SOME ASPECTS OF BIOELECTRICAL PHENOMENA.*

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This brief sketch is intended as an introduction to a series of articles on bioelectrical phenomena, its purpose being to present certain fundamental facts and underlying conceptions. Early in the course of the investigation it became evident that there are great advantages in using single cells in place of tissues. The experiments were accordingly made with single (multinucleate) cells of Valonia and Nitella, which are large enough to permit leading off simultaneously from several places on the same cell. This has important technical advantages and eliminates certain complications which always arise in the study of tissues. In addition it enables us to find out to what extent changes in any part in the cell may affect other parts. A study of such effects and of their transmission in protoplasm may be expected to throw some light on the propagation of stimuli in general and on the constitution of living matter.

Another advantage attending the use of these cells may be mentioned here. The study of bioelectrical phenomena has been hampered because nothing could be measured except potential differences between selected spots, and it has been impossible to determine the

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2 The cells of the marine alga Valonia reach a length of 2 inches or more and those of the fresh water Nitella a length of 5 inches or more. The cells consist of a thin layer of protoplasm (containing numerous chloroplasts and nuclei) outside of which lies the cell wall and inside of which is the very large central vacuole filled with cell sap.

3 For example, in a tissue the circuit includes a number of cells between which is intercellular material of some sort. If a cell is injured cell sap comes out and alters the intercellular material which in turn alters the potential difference of the uninjured cells. With single cells this cannot occur.
absolute value of the potential difference across the protoplasm at any one point. Since it is highly desirable to obtain such absolute values an attempt was made to do so. It would not be possible to make such determinations in tissues or in cells of ordinary size, but the use of very large cells enables us to reach the desired end.

In the case of *Valonia* this was done by piercing the cell with a capillary glass tube filled with cell sap (Fig. 1). On leading off from the interior of this tube to the outside of the cell we obtain a circuit which passes only once through the protoplasm (as indicated by the dotted line), and hence the measured E.M.F. gives the potential difference across the protoplasm at any point where an external contact is applied. In many cases the protoplasm attaches itself to the capillary at $F$ so as to form an electrical seal, thus preventing any short circuit through the wall (between $F$ and $G$) and along the outside of the capillary into the sap, and only such cells were employed in the experiments.

In the case of *Nitella* the same purpose was accomplished by reducing the potential difference at one point approximately to zero, by killing the protoplasm in such fashion as not to affect other points some distance away on the same cell (at least for some time). In leading off from the killed point to a normal region the circuit passed once through the killed spot and once through living protoplasm, and the results justify the conclusion that when the experiments are made under the proper conditions the observed electromotive force is practically all due to the potential difference across the living protoplasm at the selected point.

In order to interpret the results of our measurements we need information regarding the structure of the protoplasm. There is some evidence to show that in general the surface of protoplasm differs from its interior, and some experiments indicate that the surface is non-aqueous. The interior of the protoplasm may be an aqueous phase consisting of sol or gel or both, or it may be an emulsion in which the outer phase is aqueous. We might therefore, as a working hypothesis, consider the protoplasm to be made up of an aqueous phase, $W$, and phases which are probably non-aqueous, forming the

*This can be done in a variety of ways which will be discussed in detail in subsequent papers.*
external (X) and internal (Y) surface. These layers may be very thin (possibly monomolecular), or if thicker they may consist of sol or of gel or of an emulsion, the outer phase of which is non-aqueous.

It should be emphasized that this conception is set up merely as a working hypothesis which may be useful for the time being (some new evidence for the existence of layers will be presented in later papers). It is quite possible that the boundary surfaces are aqueous in character, and if the protoplasm really consists of layers it is quite possible that there are more than three. For the present, however, we shall adhere to the hypothesis in the form presented above.

Let us now consider under what conditions bioelectric effects may be expected to arise. If the protoplasm is made up of layers it may, for convenience, be represented as in Fig. 1 (in which G represents a salt solution applied to the cell wall and quickly penetrating through it to the surface of the protoplasm). We shall discuss certain possibilities on the assumption that these layers exist. It will then be evident what conditions would obtain if the protoplasm were not made up of layers.

Let us first consider the cell wall. This is of cellulose, and the experiments show that it is readily permeable to salts; so that an applied salt solution quickly penetrates the cell wall and comes in contact with the external surface of the protoplasm. If the salt solution has ions which move at different rates in the cell wall a diffusion potential will be set up. This however would not last long if the salt diffused only at right angles to the surface since the cell wall is very thin and very permeable; but a potential difference due to diffusion along the wall (from G toward F) might last a long time, but this would have little or no effect on the E.M.F. in the cell, measured as shown in Fig. 1, since in the experiments only those cells were used in which the protoplasm had made an electrical seal at F so that no current

5 It is an easy matter to tell whether this seal is made. If we place 0.6 M KCl at G and lead off from G to a drop of 0.6 M KCl placed on the outside of the cell at F (i.e. at the point where the capillary enters) it is evident that if there is a leak around the capillary we shall get the same potential difference as if we led off from G to the interior of the capillary; when the seal is made we actually observe a very different value. We arrive at the same result if we first lead off as shown in Fig. 1 and then immerse the cell completely in the solution applied at G. This will be discussed in later papers.
could leak along the outside of the capillary; hence the wall did not form a short circuit between \( F \) and \( G \) and any potential difference due to the diffusion of solution in the wall from \( G \) toward \( F \) would probably have only a negligible effect on the measured E.M.F.

Fig. 1. Diagram of a cell of *Valonia* with inserted capillary. The thickness of the protoplasm and cell wall is exaggerated, being only a few microns, while that of the vacuole may be over an inch: \( a, b, c, \) and \( d \) are the seats of phase boundary potentials, and \( f, g, h, \) and \( i \) the seats of diffusion potentials. The circuit is supposed to follow the course of the dotted line.

What has been said about diffusion in the cell wall might apply to any of the other layers which are readily permeable to salts; but \( X \) and \( Y \) may be nearly or quite impermeable.
In addition to diffusion potentials we may consider phase boundary potentials, which may arise for example at a, b, c, and d (providing X, Y, and W represent distinct phases). The cell wall is here omitted from consideration since it appears too permeable to be the seat of phase boundary potentials.

The outer layer X is probably permeable to some extent to certain ions, which may give rise to potential differences when brought in contact with it: in this case current must be able to pass through X. We must consider the possibility that Y may be almost or quite impermeable to ions, a possibility which is indicated by the situation in Valonia. Little or no Mg or SO₄ penetrates the vacuole, yet it seems probable that the protoplasm contains S and the chlorophyll bodies embedded in the protoplasm must contain Mg. It might therefore seem possible that Mg⁺⁺ and SO₄⁻⁻ penetrate X but not Y (unless MgSO₄ penetrates X only in the form of undissociated molecules). (It is also possible that the continuity of X is interrupted over each chlorophyll body so that Mg can gain access to it without passing through X.) If the layer Y is impermeable to ions generally, it is evident that the potential at both its surfaces, i.e. at c and d (Fig. 2), might under certain conditions be zero.

If Y were almost or quite impermeable to ions this would explain certain facts which indicate that in general ions cannot penetrate readily into the vacuole. If we suppose that all the layers are permeable to ions we should assume that under normal con-

6 The mere fact that E.M.F. produced at B and C can affect the measuring instrument does not prove that X conducts much current, since a very minute current can keep the electrometer charged, as can be shown by inserting a condenser in series with the cell.
7 If X were aqueous it would of course conduct.
8 This evidence has been gathered chiefly from studies by a number of investigators on the penetration of weak acids (for references see Osterhout, W. J. V., J. Gen. Physiol., 1925–27, viii, 131; Osterhout, W. J. V., and Dorcas, M. J., J. Gen. Physiol., 1925–26, ix, 255) and of bases, as well as of dyes (cf. Irwin, M., J. Gen. Physiol., 1925–26, ix, 561), which show that ions penetrate very slowly or not at all. The experiments of several investigators, especially unpublished results of Dr. Blinks, show that the resistance of the protoplasm is very high and unless this is due to polarization it must indicate a very low degree of permeability to ions on the part of some or all of the layers.
ditions this permeability is very slight. What has previously been said regarding the protoplasm applies especially to the marine alga *Valonia macrophysa*. Let us now consider the situation in the fresh water plant *Nitella*. In this case it is difficult to insert a capillary on account of the small size and the delicacy of the cells. We therefore perform the experiments by leading off from two places, as at *B and C*, Fig. 2. For convenience we shall postulate during the present discussion that the current flows chiefly in the circuit indicated by the dotted line. There may, however, be a short circuit in any layer. It seems probable that the only layer in which such short circuiting is important is the cell wall (the other layers being probably too thin or too resistant to permit much current to flow), and even in the cell wall this effect is apparently very small when it is imbibed with distilled water or with tap water, as in *Nitella*. In *Valonia*, where the cell wall is imbibed with sea water, the short-circuiting effect would become important if the protoplasm did not attach itself to the capillary to form an electric seal (at *F*, Fig. 1).

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**Fig. 2.** Hypothetical diagram of a portion of a cell. *B and C* represent drops of solution applied to the exterior: *a, b, c,* and *d* represent the surfaces of the layers and are the seats of phase boundary potentials; *e, f, g, h,* and *i* represent the seats of diffusion potentials in the cell wall and in the layers of protoplasm. The main circuit is supposed to follow the course of the dotted line. The thickness of the cell wall and of the protoplasm is only a few microns.

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9 But the diffusion potentials in the cell wall due to the solutions at *B and C* might be greater in some cases when the wall was imbibed with distilled water than when imbibed with a salt solution.
which prevents short circuiting; this seal was secured in all the experiments.

If the solution applied at B differs from the solution with which the cell wall is imbibed, potentials may arise at e and f, which may set up "eddy" currents flowing through the cell wall and through the protoplasm back to the drop applied at B. Their magnitude would depend on the potential differences as well as on the resistances involved. It is difficult to say what effect they would have upon the current which follows the course indicated by the dotted line in Fig. 2, but it seems probable that any effect will be of brief duration. Similar "eddy" currents might be set up in any of the layers.

When identical solutions are placed on B and C it frequently happens that little or no potential difference is observed. Under these circumstances it seems reasonable to assume that the potential difference at a is equal and opposite to that at a', etc., and that diffusion potentials likewise cancel out. If the solutions applied to B and C in such cells are not identical it is probable that all the values except those at a and a' and at g and g' are equal and opposite. This would, of course, differ from the circuit in Valonia, as shown in Fig. 1. In other respects, however, what is said of Valonia applies to Nitella, and the following discussion applies to both.

Solutions applied to the surface will probably not affect the deeper layers for some time (unless they are very toxic solutions which break down or alter X), so that in brief experiments with non-toxic solutions we may consider that any observed changes depend only on the effect upon X and it will make no difference in the interpretation of the results whether we regard the protoplasm as consisting of one or of many layers. The hypothesis that there are several layers becomes important when we deal with toxic effects or other alterations in the protoplasm.

Polarization may, of course, be expected at any of the layers with a consequent diminution of the current.

The observed potential difference may be made up of the phase

There is, of course, a current flowing from B to C through the cell wall and back through the galvanometer, as already mentioned.

The experiments show that in general when one solution is substituted for another the observed changes are completed in a few seconds unless injury occurs.
boundary potentials at \(a, b, c,\) and \(d,\) and of the diffusion potentials in \(X, W,\) and \(Y.\) In addition there may be a diffusion potential in the cell wall, but this will be of short duration if it is due solely to diffusion across the wall, since the wall is very permeable: if it is due to diffusion along the wall it may last for some time.

A potential difference is usually observed when we lead off from \(B\) and \(C\) (Fig. 2) with solutions of the same salt at different concentrations (concentration effect), or with solutions of different salts (chemical effect). In general we observe both effects with protoplasm but the experiments show that in the cell wall with the solutions thus far employed only the concentration effect is of importance. It is possible to arrange the experiments in such fashion that the effect due to the protoplasm can be ascertained, at least approximately. Throughout this paper the effects discussed are those on the protoplasm unless otherwise stated.

It is commonly observed that when a solution of KCl is applied at one point and a solution of NaCl of the same molar concentration at another point, KCl is negative to NaCl. How is this to be explained?

If we regard the whole effect as due to diffusion potential we may say that the mobility of K in the outer protoplasmic layer \(X,\) Fig. 1) is greater than that of Na. This is to be expected if the layer \(X\) behaves, for example, like phenol, as described by Nernst and Riesenfeld,\(^{14}\) or like the collodion membranes studied by Michaelis and Perlzweig.\(^{15}\)

The fact that a concentrated solution of KCl is negative to a dilute solution would mean that K penetrates more rapidly than Cl. This would leave the solution negatively charged, the effect being greater

\(^{13}\) The corresponding experiment is performed with Valonia, as in Fig. 1, by leading off from \(G,\) first with one solution, then with the other, and taking the difference between the two measurements.

\(^{14}\) In the brief experiments here referred to it is not probable that any of the deeper layers are involved since the potential differences with which we are here dealing are established within a few seconds. The cell wall appears to play little or no rôle in connection with the chemical effect.


where references to earlier papers are given.
as the concentration increases: hence the concentrated solution would be negative to the dilute solution. Since in general in biological experiments dilute solutions of salts are usually positive to more concentrated solutions of the same salt, we might conclude that in general cations tend to penetrate more rapidly than anions (this interpretation would not necessarily hold if the potential differences were due to phase boundary potentials).

Let us now consider phase boundary potentials. The foundation of the theory of these potentials was laid by Nernst. He assumes that the tendency to enter is not the same for all ions. Thus, let us suppose that we have to do with LiCl, and that the concentration of Li in the external solution is \( C_{\text{Li}} \) and that it tends to enter \( X \) and to reach the concentration \( A_{\text{Li}} C_{\text{Li}} \) in \( X \), \( A_{\text{Li}} \) being the "true" partition coefficient\(^{17}\) of Li. The corresponding coefficient of Cl is \( A_{\text{Cl}} \), and if this is less than \( A_{\text{Li}} \) (i.e. if Cl is less soluble in \( X \) than Li is) Li will be unable to reach its "true" value, since it cannot enter in excess of Cl (except perhaps at the very surface), but Cl will enter in excess of its "true" value. The actual concentrations reached in \( X \) may be called \( C'_{\text{Li}} \) and \( C'_{\text{Cl}} \) and these must be equal. Nernst shows that this leads to the equation

\[
\text{P.D.} = RT \log \frac{C_{\text{Li}} A_{\text{Li}}}{C'_{\text{Li}}} = -RT \log \frac{C_{\text{Cl}} A_{\text{Cl}}}{C'_{\text{Cl}}}.
\]

Hence

\[
\log \frac{C_{\text{Li}} A_{\text{Li}}}{C'_{\text{Li}}} = - \log \frac{C_{\text{Cl}} A_{\text{Cl}}}{C'_{\text{Cl}}} \quad \text{and} \quad \frac{C_{\text{Li}} A_{\text{Li}}}{C'_{\text{Li}}} = \frac{C_{\text{Cl}} A_{\text{Cl}}}{C'_{\text{Cl}}}.
\]

Multiplying both sides by \( C_{\text{Li}} A_{\text{Li}} / C'_{\text{Li}} \) and substituting the values

\[
C_{\text{Cl}} = C_{\text{Li}} \quad \text{and} \quad C'_{\text{Li}} = C'_{\text{Cl}},
\]

we obtain

\[
\frac{C_{\text{Li}} A_{\text{Li}}}{C'_{\text{Li}}} = \sqrt{\frac{A_{\text{Li}}}{A_{\text{Cl}}}}.
\]


\(^{17}\) The "true" partition coefficient is that which would be observed if Li could enter unhindered by Cl: this would be the case if the "true" partition coefficients of Li and Cl were equal.
Hence

\[ \text{p.d.} = RT \log \frac{A_{Li}}{A_{Cl}} = \frac{RT}{2} \log \frac{A_{Li}}{A_{Cl}} \]

Haber\textsuperscript{18} arrives by a different route at a formula which reduces to the same thing. Haber’s formula is

\[ \text{p.d.} = RT \log \left( \frac{C_{Li}}{C_{Li}} \right) \left( K_{Li} \right) \]

where \( K_{Li} \) is the solution tension of an imaginary Li electrode in \( X \) divided by its solution tension in water. It is evident\textsuperscript{19} that \( K_{Li} \) is equal to the \( A_{Li} \) of Nernst’s formula.

Hence we may write

\[ \text{p.d.} = \frac{RT}{2} \log \frac{A_{Li}}{A_{Cl}} = \frac{RT}{2} \log \frac{K_{Li}}{K_{Cl}} \]

If we apply LiCl at one point and NaCl at another the e.m.f. will be

\[ \text{p.d.} = \frac{RT}{2} \log \frac{A_{Li}}{A_{Cl}} - \frac{RT}{2} \log \frac{A_{Na}}{A_{Cl}} \]

\[ = \frac{RT}{2} \log \frac{A_{Li}}{A_{Na}} = \frac{RT}{2} \log \frac{K_{Li}}{K_{Na}}. \]

Hence it is evident that the p.d. depends only on the difference in the “true” partition coefficients\textsuperscript{20} or the solution tensions, and that if


\textsuperscript{20} The formula of Nernst has been extended to solutions containing more than one salt by Michaelis, L., and Fujita, A., \textit{Z. physik. Chem.}, 1924, cx, 270, and by Horovitz, K., \textit{Z. physik. Chem.}, 1925, cvii, 424. Thus for a mixture of NaCl and KNO\textsubscript{3} in water the formula would be

\[ \text{p.d.} = \frac{RT}{2} \log \frac{A_{Na} C_{Na} + A_{K} C_{K} + A_{H} C_{H}}{A_{Cl} C_{Cl} + A_{NO\textsubscript{3}} C_{NO\textsubscript{3}} + A_{OH} C_{OH}}. \]
Li has a greater tendency to enter than Na, LiCl will be negative to NaCl.\textsuperscript{21}

That these formulae do not account for the concentration effect may be made clear by an illustration. If we apply LiCl at one spot in the concentration $C_1$ and at another in the concentration $C_2$ we have at one place P.D. $= \frac{RT}{2} \log \frac{C_{1}\text{Li}}{C_{1}\text{Cl}}K_{\text{Li}}$ and at the other P.D. $= \frac{RT}{2} \log \frac{C_{2}\text{Li}}{C_{2}\text{Cl}}K_{\text{Li}}$. The total P.D. will be the difference between these or

\[ \text{P.D.} = \frac{RT}{2} \log \frac{C_{2}\text{Li}}{C_{1}\text{Li}} - \frac{RT}{2} \log \frac{C_{1}\text{Li}}{C_{2}\text{Li}} = \frac{RT}{2} \log \frac{C_{1}\text{Li}}{C_{2}\text{Li}} K_{\text{Li}}. \]

\textsuperscript{21} In order to visualize the situation it may be convenient to assign fictitious values which satisfy the requirements. This may be done as follows:

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>In $X$.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;True&quot; or &quot;ideal&quot; concentration (= $C'$)</td>
<td>Li = 100, Cl = 25</td>
<td>Na = 36, Cl = 25</td>
</tr>
<tr>
<td>Actual concentration (= $C$)</td>
<td>Li = 50, Cl = 50</td>
<td>Na = 30, Cl = 30</td>
</tr>
<tr>
<td>In external solution. Actual concentration (= $C$)</td>
<td>Li = 1, Cl = 1</td>
<td>Na = 1, Cl = 1</td>
</tr>
</tbody>
</table>

In this case LiCl is applied at $B$ (concentration $= 1$) and NaCl (concentration $= 1$) at $C$: $a_{\text{Li}} = 100$, $a_{\text{Na}} = 36$, and $a_{\text{Cl}} = 25$. At $B$ the p.d. $= \frac{RT}{2} \log 100/50$ and the positive current tends to flow from the external solution into $X$ since the concentration of Li in $X$ is only 50 and its tendency is to push in until the "true" value of 100 is reached; on the other hand Cl tends to leave $X$ since its concentration is 50 and it tends to move out to attain its "true" value of 25, and in consequence the p.d. $= -\frac{RT}{2} \log 25/50$. Na at $C$ acts in the same way as Li at $B$, but the p.d. $= \frac{RT}{2} \log 36/30$. The total p.d. will be found by subtracting that at $C$ from that at $B$, or

\[ \text{Total p.d.} = \frac{RT}{2} \log \frac{C_{1}\text{Li}}{C_{1}\text{Cl}}A_{\text{Li}} - \frac{RT}{2} \log \frac{C_{1}\text{Na}}{C_{1}\text{Cl}}A_{\text{Na}} = \frac{RT}{2} \log \left( \frac{100}{50} \right) \left( \frac{30}{36} \right) = \frac{RT}{2} \log \frac{5}{3}. \]

This is evidently equal to $\frac{RT}{2} \log \frac{A_{\text{Li}}}{A_{\text{Na}}} = RT \log \frac{100}{36} = RT \log \frac{10}{6} = RT \log \frac{5}{3}$ as above. In this instance $A_{\text{Li}}$ and $A_{\text{Na}}$ are for convenience put greater than unity, but in an actual case we should expect them to be very much less than unity.
We should expect \( \frac{C_{1Li}}{C'_{1Li}} \) to equal \( \frac{C_{2Li}}{C'_{2Li}} \) and \( \frac{C_{1Li}}{C'_{1Li}} \) to equal \( \frac{C'_{1Li}}{C'_{2Li}} \), so that the P.D. would be zero. It is evident that this would be the case since we can write

\[
P.D. = RT \log \frac{A_{Li}}{A_{Cl}} - RT \log \frac{A'_{Li}}{A'_{Cl}} = 0.
\]

This gives no concentration effect.

According to Wosnessensky it is possible to account for the concentration effect by supposing that the partition coefficients of the ions are not constant but vary independently with the concentration. In this case \( \frac{C_{1Li}}{C'_{1Li}} \) would not be equal to \( \frac{C_{2Li}}{C'_{2Li}} \).

If we use the formula of Nernst it is easy to show that the sign of the dilute solution will depend on the relation between \( A_{Li} \) and \( A_{Cl} \). If we assume for convenience that \( A_{Li} \) is always greater than \( A_{Cl} \) and that the latter remains constant while the former varies with concentration it is a simple matter to demonstrate that when \( A_{Li} \) is greater in the concentrated than in the dilute solution the latter will be positive (and vice versa).

Michaelis states that a concentration effect is possible when a second electrolyte is present.

Since Haber and Klemensiewicz found a concentration effect with \( H^+ \) ions in the case of certain kinds of glass they assumed that a small amount of water is present in the glass giving a constant concentration of \( H^+ \) and \( OH^- \) ions. In that case we should have inside the glass \( C'_{1H} = C'_{2H} \) and the equation would become

\[
P.D. = RT \log \frac{C_{1H}}{C_{2H}}
\]

which would explain the concentration effect.

To account for the concentration effect of various non-aqueous

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23 Michaelis, L., Hydrogen ion concentration, Baltimore, 1926, i, 205.
24 According to Horovitz this is not equally true of all kinds of glass (see Footnote 28).
liquids Beutner\textsuperscript{25} assumes that an approximately constant concentration of certain ions results from a chemical reaction. Thus if we suppose \( X \) to contain an organic acid \( HA \) in very small amounts, the reaction
\[
LiCl + HA \rightarrow LiA + HCl
\]
might occur.\textsuperscript{26} If \( X \) contained equal numbers of \( Li^+ \) and \( Cl^- \) ions there would be no resulting P.D., but if \( HCl \) is less dissociated in \( X \) than is \( LiA \) the number of \( Cl^- \) ions would be less and a P.D. would result, which according to Beutner could be calculated by means of the formula
\[
P.D. = RT \log \frac{C_{Li}}{C_{2Li}}
\]
In order to employ this formula it is necessary to assume that \( HA \) is present in such small amounts\textsuperscript{27} that practically all of it is converted to \( LiA \) even when the cation is present in the external solution in exceedingly low concentrations. This would give an approximately constant concentration of \( Li^+ \) in \( X \).

The scheme proposed by Beutner involves a number of assumptions, in part tacit, which cannot be discussed here. Some of these assumptions are of very doubtful validity.\textsuperscript{28}

If Beutner’s scheme\textsuperscript{29} (as presented by Michaelis\textsuperscript{30}) should be applied to a series of chlorides, \( A, B, C \) (of the same molar concentration) such that \( A \) is negative to \( B \), and \( B \) is negative to \( C \), it would be said that the cation of \( A \) tends to be taken up more than that of \( B \) (since \( A \) is nega-


\textsuperscript{26}Michaelis and Perlzweig have raised a serious objection to this assumption (cf. Michaelis, L., and Perlzweig, W. A., J. Gen. Physiol., 1926–27, x, 575). There are other serious objections to Beutner’s scheme.

\textsuperscript{27}It is assumed that \( A \) comes out into the water to a slight extent only.

\textsuperscript{28}In applying the equations for phase boundary potential we do not assume that the cell has reached complete equilibrium with the exterior, since in a living growing cell this is not to be expected, but it is possible to assume that the penetrating substance very quickly reaches approximately the equilibrium concentration at the surface or just inside the surface of \( X \), in which case we should have approximately the value demanded by the equations.

\textsuperscript{29}Michaelis, L., Hydrogen ion concentration, Baltimore, 1926, i. 191 ff. Cf. also Foot-note 26.
tive to B) and that of B tends to be taken up more than that of C. (This is on the assumption made by Beutner that diffusion potentials are negligible.) Hence we must suppose that the cations of A and B penetrate X even if that of C is unable to do so. In this way it might be possible to determine what ions enter X.

The glass used by Haber and Klemensiewicz acted as a hydrogen electrode only, but Horovitz has found glasses which can take up silver and other cations and act as reversible silver electrodes, etc. This reminds us of the behavior of protoplasm which can act as a reversible electrode for many kinds of ions. The question arises whether the theory formulated by Horovitz for these glasses can be applied to protoplasm. It would require us to assume that the concentration of ions in X cannot exceed a certain constant value which is independent of the nature of the ions and that no anions enter except combined with cations in the form of molecules which cannot dissociate in X (this does not imply that the substances in question are not wholly dissociated in the external solution, since we may assume that ions combine at the surface of X to form molecules and so pass through X). We should have to assume that the cell gives out as many cations as it takes up, but it is of course possible that it can produce enough $H^+$ ions for this purpose.

The conclusions already drawn regarding the series A, B, C would remain unchanged on the basis of the scheme proposed by Horovitz, providing diffusion potentials are neglected; this however is not permissible, according to Horovitz, so that we cannot tell which cation tends to be taken up to a greater degree, but we can say in regard to the series A, B, C that the cation of A tends to be taken up more than that of B or else has a greater mobility in X (or that both statements are true). On either basis we should conclude that the cation of A is able to enter X.

Whenever the entrance or taking up of ions is mentioned it is of course understood that effects may be produced by the exit of these ions.


This is still unpublished. I am indebted to Dr. Horovitz for the privilege of seeing his manuscript in advance and for discussion of the theories here considered.
Let us now consider the Donnan potential. As already stated, it may be doubted whether any part of an actively growing cell can come into a condition of real equilibrium with its surroundings and it could not very well be in equilibrium with two different solutions applied at different places. The question arises whether an approximate local Donnan equilibrium might be set up at two different points in contact with different concentrations of the same salt, so that we could calculate the P.D. by means of the usual formula

\[ \text{P.D.} = RT \log \frac{C_1}{C_2} \]

where \( C_1 \) is the concentration of a diffusible cation in the external solution and \( C_2 \) its concentration inside the membrane.

If this were the case we might expect a concentration effect which would fall off with increase of concentration (as is the case with protoplasm). But, as has been pointed out by Michaelis, we should not expect this to be as large as that observed in the cell. If such an effect exists it seems very doubtful whether it can be calculated in this way since there are disturbing factors, such as movement of water due to osmotic pressure, etc.

On the other hand, it is difficult to see how a chemical effect could arise since at equilibrium all the diffusible cations would be expected to behave alike. They might, of course, differ in speed of penetration or in activity, but it is a question to what extent a temporary chemical effect could arise in this way. If it exists we should still conclude that if \( A \) is negative to \( B \) (in the series mentioned above) it means that more cations of \( A \) are taken up.

\(^{33}\) This is variously classified by different authors but may for convenience be placed in a separate category. Cf. Michaelis, L., Hydrogen ion concentration, Baltimore, 1926, i. Michaelis, L., and Perlzweig, W. A., J. Gen. Physiol., 1926–27, x, 575.

\(^{34}\) The (unpublished) formula proposed by Horovitz, as well as that employed by Beutner, would lead us to expect an increase in concentration effect (i.e. an increased increment in potential difference for a fivefold dilution) as the concentration increases from zero, but after a certain point is reached no further increase in the concentration effect would be expected. It is found both with protoplasm and with the organic liquids immiscible with water studied by Beutner that the concentration effect falls off as the concentration increases above a certain point.

\(^{35}\) Michaelis, L., Hydrogen ion concentration, Baltimore, 1926, i.
We cannot decide at present to what extent bioelectric effects are to be attributed to diffusion potentials, to phase boundary potentials, or to Donnan potentials. It seems probable that in most cases two or more of these act simultaneously. We can, however, arrive at certain conclusions regarding the penetration of ions, provided we adhere to any of the schemes discussed above. Regarding the series of chlorides A, B, C previously referred to, we can say that the cations of A and B must be able to penetrate. For on the basis of any of the hypotheses outlined above we can say that even if the cations of C cannot enter, those of B must go in in order that B may be negative to C. Conversely, if we have a series of K salts D, E, F (of the same concentration), with D positive to E and E positive to F, we can say that the anions of D and E penetrate even if those of F do not.

We can also say that where there is a concentration effect not due solely to the cell wall ions must be able to enter the protoplasm.

Let us now consider the possibility of measuring the absolute values of certain potential differences. It seems probable that in brief experiments the applied salt solution does not penetrate through X into the deeper layers, and that in consequence any changes observed are due to changes in X. Let us suppose that we lead off from two places, B and C, and measure the potential difference of C against B 12. Since the potential difference of B is opposite to that of C in the circuit, we may write

\[ \text{Observed P.D. of C} = (\alpha_c + Z_c) - (\alpha_B + Z_B), \]

where \( \alpha_c \) is the absolute value of the potential difference at the surface of X (\( \alpha \), Fig. 2) at the point in contact with C, and \( Z_c \) is the sum of the remaining values in X and in the deeper layers (the values of \( \alpha_B \) and \( Z_B \) have corresponding significance).

If at the point in contact with B anions and cations tend to enter X to about the same degree, the value of \( \alpha_B \) may be negligibly small and we shall have

\[ \text{Observed P.D. of C} = \alpha_c - Z_c - Z_B. \]

If the values of Z are the same at all points in the cell (assuming that the applied salt solution has not yet penetrated through X) this reduces to

\[ \text{Observed P.D. of C} = \alpha_c. \]
In this case we might be able to approximate the absolute value of $a_C$.

If we obtain the absolute value of the potential difference across the protoplasm at $C$, and if, as before, we write $P.D. = a + Z$, it is evident that if the value at $a$ is negligibly small we may be able to approximate the value of $Z$. If it should happen (a possibility suggested above) that neither anions nor cations enter $Y$, the potential difference at both $c$ and $d$ might be zero and we should be able to approximate the value of $b_C + g + h$.

It seems evident from what has been said that bioelectrical investigations may throw some light upon the structure and properties of protoplasm. An especial advantage of this method of study is that it enables us to detect and record changes which last only a fraction of a second. It may thus uncover important activities of the protoplasm which would otherwise escape observation on account of the crudity of our methods of observation. This will be fully discussed in later reports.

**SUMMARY.**

It is pointed out that there are great advantages in using single cells instead of tissues in the study of bioelectrical phenomena.

Certain bioelectrical phenomena are discussed in relation to the structure of protoplasm.

Under certain circumstances measurements of potential differences may enable us to determine what ions enter the protoplasm.

Under suitable conditions we are able to ascertain the potential differences across the protoplasm at single points, instead of being obliged merely to measure the differences between two points.