CALCULATIONS OF BIOELECTRIC POTENTIALS

V. POTENTIALS IN HALICYSTIS

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Interest in Halicystis and in Valonia has been stimulated by discoveries of marked contrasts and striking similarities existing side by side.

Although originally regarded as similar and placed in the same genus they are now classified in separate orders: they show great differences in morphology and reproduction, in composition of sap, and in bioelectric behavior. As examples of the last may be mentioned:

1. The normal P.D. is outwardly directed (positive) in Halicystis (65 mv.), but inwardly directed (negative) in Valonia (10 mv.). When these cells are placed in their own sap so as to set up the chain

\[ \text{sap} \rightarrow \text{protoplast} \rightarrow \text{sap} \]

the P.D. is negative in Valonia (60-70 mv.) and positive in Halicystis (40-50 mv.). These P.D.'s must be due to conditions in the protoplasm, which are strikingly different in the two plants.

2. Guaiacol changes the P.D. of Valonia in a positive direction and that of Halicystis in a negative direction. It decreases the apparent mobility of K⁺ in Valonia but not in Halicystis.

3. Lowering the pH of sea water changes the P.D. of Halicystis in a negative direction, but has practically no effect on the P.D. of Valonia.

4. Very low concentrations of NH₃ produce drastic changes in the

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3 The P.D. is called positive when the positive current tends to flow from the sap across the protoplasm to the external solution; i.e., it is outwardly directed.
4 In this paper when Valonia is mentioned V. macrophysa, Kütz., is meant and Halicystis means H. Osterhoutii, Blinks and Blinks.
5 Blinks, L. R., J. Gen. Physiol., 1934-35, 18, 409.

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p.d. of *Halicystis* (including reversal of the sign) but have little or no effect on *Valonia*.

Resemblances between the two plants are illustrated by their behavior toward alkali cations. The situation in *Halicystis* is illustrated in Table I. The p.d. in sea water is about 65 mV. positive. We see that partial or complete replacement of Na⁺ by K⁺ or Rb⁺ changes the p.d. in a negative direction. This is also the case with *Valonia*, and the extent of the change is similar in both forms. Replacement of Na⁺ by Li⁺ produces no change, whence we conclude that the apparent mobility of Li (or uLi) in the outer protoplasmic surface is equal to that of Na (or uNa). The latter has been found to be 0.2 in *Valonia* and the concentration effect 1° shows that it is about the same in *Halicystis*.

We also see differences in experiments with the alkali metals. In *Halicystis* uCs is higher than vCl and higher than uNa, but in *Valonia* uCs is lower than vCl and about equal to uNa.\(^9\)


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### Table I

**Changes in P.D. Caused by Substituting Various Chlorides (MCI) for Sea Water (S.W.)**

The number of observations is given in parenthesis. Sea water was positive in the external circuit in all cases where a difference of p.d. existed.

<table>
<thead>
<tr>
<th>MCI</th>
<th>Sea water to 0.5 M MCI, 1 part + S.W. in 1 part</th>
<th>u</th>
<th>Sea water to S.W. containing 0.5 M MCI</th>
<th>u</th>
<th>Sea water to S.W. containing 0.5 M MCI, 1 part + mannite solution, 1 part</th>
<th>u</th>
<th>Average for u</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>48.0 ± 2.1 (11)</td>
<td>uK = 16</td>
<td>63.0 ± 1.3 (14)</td>
<td>uK = 18</td>
<td>51.0 ± 3.3 (4)</td>
<td>uK = 19</td>
<td>uK = 17.7</td>
</tr>
<tr>
<td>RbCl</td>
<td>48.4 ± 3.2 (15)</td>
<td>uRb = 16</td>
<td>62.6 ± 1.2 (7)</td>
<td>uRb = 18</td>
<td>46.5 ± 2.9 (7)</td>
<td>uRb = 15</td>
<td>uRb = 16.3</td>
</tr>
<tr>
<td>CaCl</td>
<td>22.2 ± 1.6 (12)</td>
<td>uNa = 4.4</td>
<td>48.8 ± 2.2 (3)</td>
<td>uNa = 9.5</td>
<td>35.8 ± 3.0 (4)</td>
<td>uNa = 8.5</td>
<td>uNa = 7.5</td>
</tr>
<tr>
<td>LiCl</td>
<td>Zero (5)</td>
<td>uLi = 0.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>uLi = 0.2</td>
</tr>
</tbody>
</table>

Note. Sea water to (0.4 M MgCl₂ 1 part plus sea water 1 part) gave no change of p.d. (5 observations) whence uMg²⁺ = 1.9.

Sea water to (0.4 M CaCl₂ 1 part plus sea water 1 part) gave a change of 56.4 ± 2.0 mV. (5 observations) in a negative direction (but no calculation can be made).
On replacing sea water by 0.4 M MgCl₂ plus an equal volume of sea water we find no change in p.d. The calculation shows that if we put \( u_{Mg} = 1.9 \) we get 0.02 mv. with sea water positive in the external circuit but if we put \( u_{Mg} = 1.8 \) we get 0.29 mv. with sea water negative. If we assign still lower values to \( u_{Mg} \) the calculated value for sea water becomes correspondingly more negative. We therefore take \( u_{Mg} \) as 1.9.

The measurements were made on *Halicystis Osterhoutii*, Blinks and Blinks, using the technique described in former papers. Some of the measurements were made by W. C. Owens, using a D'Arsonval galvanometer in place of the string galvanometer employed in previous work.

No evidence of injury was observed in these experiments.

The temperature was 20 to 25°C.

The RbCl and CsCl were obtained from Theodor Schuchardt, the remaining salts from Kahlbaum.

In column II of Table I are shown the changes in p.d. produced by replacing Bermuda sea water by a solution made by mixing equal parts of Bermuda sea water and 0.6 M solution of a chloride. The mixture was brought to the p\( \text{H} \) of sea water (8.2); this applies to all the solutions used in this investigation unless otherwise stated.

Column IV gives the changes in p.d. produced by replacing the sea water by an artificial sea water containing 0.52 M of a chloride in place of the 0.488 M NaCl found in Bermuda sea water.

Column VI gives the changes observed when the solutions of column IV were diluted with an equal volume of a solution containing 1.1 M mannite\(^6\) + 0.012 M KCl + 0.02 M CaCl₂. The note appended to the table gives the results obtained by replacing sea water by a mixture\(^7\) of 0.4 M MgCl₂ or of 0.4 M CaCl₂ with an equal volume of sea water (both at p\( \text{H} \) 7.8).

The calculations of the apparent mobilities \( u_{K} \), \( u_{Rb} \), \( u_{Cs} \), and \( u_{Li} \) were made as in former papers by means of Henderson's equation, putting \( v_{Cl} = 1 \), and disregarding

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\(^6\) In the experiments here described the p.d. at the start (in sea water) was normal in all cases.


\(^8\) The p.d. is determined as in Damon's experiments (Damon, E. B., *J. Gen. Physiol.*, 1931–32, 15, 525; 1932–33, 16, 375; 1937–38, 21, 383) by taking the first maximum of the p.d. time curve.

\(^9\) For a time curve of the changes due to sea water containing 0.52 M KCl see Blinks, L. R., *J. Gen. Physiol.*, 1932–33, 16, 147, and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937–38, 21, 631, 707. The time required to reach the maximum was about 30 seconds for the cases dealt with in columns IV and VI and about 3 or 4 times as long for those dealt with in column II.


\(^11\) These mixtures were approximately isotonic with the sea water.

Mg$^{++}$ and Ca$^{++}$. The value of $u_{K}$ was taken as 0.2 as in Valonia and the value of $u_{K}$ in each column was taken as that given in the upper part of the column. Thus in column IV it was found by trial that $u_{K} = 18$ (putting $v_{Cl} = 1$). We then have for RbCl the following

\[
U_{I} = u_{Rb} \cdot C_{Rb} = u_{Rb} (0.52)
\]

\[
V_{I} = v_{Cl} \cdot C_{Cl} \text{ is taken for convenience as 0.5}
\]

\[
U_{II} = u_{Na} \cdot C_{Na} + u_{K} \cdot C_{K} = 0.2(0.488) + 18(0.012) = 0.314
\]

\[
V_{II} = v_{Cl} \cdot C_{Cl} = 1(0.5)
\]

We find by trial that $u_{Rb} = 18$.

The calculations have been made (as for Valonia in former papers) on the assumption that the partition coefficient $S$ ($S = \text{concentration in the external non-aqueous protoplasmic surface} + \text{concentration in the external solution}$) is the same for all the salts. This is probably not correct since it does not hold for Nitella but it is the best that can be done under the circumstances since the mobilities of K$^{+}$, Rb$^{+}$, Cs$^{+}$, and Li$^{+}$ cannot very well be obtained by measuring the concentration effect. The difficulty may be illustrated as follows. In an artificial sea water containing KCl in place of NaCl (which will be called "K-sea water" for convenience) the P.D. changes first in a negative and then in a positive direction. A similar situation exists in Valonia. It is customary in these cases to take the apex of the curve as indicating the full effect of KCl but if no penetration took place a higher value might be obtained.

If we wait until the apex of the curve is reached and then substitute for "K-sea water" a solution containing "K-sea water" + 1 part of isotonic mannite solution the curve begins to fall but as it would also fall in case no change of solution were made we cannot measure the concentration effect. This difficulty is also found with RbCl and CsCl.

It is of interest to note that the apparent mobility of Cs$^{+}$ in the outer protoplasmic surface is lower than that of K$^{+}$ whereas in water it is higher. In general the values of the mobilities do not agree with those found in water. To what extent this is due to solvation or to the formation of complexes is uncertain.

It is also of interest that the values of the apparent mobilities in the outer protoplasmic surface agree so well when calculated from different combinations.

Thus far the behavior of Halicystis, as summarized in Table I, agrees very well with that of Valonia; the greatest difference being found with CsCl which with Valonia acts very much like NaCl but with Halicystis behaves as though $u_{Cs}$ were greater than $u_{Na}$ and $v_{Cl}$.

19 According to Damon and Osterhout (footnote 10) the change in P.D. on replacing sea water by sea water plus an equal volume of isotonic glycerol solution amounts to 11.6 mv. With Halicystis the same dilution, using mannite solution (footnote 15) produces a change of 11.8 ± 0.1 (27 observations).


21 Osterhout, W. J. V., J. Gen. Physiol., 1937–38, 21, 632 (Fig. 1).

In Valonia $u_{\text{NH}_4}$ is greater than $u_K$ or $u_{\text{Rb}}$;\(^{21}\) in Halicystis the value of $u_{\text{NH}_4}$ is uncertain because of the disturbing action of $\text{NH}_4$. If we replace sea water by 0.6 M $\text{NH}_4\text{Cl}$ plus an equal volume of sea water the concentration of $\text{NH}_4$ is great enough even at low pH to produce the reversal of sign described by Blinks.\(^8\) But according to Blinks at pH 5 low concentrations of $\text{NH}_4\text{Cl}$ may act very much like KCl.\(^8\)

With respect to $\text{MgCl}_2$ the two plants act similarly but with $\text{CaCl}_2$ a very different situation develops. Replacement of sea water by 0.4 M $\text{CaCl}_2$ plus an equal volume of sea water makes a change in the negative direction of 56.4 mv. But, as Blinks has shown, a similar change is produced by absence of $\text{CaCl}_2$.\(^{24}\) Hence it is evident that the protoplasmic surface undergoes alteration and that Henderson's equation is not obeyed. In Valonia absence of calcium has little or no effect.\(^{25}\)

**SUMMARY**

Interest in the study of Halicystis and of Valonia has been stimulated by discoveries of marked contrasts and striking similarities existing side by side. This is illustrated by new experiments with the alkali metals and alkaline earths.

In Halicystis the apparent mobilities of $K^+$, $\text{Rb}^+$, $\text{Cs}^+$, and $\text{Li}^+$ (calculated by means of Henderson's equation from changes in p.d. produced by replacing sea water by a mixture of equal parts of sea water and 0.6 M of various chlorides) are as follows. $u_K = 16, u_{\text{Rb}} = 16, u_{\text{Cs}} = 4.4$, and $u_{\text{Li}} = 0.2$; $u_{\text{Na}}$ is taken as 0.2. These values resemble those in Valonia except that in the latter $u_{\text{Cs}}$ is about 0.2.

No calculation is made for $u_{\text{NH}_4}$ because in these experiments even at low pH so much $\text{NH}_4$ is present that the sign of the p.d. may reverse. This does not happen with Valonia. According to Blinks, $\text{NH}_4^+$ at pH 5 in low concentrations acts like $K^+$.

The calculation gives $u_{\text{Na}} = 1.9$ which is similar to the value found for Valonia.

No calculation can be made for $\text{CaCl}_2$ since it produces protoplasmic alterations and in consequence Henderson's equation does not apply. This differs from Valonia.

Evidently these plants agree closely in some aspects of electrical behavior but differ widely in others.


\(^{25}\) Transferring from sea water to 0.6 M NaCl has little or no effect on the p.d. of Valonia. *Cf.* footnote 23.