimating their volume gave results which were too low.

Fricke's formulae have been applied to some of the writer's data, with the results given in Table II. Assuming that the cells retain their natural shape and that this is approximately that of an oblate spheroid the ratio of whose axes \( \frac{a}{b} = \frac{1}{5} \), values were assumed for the conductivity of the cells \( \frac{k_2}{k_1} \), corresponding values of the constant \( \beta \) deduced from Fricke's Fig. 1, and the volume concentration \( \rho \) calculated therefrom. Values of \( \frac{k_2}{k_1} \) and \( \beta \) were thus found which led to values of \( \rho \) very close to those observed. These are the values given in Table II.

In the last two columns are given the values which must be assumed for \( \beta \) and \( \frac{a}{b} \) if the cells are assumed to be non-conductive. The assumption is seen to lead to rather strange or impossible conclusions.
Change of shape probably compensates for part of the observed differences in the ratio \( \frac{k_2}{k_1} \), but these values fit the facts much better than the value \( \frac{k_2}{k_1} = 0 \) assumed by Stewart, Fricke, and others.

One great service of Fricke is that he has shown, theoretically at least, the great influence which the shape of suspended particles exerts on the conductance of their suspensions. Ponder has shown that erythrocytes in swelling undergo great change of shape, and it follows that no study of the conductance of red blood cells is of any value unless swelling or shrinkage of the cells is shown to be absent, or properly taken into account.

All the methods of estimating cell volume have been shown to rest upon more or less dubious assumptions. To the writer the assumption underlying the hematocrit method, namely that translucence of the sediment indicates freedom from serum, seems to be the most objective, and the least dubious of all. This is also the conclusion reached by Ege who has already called attention to many of the points noted in the present paper.

It is, therefore, evident that we are still at liberty to assume that red blood cells are conductive, which seems to be almost unavoidable when one considers their great permeability to anions and hydriions. Further, one may assume that the hematocrit or an equivalent centrifugation method gives the most nearly correct values of the volume of red blood cells present in a suspension.

In practice, a centrifugation of 5 minutes with a precipitating force at the bottom of the 15 cc. centrifuge tube equal to about 1,600 \( \times \) gravity was usually enough to bring the volume of the cells to within about 1 per cent of the volume when the cells were centrifuged to translucence. Some procedures, such as heating, so reduce the specific gravity of the cells that this method of determining the volume is useless. Under most conditions, erythrocyte volume can be, and has in my work been determined by centrifugation.

26 For the literature see Höber, R., Physikalische Chemie der Zelle und der Gewebe, Leipzig, 5th edition, 1922, pp. 443–54.
b. Bacteria.—When we study bacteria, however, we find that sedimentation depends so much upon the surface charge of the cells that centrifugation is less satisfactory than for erythrocytes. Apparent changes in volume may be due to changes in the reaction of the medium, or to the addition of some new substance, and all endeavors to determine the actual conductivity of bacteria must be made under such conditions as to keep their surface potential constant. This must be considered in addition to the factors mentioned in the discussion of erythrocyte volume, factors, which in the case of bacteria, are of much less relative importance. Certain bacteria have more than one point of no potential, e.g. Bacillus cereus, at pH 3.0 and 10.0 but in any event these reactions represent highly unfavorable conditions under which the cells cannot long survive. In the case of some bacteria, however, a pH can be chosen and maintained at which the potential of the cells is low enough to allow fairly efficient centrifugation, and yet have no serious effects on the viability of the bacteria during the course of the experiment.

The absolute volume of bacterial cells is inaccessible to determination during ordinary experiments, but with proper precautions, the relative volume, and hence, to a limited extent, the changes of electrical conductivity, can be followed by the writer's method of centrifugation and resuspension.

II.

The Viability of Bacteria in Suspensions.

In experiments to determine the effect of reagents on the electrolytic conductance of living cells it is important to know that the cells are really living. This is simple in the case of red blood cells which may be considered to be living (as much as any red blood cell may be considered alive) unless or until it is hemolyzed.

Bacteria may be plated on suitable media and the number of colonies compared with the number of organisms present in a stained smear. Using known volumes and dilutions it is to be expected that clumping of bacteria would make the number of colonies growing on the plates

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less than that expected by calculation from the number of stained organisms countable in the smears. This was found to be the case when four independent determinations were made in the case of a suspension of Escherichia communior: (Bacillus coli communior, fg strain): the number of colonies was 52.0 ± 1.2 per cent of the calculated number.

Apparently a large proportion of the bacteria were viable; the possible effects of any dead bacteria present can only be guessed at, since the manner of killing affects the subsequent characteristics of the cells.

III.

Interpretation of Data during Progressive Change in the Conductance of Cells and Surrounding Fluid.

Still another difficulty is often encountered in studying the conductance of suspended cells: the conductance of both cells and suspending fluid may be undergoing rapid change. It is misleading to determine the net conductance from determinations of the resistances of the suspending fluid and suspension when they are separated by an interval of several minutes during which changes are occurring in both. Apparently the best approximations are to be made by assuming that the conductance found for the suspending fluid is that proper to some time during centrifugation, presumably when about half the cells are already gathered into a sediment. Plotting the observed resistances at these times, and the resistances of the suspension at the moment of observation, the most probable values of the net conductance can be determined by interpolation.


The suspension contained cells grown on pea extract trypsin agar; slants were inoculated from stock cultures on nutrient agar, grown 24 hours at 37.5°C., the growth of each tube then transferred to fresh medium in Kolle flasks and grown 18 to 20 hours more at 37.5°C. The growth was then taken up in unbuffered Ringer-Locke solution, washed several times with the same, and finally suspended in about four times its volume of the same solution. The pH of the suspension was 8.8 to 9.0, varying slightly on different days.
Experience has shown that these interpolations usually follow with very little uncertainty. The permissible range of variation in the placement of the smoothed curves is so limited that any error involved is obviously smaller than other experimental errors. Such interpolations as these will be considered in a forthcoming publication.

IV.

The Interpretation of Conductance Data in Terms of Permeability.

Let us finally examine what is meant by the term permeability of a membrane. By derivation and in its physicochemical sense it may be defined as the freedom with which the membrane allows a substance or substances to pass through it; the membrane may be thought of as hindering in some way the free diffusion of ions; the greater the hindrance the less the permeability. It would seem possible to measure permeability by the rate at which the substance in question passes through the membrane relative to the rate of free diffusion in the absence of a membrane. If permeability remained constant, then doubling the concentration gradient should double the rate of diffusion both with and without the presence of the membrane, but it should leave the ratio between the two unchanged. In general, however, changes in the constitution of the solutions bathing a membrane produce changes in its permeability; this is particularly true of living cells, whether their semipermeability be considered to be due to the presence of a plasma membrane, or be referred to the protoplasm as a whole, considered as a membrane.

Practically, the permeability of a cell is that property which determines how rapidly, if at all, substances pass into and out of the cell. The definition given above is the only one which is satisfactory from this point of view, and the word permeability will be so understood in this and the following papers by the writer.

When this definition is applied to the use of conductance as a measure of permeability it is apparent that changes in conductance of the cell or any part of it in which its semipermeability is supposed to reside, will change the conductance of a mass or suspension of cells whether or not the permeability as just defined undergoes any change. Electrolytic conductance measures two independent quantities—per-
meability and conductivity, the latter being thought of as a function of the dissociation constants, ionic mobility, and concentration of the electrolytes present. It will, of course, be recognized that the semi-permeable region may owe some of its semipermeability to an effect which it produces on the mobility, dissociation, or other characteristics of an electrolyte, but these can also change independently of the semi-permeable structure or phase. Therefore, electrolytic conductance cannot be used as a measure of permeability unless such independent changes in conductivity can be excluded.

The writer has shown that such changes probably occur and has called attention to the misleading conclusions as to permeability which might have been drawn from the data presented.

The conclusion that electrolytic conductance does not necessarily measure permeability has been stated independently by Robbins. The number of pitfalls in the interpretation of the measurements of electrical conductance of cell suspensions is impressive. It is not to be wondered at that so little of permanent value has been achieved by such studies. We may take as one last example the preliminary report by McClendon on the conductance at different frequencies of a sediment of erythrocytes "containing a small percentage of serum." The observed specific conductance at $10^{9} \ \infty$ was 0.0010 ohms $^{-1}$, and at $10^{9} \ \infty$ it was 0.0014 ohms $^{-1}$, an increase of 40 per cent, and one which is apparently very striking. Suppose, however, that the volume of the erythrocytes had changed by less than 5 per cent, e.g. from 95.0 per cent to 90.5 per cent of the total volume of "sediment," then, without change in any other factor such as shape or conductivity of cells or serum, this slight change alone would account entirely for the observed difference in conductance. While it may justly be claimed that there is no a priori reason for supposing any such change to occur, the fact remains that the data as presented are not adequate proof that there is any difference in the conductance of the erythrocytes at the two different frequencies.

34 McClendon, J. F., Science, 1924, lx, 204.
Suspended cells are living things in a state of labile equilibrium with their environments; and no attempt at physical analysis of their electrolytic behavior, no matter how excellent it may be as an analysis, can interpret living cells if they are treated as inanimate, unresponsive particles.

SUMMARY.

The problem of determining by means of measurements of electrolytic conductance the permeability of living cells in suspension is considered in some detail and it is pointed out that several factors, usually neglected, have an important influence on the interpretation of such studies. These are:

1. The relative volume and the shape of cells, which are responsive to changes in osmotic pressure and constitution of the surrounding solution. The sources of error in various methods of determining the true volume of red blood cells in a suspension are explained. The hematocrit method appears to be the most reliable method in this case.

2. The proportion of living cells, which is especially to be regarded in the case of suspensions of bacteria. It is shown that this may be very high when appropriate cultural methods are used. The conductance of the dead cells must also be taken into account.

3. The progressive nature of the changes occurring during the course of an experiment. Approximate accuracy may be obtained by proper interpolation.

4. The conductivity of the protoplasm itself, which varies in response to variations is that of the surrounding fluid.

It is emphasized that cells, and in particular red blood cells, are not to be regarded as stable non-conducting particles, but rather as labile and as permeable to electrolytes. It is shown that the available data support this interpretation.