BIOELECTRIC POTENTIALS IN VALONIA

THE EFFECT OF SUBSTITUTING KCl FOR NaCl IN ARTIFICIAL SEA WATER

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In a study of bioelectric potentials in Valonia macrophysa, the effects produced by KCl are especially interesting in view of the remarkable degree to which this salt is accumulated in the cell sap. The present report deals with changes in the P.D. across the protoplasm when a cell is transferred from natural sea water to certain artificial solutions resembling sea water, in which the concentration of KCl is varied from 0 to 0.500 mol per liter.

These solutions had the following composition, based on a recipe for artificial sea water recommended by McClendon, Gault, and Mulholland:

<table>
<thead>
<tr>
<th>Ion</th>
<th>Molar Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>0.570</td>
</tr>
<tr>
<td>K + Na</td>
<td>0.500</td>
</tr>
<tr>
<td>Br</td>
<td>0.001</td>
</tr>
<tr>
<td>Ca</td>
<td>0.011</td>
</tr>
<tr>
<td>SO₄</td>
<td>0.028</td>
</tr>
<tr>
<td>Mg</td>
<td>0.054</td>
</tr>
<tr>
<td>HCO₃</td>
<td>0.003</td>
</tr>
</tbody>
</table>

An artificial sea water made up in accordance with this formula, taking 0.012 as the molar concentration of KCl, has proved to be a satisfactory imitation of natural sea water for the purposes of these experiments. Valonia cells have been kept in this solution for weeks, and apparently will live in it indefinitely. The P.D. across the protoplasm of Valonia cells immersed in this solution is in good agreement with the values observed in natural sea water.

Measurements of e.m.f. were made by a compensation method, using a simple type of potentiometer and a Compton quadrant electrometer. By using this combination as a deflection potentiometer, as many as three readings per minute could be taken provided that the P.D. was not changing so rapidly as to require

1 For analyses of Valonia sap and a bibliography, see Osterhout, W. J. V., Biol. Rev., 1931, 6, 155-216, particularly Table I, p. 158, and Footnotes 1 and 2, p. 156.

resetting the potentiometer. The sensitivity of the electrometer permitted reading to 0.1 mv.

The technic of measuring P.D. with Valonia has been described in detail in earlier papers. The cell, supported by a four-pronged cork mount, is impaled on a glass capillary filled with artificial sap, through which electrical connection with the vacuole is established. Contact with the outside of the cell is made through a strip of wet filter paper touching its highest point; the solutions applied to the cell flow down the filter paper and over the entire cell surface. The rate of flow is usually 2 to 3 cc. per minute. When the solution applied to the cell is changed, the cell is rinsed with about 5 cc. of the new solution delivered rapidly from a pipet. This rapid rinsing is a small but important improvement in technic in cases where the P.D. passes through a maximum. It has been shown that the P.D. may be decreased considerably by the presence of a second solution wetting a part of the surface of the cell; consequently, too slow rinsing may prevent the P.D. from reaching its full maximum value.

The following experiment furnished a particularly striking example of the effect of a second solution wetting a part of the cell surface. The arrangement of the cell is shown in Fig. 1. Strips of wet filter paper, a and b, served to make electrical contact with opposite ends of a rather long cell, impaled with its long axis nearly horizontal. The P.D. measured directly between the ends of the cell could then be compared with the difference between the P.D.'s measured between

Fig. 1. Diagram showing the arrangement of an impaled Valonia cell (supported on a cork mount) in an experiment to demonstrate the effect of a second solution wetting a part of the cell surface.

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the interior of the cell and the opposite ends. When natural sea water was applied at both ends, the P.D. between the interior of the cell (positive in the external circuit) and $a$ was found to be 4.6 mv., that between the interior and $b$, 5.4 mv. The difference, 0.8 mv., was in good agreement with the value, 0.9 mv., measured directly between $a$ (positive) and $b$. This is in accord with the behavior of intact cells, where the more pointed end, at which the cell had previously been joined to the parent cell, is generally found to be slightly positive with respect to the opposite end. When natural sea water at $a$ was replaced by KCl-sea water (i.e., the artificial sea water described above with all NaCl replaced by KCl), $b$ became positive with respect to $a$, the P.D. varying between 3.8 and 5.8 mv. This agrees with the usual behavior of intact cells in such experiments. Measurement between the interior of the cell (positive) and KCl sea water at $a$ showed 12.5 mv., between the interior and natural sea water at $b$, 7.1 mv., difference, 5.4 mv. KCl-sea water was then applied at both ends of the cell, the entire surface was rinsed with KCl-sea water, and the P.D. was measured between the interior of the cell and the two ends, $a$ and $b$, connected together. This P.D. rose rapidly, passing through a maximum value of over 70 mv., which we shall see agrees with the usual behavior of impaled cells measured with KCl-sea water. From this and similar experiments we conclude (1) that these measurements were not affected by impalement, and (2) that the P.D. with KCl-sea water may be greatly diminished if a part of the cell surface remains wet with natural sea water.

Electrical effects observed when Valonia cells are exposed to artificial sea waters containing different concentrations of KCl are illustrated by the P.D.-time curves, Fig. 2. These seven curves represent a series of measurements on the same cell using samples of modified artificial sea water in which the concentrations of KCl (in the order in which the measurements were carried out) were 0.400, 0.500, 0.300, 0.200, 0.100, 0.050, and 0.000 mol per liter. The pH of the solutions used in this series was adjusted to the same value as that of natural sea water, as indicated by the color of cresol red.

The P.D.-time curves with KCl-rich sea waters closely resembled the curves obtained with natural and artificial Valonia sap, and are undoubtedly to be interpreted in the same way. In both cases there was an initial rise to a maximum, followed by a rapid fall to a minimum, and then a gradual rise to a second maximum. This behavior has

4 In all experiments, the impaled cells are allowed to stand in sea water for at least 2 days before the first measurement, to permit the cell to form a good seal between the protoplasm and glass, and to recover from any temporary effects of impalement.

FIG. 2. P.D.-time curves, obtained from a series of measurements on the same Valonia cell, showing the changes in the P.D. across the protoplasm which are observed when the external solution is changed from natural sea water (shaded circles) to artificial modified sea waters (open circles) in which the concentration of KCl is varied from 0 to 0.500 mol per liter. The scale of ordinates at the left applies to the 4 lower curves; the scale at the right to the 3 upper curves. The sign is that of the inside of the cell; i.e., for positive values of P.D., positive current tends to flow in an external circuit from the capillary through the measuring instrument to the solution applied externally.
been interpreted as due to an increase in the concentration of KCl in the main body of the protoplasm.\textsuperscript{3,4} If this explanation is correct, the shape of the P.D.-time curves should serve as a rough measure for comparing the rates at which KCl enters the protoplasm under different conditions; \textit{i.e.}, other things being equal, more rapid penetration of KCl should cause the curve to fall earlier and more sharply from its first maximum, and also to rise again more rapidly after passing through a minimum.

Application of this hypothesis to experiments in which the pH of the external solution was varied brings up some interesting questions. It has been found that the P.D. across the protoplasm of \textit{Valonia} cells in natural sea water is not affected appreciably by changes in the pH within the range pH 5 to pH 10. We may therefore expect that within these limits changes in the pH of KCl-rich sea water will not alter the value of the initial maximum P.D. Certain theories\textsuperscript{4} which have been proposed to account for the accumulation of KCl in \textit{Valonia} sap, however, lead to the prediction that varying the pH of KCl-rich sea water applied to the cell should change the rate at which KCl enters the protoplasm, and hence should affect the shape of the P.D.-time curve. These theories, while differing in several important respects, are alike in connecting the mechanism of accumulation with the difference between the pH of the sap and that of the external sea water, a difference which is supposed to be maintained by the production of H\textsubscript{2}CO\textsubscript{3} or some other acid in the cell. Increasing this difference by raising the pH of the external solution should enhance the rate at which KCl enters the protoplasm; conversely, lowering the pH should inhibit the entrance of KCl.

These conclusions were tested by comparing the P.D.-time curves observed when a cell was exposed to samples of KCl-rich sea water of different pH. Effects due to variations in pH can be detected more easily with solutions containing a relatively low concentration of KCl, since with these solutions the characteristic fluctuations in P.D. are ordinarily not very rapid. In the artificial sea water used in this experiment the concentration of KCl was 0.050 molar. The pH of

the more acid sample, determined by an indicator method, was about 5 at the beginning of the experiment; at the end, 3 days later, it had changed to about 6. The alkaline solution was initially at about pH

![Graph showing P.D.-time curves, illustrating the effect of changing the pH of KCl-rich sea water containing 0.050 mol of KCl per liter from pH 5 (triangles) to pH 10 (circles). The shaded triangles or circles represent the last of the three measurements of each series. As ordinates are plotted the differences between the P.D. across the protoplasm in the KCl-rich sea water and the P.D. previously observed in natural sea water. The curves are displaced horizontally to prevent confusion.](image-url)
10, as shown by incipient precipitation of Mg(OH)$_2$, but probably became less alkaline before the end of the experiment. In order to distinguish between effects due to possible alteration in the cell during the experiment and effects actually produced by varying pH, three measurements were made on each cell, applying the acidified and alkaline solutions alternately. The cell was allowed to stand overnight in natural sea water between measurements. Results of such experiments with two different cells are shown in Fig. 3. It will be seen that in every case the first peak of the P.D.-time curve is broader in the measurements with the more acid solution, and that the rise after passing through a minimum is more moderate. This might be interpreted as evidence that KCl enters the protoplasm less rapidly from solutions of lower pH. To this extent, the experiment is in agreement with the theories for accumulation of KCl.

In the vacuolar sap, however (where the concentration of KCl is about 0.5 molar and the pH is about 6), the concentration of potassium is ten times as great as in the KCl-rich sea water here employed at pH 5, while the concentration of H$^+$ is only about 1/10 as great. According to the theories for accumulation of KCl, we should expect potassium to come out of the cell under these conditions. The P.D.-time curves with this acidified KCl-rich sea water, on the contrary, have the characteristic shape which has been explained as due to the entrance of KCl. Furthermore, the P.D. in natural sea water remains constant when the pH is lowered to 5, showing no such changes as might be expected if KCl were coming out of the cell. This conflict between the theories dealing with the accumulation of KCl and the interpretation of these P.D.-time curves can probably be decided by a study of the composition of the sap of *Valonia* cells which have been exposed to acidified sea water.$^7$

Since lowering the pH of KCl-rich sea water broadens the first peak of the P.D.-time curve without apparently affecting the value of the maximum P.D., the probability of observing the full value of this maximum can be increased by using acidified solutions. Advantage is taken of this in some of the later measurements described in this paper.

It is evident from inspection of Fig. 2 that the first maximum in

$^7$ Such experiments have been under way for some time at this laboratory, and will be published in the near future.
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Natural sea water

\[
X \quad \text{(in equilibrium with natural sea water)} \quad a
\]
\[
W \quad b
\]
\[
Y
\]

Vacuolar sap

KCl-rich sea water

\[
X \quad \text{(in equilibrium with KCl-rich sea water)} \quad a'
\]
\[
W 
\]
\[
Y \quad \text{(in equilibrium with natural sea water)} \quad b
\]

Vacuolar sap

**Fig. 4.** Hypothetical diagrams illustrating the theory of protoplasmic layers. The middle layer, \(W\), represents the main body of the protoplasm, assumed to be aqueous. \(X\) and \(Y\) represent the external and internal surface layers of the protoplasm, which are assumed to have the properties of two different non-aqueous liquids, immiscible with water. The observed P.D.'s are accordingly considered as made up of phase-boundary potentials at the surfaces of \(X\) and \(Y\), plus diffusion potentials within the different layers.

The upper diagram represents conditions when the cell has been exposed to natural sea water for a long time, so that the external layer, \(X\), is in distribution equilibrium with natural sea water. For the purposes of the present discussion, the P.D. at \(b\) (the inner surface of \(X\)) and all other P.D.'s located between \(b\) and the vacuolar sap may be lumped and called P.D.\(_0\). The P.D. with natural sea water may then be considered as the sum of P.D.\(_0\) plus the phase-boundary potential at \(a\):

\[
P.D._{\text{natural s.w.}} = P.D.a + P.D.0
\]

The lower diagram represents conditions supposed to correspond to the first maximum in the P.D.-time curve, soon after KCl-rich sea water has replaced natural sea water as the external solution. The outer region of the \(X\) layer, between \(a'\) and \(c\), is now in distribution equilibrium with KCl-rich sea water, but between \(c\) and \(b\) concentrations still remain the same as when the cell was in natural sea water. At \(b\), and below, everything remains unchanged; the value of P.D.\(_0\) is therefore the same as in the measurement with natural sea water. At the outer surface of \(X\), however, conditions have changed from the situation represented at \(a\) to that at \(a'\), and at \(c\) a new diffusion potential has been set up. Hence we now have:

\[
P.D._{\text{KCl-rich s.w.}} = P.D.a' + P.D.c + P.D.0
\]

The change in P.D. across the protoplasm when natural sea water is replaced by KCl-rich sea water is accordingly:

\[
P.D._{\text{KCl-rich s.w.}} - P.D._{\text{natural s.w.}} = P.D.a' + P.D.c - P.D.a
\]
In other words, the initial rise in P.D. corresponds to the P.D. of the hypothetical chain:

<table>
<thead>
<tr>
<th>KCl-rich sea water</th>
<th>region of the outer surface layer of the protoplasm in equilibrium with KCl-rich sea water</th>
<th>natural sea water</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha' )</td>
<td>( \alpha )</td>
<td></td>
</tr>
</tbody>
</table>

the time curve represents the only value of the P.D. which can be correlated with the concentration of KCl in the external solution. From examination of these curves and other similar data it appears that greater regularity is found if we subtract from this value the P.D. previously observed in natural sea water. (Similarly, in studying the concentration effect with natural sea water it was found that it is the difference between the P.D.'s in natural sea water and in diluted sea water which is proportional to the logarithm of the dilution.) In terms of the theory of protoplasmic layers, illustrated in Fig. 4, we may assume that only the outer surface layer of the protoplasm, \( X \), and the solutions applied externally are concerned in this first sharp rise in P.D. The difference between the initial maximum in the P.D.-time curve and the P.D. previously observed in natural sea water then represents the E.M.F. of the ideal system:

<table>
<thead>
<tr>
<th>KCl-rich sea water</th>
<th>outer surface layer of the protoplasm</th>
<th>natural sea water</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha' )</td>
<td>( \alpha )</td>
<td></td>
</tr>
</tbody>
</table>

In cases where this assumption is incorrect, some KCl having diffused through the external surface layer before the P.D. attains its maximum, the observed value will be lower than that corresponding to this ideal system. Other things being equal, therefore, the higher of two values

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is probably the more reliable; extremely low values must be viewed with suspicion.

While the variations in the shape of the p.d.-time curves were less pronounced with KCl-rich sea water than with Valonia sap,\textsuperscript{5,6} the shape of the curve did not always permit assigning a definite value to the first maximum. In some cases the first peak was so sharp that the p.d. had begun to fall before the first measurement could be made. In other cases, the rise to the first maximum was so slow as to lead to suspicion that some KCl had diffused through the outer surface layer before the maximum p.d. was reached. This difficulty was generally found in cases where the cell had become coated with a gelatinous film of marine bacteria; it may have been caused by bacteria also in cases where this film was not detected. Occasionally the time curve passes through a point of inflection instead of a maximum;\textsuperscript{10} in such cases the assignment of a definite value to the p.d. would obviously be more or less arbitrary.

In Fig. 5, differences between the p.d. in natural sea water and the first maximum in the p.d.-time curve with KCl-rich sea water are plotted against the concentration of KCl in the modified sea water. From a series of thirty-five measurements (including the ones reported in Fig. 2) which had been carried out primarily to study the form of the p.d.-time curve, twenty-nine curves were obtained which permitted assigning definite values to the first maxima. These values are represented by shaded circles in Fig. 5. The five points represented by triangles were taken from some earlier exploratory measurements, and may not be strictly comparable with the more recent data, since with these solutions, as with Valonia sap,\textsuperscript{6} the p.d. may be considerably affected by the condition of the cells. Measurements with KCl-free sea water were not included in Fig. 5 because this solution seemed to be injurious to Valonia. While in some experiments the behavior of KCl-free sea water agreed with the curve marked "C\textsubscript{K} = 0" in Fig. 2, in others secondary changes occurred similar to those observed when Valonia cells are exposed to hypotonic diluted sea water for a short time, or to isotonic diluted sea water for a longer time.\textsuperscript{8} These

\textsuperscript{10}A p.d.-time curve of this form (with artificial sap) is shown in an earlier report, J. Gen. Physiol., 1929-30, 13, 215, Fig. 6, Curve B.
changes are evidenced by erratic fluctuations in the P.D., and sometimes by high P.D.'s of opposite sign (inside of the cell negative). It now appears that a moderate reduction in the concentration of KCl does not necessarily involve injury to the cell, since Jacques and

![Graph showing the relation between the concentration of KCl in modified sea water and the P.D. across the protoplasm in Valonia.](image)

**Fig. 5.** Curve showing the relation between the concentration of KCl in modified sea water and the P.D. across the protoplasm in *Valonia*. The ordinates represent differences between the P.D. in natural sea water and the first maximum in the P.D.-time curve when natural sea water was replaced by KCl-rich sea water. Each group of points represents measurements with a single concentration of KCl but in order to show all the observed values some of the points have been displaced to the right or left of their true abscissae.

Triangles represent five measurements on four *Valonia* cells, collected Nov. 25, 1929, measured Jan. to Feb., 1930. Shaded circles represent twenty-nine measurements on fifteen different cells, collected Nov. 14, 1930, measured Nov. 14, 1930, to Jan. 1, 1931. In these measurements the modified sea water had the same pH as natural sea water. Open circles represent fifty-one measurements on ten individuals, collected May 25, 1931, measured June to July, 1931. In these measurements, the modified sea water was acidified to pH 6.

The curve drawn through these groups of points is identical with the curve drawn in Fig. 6.
Osterhout\textsuperscript{11} have recently found that \textit{Valonia} cells survived exposure for 20 days to modified sea water containing one half the usual amount of KCl.

While these data with KCl-rich sea water showed that the initial change in P.D. varies in a regular manner with the concentration of KCl, they seemed hardly adequate for determining the exact relation between P.D. and concentration. Since these thirty-four accepted values represented measurements on nineteen different cells, an average of less than two measurements per cell, possible variations among the individual cells might well prove very misleading. Furthermore, the rejection of a number of unsatisfactory observations introduced an undesirable subjective factor. Accordingly, it seemed worth while to carry out a new series of measurements for the express purpose of determining the value of the initial change in P.D. when sea water is replaced by KCl-rich sea water.

In the earlier experiments, the cells had been exposed to modified sea water for an hour or more in each measurement; the solutions had been adjusted to the same pH as natural sea water. In the new series, the pH of the KCl-rich sea waters was lowered to 6 in order to broaden the first peak of the P.D.-time curves, and thus increase the probability of observing the full value of the P.D. change. To reduce to a minimum any possible alteration in the cell produced by exposure to KCl-rich solutions, the cells were left in contact with these solutions only just long enough to determine the full value of the P.D. change; as soon as the P.D. had started to fall from its first peak (generally within about 3 minutes) the cells were replaced in natural sea water and allowed to stand overnight or longer before the next measurement. No evidence of marine bacteria in quantities which could be detected by macroscopic observation was noted during these experiments.

The data obtained in this series, fifty-one measurements on ten cells, are shown plotted as open circles in Fig. 5. In a number of cases, where the cell was measured more than once with a given solution, the highest observed value of the P.D. is reported. The agreement of this series with the earlier data confirms the assumption that the values of the first maxima are not altered by moderate changes in the pH of KCl-rich sea water.

The new data are free from some of the defects of the earlier series. Due to the larger number of measurements with each cell, variations among the individual cells affect all parts of the P.D.-concentration curve more or less equally. Since doubtful measurements could be repeated, it proved unnecessary to reject any measurement except in favor of a similar measurement with the same cell. The personal factor was thus greatly decreased, although not wholly eliminated. Another uncertainty still remained: it became apparent that the behavior of some of the cells was changing considerably during the series of measurements. It was difficult to estimate how nearly such changes in different cells would compensate one another.

It was concluded that the most reliable data for determining the relation between P.D. and concentration of KCl would consist in a series of measurements in duplicate on a single cell, in which the agreement between the first and second values observed with each solution would indicate to what extent the cell had changed during the experiment. Several series of measurements in duplicate were carried out in accordance with this plan. The most successful of these, in which the cell evidently suffered no significant alteration, is recorded in Fig. 6 (upper curve).

In accordance with the theory of protoplasmic layers illustrated in Fig. 4, the P.D.'s plotted in Fig. 5 and Fig. 6 may be interpreted as equivalent to the P.D. of the ideal system:

\[
\begin{array}{c|c|c}
\text{KCl-rich sea water} & \text{region of the outer surface layer of the protoplasm in equilibrium with KCl-rich sea water} & \text{region of the outer surface layer of the protoplasm in equilibrium with natural sea water} \\
\hline
\text{outer surface layer of the protoplasm in equilibrium with KCl-rich sea water} & a' & c \\
\text{outer surface layer of the protoplasm in equilibrium with natural sea water} & a &
\end{array}
\]

where the outer surface layer of the protoplasm is assumed to have the properties of a non-aqueous liquid immiscible with water. According to various theories which have been proposed for calculating such P.D.'s, the observed value may be made up of (1) phase-boundary potentials at \(a\) and \(a'\) and (2) a diffusion potential in the non-aqueous layer at \(c\). Since the calculation of such mixed potentials is a formida-
ble task, the assumption is often made that the relative mobilities of ions in the non-aqueous layer are not greatly different from their values in aqueous solutions, and hence that the diffusion potential at $c$ will

![Graph showing the relation between KCl concentration and P.D. across protoplasm in Valonia.]

**Fig. 6.** Curves showing the relation between the concentrations of KCl in modified sea water (plotted as abscissae) and the P.D. across the protoplasm in *Valonia*.

The ordinates of the upper curve (scale at left) represent differences between the P.D. in natural sea water and the first maximum in the P.D.-time curve when natural sea water was replaced by KCl-rich sea water. The data represented by open circles were obtained in a series of measurements in duplicate using the same *Valonia* cell, the numbers adjacent to these circles indicating the order in which the measurements were made. Measurements were at room temperature, which varied between 23.5° and 26.5°, average, 25°C. The *Valonia* cell was collected May 25, 1931, measured June 16 to July 3, 1931.

The ordinates of the lower curve (scale at right) represent the antilogarithms of the quotients of these P.D. changes divided by $\frac{RT}{0.434F}$ (which at 25°C. has the value 59.1 mv.). The values plotted as shaded circles were calculated from the averages of the two P.D.'s plotted as open circles directly above. The upper curve was obtained from the straight line drawn through these shaded circles: each ordinate of the upper curve is equal to 59.1 multiplied by the logarithm of the corresponding ordinate of the straight line.
be negligibly small as compared with the phase-boundary potentials at $a$ and $a'$. If so, phase-boundary potentials calculated by assuming suitable values for the hypothetical ionic partition coefficients should agree with the observed P.D.'s. But since these ionic partition coefficients and the mobilities in the non-aqueous layer are alike unknown, it is just as reasonable to assume that the partition coefficients of the various ions are approximately equal, in which case the phase-boundary potentials at $a$ and $a'$ will be negligibly small. The observed P.D. would then represent only the diffusion potential at $c$. It is interesting to compare the observed P.D.'s with the values calculated in accordance with each of these mutually contradictory assumptions.

The formula for calculating phase-boundary potentials with mixed electrolytes has been derived by Michaelis and Fujita\textsuperscript{12} and by Horovitz.\textsuperscript{13} According to their equation, the P.D. at either phase-boundary, $a$ or $a'$, is given by the expression:

$$
P.D. = \frac{RT}{2F} \ln \frac{A_K C_K + A_{Na} C_{Na}}{A_{Cl} C_{Cl}}
$$

where $C_K$, $C_{Na}$, and $C_{Cl}$ represent the concentrations\textsuperscript{14} of these ions in the natural or modified sea water, and $A_K$, $A_{Na}$, and $A_{Cl}$ the "true" ionic partition coefficients. The Ca, Mg, and SO$_4$ ions are omitted because it has been shown that P.D.'s observed with diluted sea water are not affected by considerable changes in the concentrations of these ions.\textsuperscript{8} It may therefore be assumed that the partition coefficients of these ions are small (an assumption which is in agreement with the low concentrations of these ions found in the vacuolar sap) and hence that the products, $A \cdot C$, for these ions may be neglected in comparison with the corresponding products for K, Na, and Cl. Other ions are not considered because their concentrations in sea water are very small. It has been shown, for example, that the P.D. is independent of rather large changes in pH. The P.D. between natural sea water and KCl-

\textsuperscript{12} Michaelis, L., and Fujita, A., Z. phys. Chem., 1924, 110, 266.

\textsuperscript{13} Horovitz, K., Z. phys. Chem., 1925, 115, 424.

\textsuperscript{14} For the sake of simplicity, concentrations have been used instead of activities in this discussion. Since the natural and modified artificial sea waters used in these measurements were all solutions of the same ionic strength, and since the activity coefficients for KCl and NaCl at this ionic strength are not very different, errors introduced by omitting the activity coefficients should be small, and approximately the same in all cases.
rich sea water, the algebraic sum of the p.d.'s at a and a', will then be
given by the following expression:

\[ \text{p.d.} = \frac{RT}{2F} \ln \frac{A_K C'_K + A_\text{Na} C'_\text{Na}}{A_{Cl} C'_{Cl}} + \frac{RT}{2F} \ln \frac{A_{Cl} C_{Cl}}{A_K C_K + A_\text{Na} C_\text{Na}} \]

where primes indicate concentrations in the KCl-rich sea water. Since
the concentration of Cl is the same on both sides, the equation
becomes:

\[ \text{p.d.} = \frac{RT}{2F} \ln \frac{A_K C'_K + A_\text{Na} C'_\text{Na}}{A_K C_K + A_\text{Na} C_\text{Na}} \]

It can be shown, however, that this equation is inadequate to account
for the p.d. changes observed with Valonia. For example, we may
consider the p.d. between natural sea water and KCl-sea water (in
which all the NaCl is replaced by KCl) for which the observed values
plotted in Fig. 5 range between 51.4 and 69.9 mv. If we insert numeri-
cal values in Equation 3:

\[ \text{p.d. (in mv.)} = \frac{59.1}{2} \log \frac{0.500 A_K}{0.012 A_K + 0.488 A_\text{Na}} \]

and then divide the numerator and denominator of the fraction by
0.500 \( A_K \) we get:

\[ \text{p.d.} = 29.6 \log \frac{1}{0.024 + 0.976 \frac{A_\text{Na}}{A_K}} \]

We may now calculate the p.d. according to this equation by assuming
different values for \( A_K \). If we try first the assumption that \( A_K \)
may be 100 times as great as \( A_\text{Na} \), the calculated p.d. is found to be
43.5 mv., which, although of the right order of magnitude, is obviously
too low. Materially better agreement with the observed values
cannot be obtained, however, by assuming a larger value for \( A_K \),
since if \( A_K \) is allowed to increase without limit we find that the p.d.
approaches the limiting value of only 47 mv. It is obvious that no
positive value which can be assigned to \( A_K \) will account for the
observed potentials.
We may now test whether the P.D. with KCl-rich sea water can be calculated more satisfactorily as a diffusion potential (at c) by assuming a reasonable value for the mobility of $K^+$ in the non-aqueous layer. This calculation is greatly simplified by the assumption that the ionic partition coefficients, $A_K$ and $A_{Na}$, are equal, and hence that the actual partition coefficients of KCl and NaCl are equal. It follows from this assumption that the sum of the phase-boundary potentials at $a$ and $a'$ is equal to zero.

In this calculation only $K^+$, $Na^+$, and $Cl^-$ are considered; other ions are neglected for the same reasons as in the above calculation of phase-boundary potentials. Either Planck's or Henderson's formula for the liquid junction potential may be taken as a starting point, since both reduce to the same expression for the case of two electrolytes with common anion at the same concentration:

$$P.D. = \frac{RT}{F} \ln \frac{c_{Na}u_{Na} + c_Ku_K + c_{Cl}u_{Cl}}{c_{Na}u_{Na} + c_Ku_K + c_{Cl}u_{Cl}}$$

where $u_{Na}$, $u_{Na}'$, and $u_{Cl}$ represent the mobilities of these ions in the non-aqueous layer, $c_{Na}$, $c_{Na}'$, and $c_{Cl}$ are the concentrations of these ions in the non-aqueous layer in equilibrium with natural sea water, and $c_{Na}'$, $c_{Na}'$, and $c_{Cl}$ are their concentrations in the non-aqueous layer in equilibrium with KCl-rich sea water. (The concentration of $Cl$ is the same on both sides.) In accordance with our assumption that the partition coefficients of $K^+$ and $Na^+$ are equal ($A_K = A_{Na} = A$) we may express the concentrations in the non-aqueous layer in terms of the concentrations in the sea waters:

$$c_{Na} = A \cdot C_{Na}, \quad c_{Na}' = A \cdot C_{Na}'; \quad c_K = A \cdot C_K, \quad c_K' = A \cdot C_K'$$

$$c_{Cl} = A (C_{Na} + C_K) = A (C_{Na}' + C_K').$$

Substituting in Equation 4:

$$P.D. = \frac{RT}{F} \ln \frac{AC_{Na}u_{Na} + AC_Ku_K + A(C_{Na} + C_K) \nu_{Cl}}{AC_{Na}u_{Na} + AC_Ku_K + A(C_{Na} + C_K) \nu_{Cl}}$$

$$= \frac{RT}{F} \ln \frac{C_{Na}u_{Na} + C_Ku_K + (C_{Na} + C_K) \nu_{Cl}}{C_{Na}u_{Na} + C_Ku_K + (C_{Na} + C_K) \nu_{Cl}}.$$
We may now substitute in Equation 5 certain numerical values: the concentrations of Na and K in natural sea water, and the value of \( RT/F \) at 25°C.; at the same time we may change from natural to common logarithms:

\[
\text{P.D. (in mv.)} = 59.1 \log \frac{C'_N a_{Na} + C'_K a_{K} + 0.500 \tau_{Cl}}{0.488 a_{Na} + 0.012 a_{K} + 0.500 \tau_{Cl}}
\]

The agreement of the data with an equation of this type can be tested more readily if Equation 6 is rearranged (using the relation, \( C'_{Na} + C'_K = 0.500 \)) into:

\[
\text{antilog} \frac{\text{P.D.}}{59.1} = \frac{a_{Na} - a_{Na}}{0.488 a_{Na} + 0.012 a_{K} + 0.500 \tau_{Cl}} \cdot \frac{C'_N}{C'_K} + \frac{0.500 \left( a_{Na} + \tau_{Cl} \right)}{0.488 a_{Na} + 0.012 a_{K} + 0.500 \tau_{Cl}}
\]

which is an equation of the form:

\[
\text{antilog} \frac{\text{P.D.}}{59.1} = B c'_K + D
\]

where \( B \) and \( D \) are constants. Accordingly, if values of antilog \( \frac{\text{P.D.}}{59.1} \) calculated from observed values of P.D. are plotted as ordinates against the corresponding concentrations of KCl in modified sea water as abscissae, the points should fall along a straight line.

In Fig. 6, averages of the P.D. values represented by open circles in the upper curve were used in computing the values of antilog \( \frac{\text{P.D.}}{59.1} \) plotted as shaded circles directly below. It is evident that the points represented by these shaded circles do adhere very closely to the straight line which has been drawn through them. The curve drawn through the open circles in Fig. 6 was obtained from this straight line; i.e., each ordinate of the upper curve is equal to 59.1 multiplied by the logarithm of the corresponding ordinate of the straight line. This upper curve therefore corresponds to Equation 6.

We conclude, therefore, that the first rise in the P.D.-time curve when natural sea water is replaced by KCl-rich sea water may be
calculated satisfactorily by substituting in Equation 6 suitable values for the relative mobilities of $K^+$, $Na^+$, and $Cl^-$ in the non-aqueous layer. From measurements of the concentration effect with natural sea water* the mobility of $Cl^-$ in Valonia protoplasm has been found to be five times as great as that of $Na^+$. While the absolute values of these mobilities are of course unknown, in these calculations we may equally well use relative mobilities referred to the mobility of $Cl^-$ taken as unity; i.e., $v_{cl} = 1.00$, $u_{na} = 0.20$. We may calculate the relative mobility of $K^+$ by inserting these values in Equation 6 or 7. For the case of the cell used in the measurements reported in Fig. 6, the relative mobility of $K^+$ is found to be 20. From the spread of the P.D. values plotted in Fig. 5, it is apparent that there is a considerable variation in the value of $u_k$ among different cells. Evidently the value of $u_k$ may also vary considerably in the same individual under different conditions. Since the curve drawn through the groups of points in Fig. 5 is identical with the curve drawn in Fig. 6, it is evident that the value, $u_k = 20$, is in good agreement with the average behavior of all the cells included in this report.

While it has been shown that the observed values of P.D. with KCl-rich sea water are accurately reproduced by Equation 6, it does not

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16 The relative mobility of $Na^+$ may also vary, but the good agreement usually found among measurements of concentration effect with natural sea water suggests that $u_{na}$ is subject to less variation than $u_k$ in cells of similar history. Valonia cells which have been kept at the laboratory for a long time, especially cells which have received relatively little illumination, show certain differences from recently collected cells. Observations made at different times using "old" cells with different histories (for which reason the data may not be strictly comparable) indicate that with such "old" cells (1) the P.D. with KCl-rich solutions is lower, and hence the value of $u_k$ is smaller; (2) the concentration effect with natural sea water is smaller, and hence the value of $u_{na}$ is larger; (3) the ratio of $K^+ : Na^+$ in the sap is lower than in recently collected cells. It is hoped that this apparent correlation between the composition of the sap and the relative mobilities of $K^+$ and $Na^+$ can be investigated more carefully.

Dr. L. R. Blinks has suggested (private communication) that the apparent variation in $u_k$ from cell to cell may actually represent the variation of some other factor, such as the thickness of cell wall or of protoplasm, which would affect the speed with which concentration changes reach $W$, and hence the height of the maximum in the P.D.-time curve. The highest calculated value of $u_k$ should accordingly be regarded as a minimum value for the relative mobility of $K^+$.
necessarily follow that the assumptions used in deriving this equation are correct. Any equation of the same general form, i.e.

$$\text{P.D.} = \frac{RT}{F} \ln (BC_K + D)$$

will of course fit the data equally well. It is possible that an equation of this type might be derived on the basis of entirely different assumptions, in which case the constants of the equation would be interpreted differently. Since, however, the equation has actually been derived on the assumption that the observed P.D. represents a diffusion potential, it is convenient to refer to the constants, $u_k$, $u_{sa}$, and $v_{oe}$, as apparent relative mobilities. It is hoped that these values, and the apparent relative mobilities of other ions obtained in the same way, may prove useful in interpreting bioelectric measurements with Valonia.

**SUMMARY**

The P.D. across the protoplasm of *Valonia macrophysa* has been studied while the cells were exposed to artificial solutions resembling sea water in which the concentration of KCl was varied from 0 to 0.500 mol per liter. The P.D. across the protoplasm is decreased by lowering and increased by raising the concentration of KCl in the external solution. Changes in P.D. with time when the cell is treated with KCl-rich sea water resemble those observed with cells exposed to *Valonia* sap.

Varying the reaction of natural sea water from pH 5 to pH 10 has no appreciable effect on the P.D. across *Valonia* protoplasm. Similarly, varying the pH of KCl-rich sea water within these limits does not alter the height of the first maximum in the P.D.-time curve. The subsequent behavior of the P.D., however, is considerably affected by the pH of the KCl-rich sea water. These changes in the shape of the P.D.-time curve have been interpreted as indicating that potassium enters *Valonia* protoplasm more rapidly from alkaline than from acidified KCl-rich sea water. This conclusion is discussed in relation to certain theories which have been proposed to explain the accumulation of KCl in *Valonia* sap.

The initial rise in P.D. when a *Valonia* cell is transferred from natural sea water to KCl-rich sea water has been correlated with the concen-
trations of KCl in the sea waters. It is assumed that the observed P.D. change represents a diffusion potential in the external surface layer of the protoplasm, where the relative mobilities of ions may be supposed to differ greatly from their values in water. Starting with either Planck's or Henderson's formula, an equation has been derived which expresses satisfactorily the observed relationship between P.D. change and concentration of KCl. The constants of this equation are interpreted as the relative mobilities of K⁺, Na⁺, and Cl⁻ in the outer surface layer of the protoplasm. The apparent relative mobility of K⁺ has been calculated by inserting in this equation the values for the relative mobilities of Na⁺ (0.20) and Cl⁻ (1.00) determined from earlier measurements of concentration effect with natural sea water. The average value for the relative mobility of K⁺ is found to be about 20. The relative mobility may vary considerably among different individual cells, and sometimes also in the same individual under different conditions.

Calculation of the observed P.D. changes as phase-boundary potentials proved unsatisfactory.