CALCULATIONS OF BIOELECTRIC POTENTIALS

VI. SOME EFFECTS OF GUAIACOL ON NITELLA

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Important changes are produced in the apparent mobilities and partition coefficients of inorganic ions by applying guaiacol to Nitella. The calculations indicate that guaiacol may increase apparent mobilities 3-fold and partition coefficients more than 50-fold. These effects are completely reversible.¹

The apparent mobilities were calculated, as explained elsewhere,² from the P.D. between 0.01 M and 0.001 M for each salt. The results³ are given in Table I (column 3).

The order of mobilities of the alkali metals follows that in water until we reach cesium.⁴ In water the mobility of Cs⁺ exceeds that of K⁺ but in Nitella it is much less. This is also true of Valonia⁵ and of Halicystis.⁶

In these cases Cs⁺ acts like Na⁺. This seems to be the case also with

¹ The experiments were made on Nitella flexilis, Ag., at 20-25°C., using the technique described in former papers (Hill, S. E., and Osterhout, W. J. V., J. Gen. Physiol., 1937-38, 21, 541). The cells had the same treatment as described in the previous paper on the effects of guaiacol (cf. Osterhout, W. J. V., J. Gen. Physiol., 1938-39, 22, 417). The concentration of guaiacol was in all cases 0.02 M.

² Osterhout, W. J. V., J. Gen. Physiol., 1938-39, 22, 417. In all cases the change was made from the dilute to the concentrated solution and vice versa; in the former case the effect of action currents must be taken into account.

³ All mobilities are calculated by putting the mobility of Cl, or vCl, equal to unity. Calculated on this basis the mobilities in water at 25°C. are as follows: Li 0.5, Na 0.65, NH₄ 0.96, K 0.96, Rb 1.03, Cs 1.04, ½ Mg 0.7, ½ Ca 0.78, Cl 1.

⁴ The behavior of cesium is exceptional in respect to permeability in Valonia where its rate of entrance is exceedingly slow (cf. Cooper, W. C., Jr., Dorcas, M. J., and Osterhout, W. J. V., J. Gen. Physiol., 1928-29, 12, 427).


muscle and nerve. If we arrange the ions in the order of their effect in producing negativity in *Nitella* we have K, Rb > Na > Li > Cs (Table I, column 6). This does not differ much from the series found in *Valonia*\(^7\) and in *Halicystis*,\(^9\) as well as in muscle and nerve.

According to Höber\(^8\) the order in frog muscle is K > Rb > NH\(_4\) > Cs > Mg > Na > Li. For the sciatic nerve of the frog\(^9\) we have K > Rb > NH\(_4\) > Cs > Na, Li. For the nerve of the spider crab\(^10\) we have K > Rb > Cs.

It is a striking fact that these cells react so differently to pairs of ions, e.g. Na\(^+\) and K\(^+\), K\(^+\) and Cs\(^+\), Rb\(^+\) and Cs\(^+\), which are chemically similar.\(^11\) This deserves further study.

Of especial interest is the increase in apparent mobilities produced by guaiacol (Table I, next to the last column). As an example we may take \(u_{Na}\) which is raised from 2.33 to 7.30 by the action of guaiacol. The p.d. between 0.01 M and 0.001 M NaCl increases from 23.2 to 44.0 when guaiacol is applied.

The question arises whether this is because the protoplasmic surface is acting more like a layer of guaiacol. To answer this some guaiacol (previously shaken with 0.001 M NaCl) was placed in a U-tube with 0.001 M NaCl at one side and 0.01 M NaCl at the other.\(^12\) On leading off from the aqueous solutions to a Compton electrometer we observed a potential of about 10 mv. (dilute solution positive in the external circuit). Hence if the protoplasmic surface acted like guaiacol we should not expect a concentration effect of 44 mv. With KCl the concentration effect with guaiacol was about 15 mv.

The chemical effect, e.g. the p.d. between 0.01 M KCl and 0.01 M NaCl presents a different picture. This chemical effect in *Nitella* amounts to 94 mv. which is reduced to 20.9 by the application of guaiacol. With guaiacol in the U-tube the corresponding value is about 14 mv. Hence

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\(^7\) Damon, E. B., *J. Gen. Physiol.*, 1938-39, 22, 819. In *Valonia*, \(u_{Ca}\) like \(u_{Na}\) is less than \(u_{Cl}\).


\(^11\) The chemical similarity is greater in the case of K\(^+\) and Na\(^+\) than in the other pairs.

in this respect the protoplasmic surface acts somewhat more like guaiacol after guaiacol is applied.

It is not surprising that we are unable to predict the effects of guaiacol in the protoplasmic surface which is undoubtedly a mixture of substances concerning which our knowledge is very limited. As might be expected, guaiacol does not affect all cells in the same way. We find that *Nitella* resembles *Halicystis* in that the mobility of Na$^+$ is increased by guaiacol but that of K$^+$ is not affected. In *Valonia* the mobility of Na$^+$ is increased and that of K$^+$ is decreased.

In addition to the alkali metals mentioned, NH$_4^+$, Mg$^{++}$, and Ca$^{++}$ were employed. Regarding NH$_4^+$ it may be said that its mobility is only about half that of K$^+$ although in water the two mobilities are nearly equal. We see that the mobility of $\frac{1}{2}$ Mg$^{++}$ and that of $\frac{1}{2}$ Ca$^{++}$ are greater than that of K$^+$ which is not the case in water. It seems probable that Mg$^{++}$ and Ca$^{++}$ do not obey very well the equations here used since it is possible that they produce alterations in the surface.

Let us now consider the partition coefficient $S$ ($S = \text{concentration in the non-aqueous protoplasmic surface layer + concentration in the external solution}$). This was determined as previously explained by measuring the P.D. of 0.01 M KCl against 0.01 M of each salt in turn. The partition coefficient was calculated as follows. In the case of NaCl, for example, we find by trial what value of $S_{\text{NaCl}} + S_{\text{KCl}}$ will give the observed P.D. of 44 mv. employing the mobilities already found; i.e., $u_K = 8.76$ and $u_{Na} = 2.33$.

We thus obtain $S_{\text{NaCl}} + S_{\text{KCl}} = 0.0263$. This is done for each salt in turn. The results are shown in Table I.

We can compare the partition coefficients before and after the application of guaiacol since, as shown in a previous paper, the partition coefficient of KCl is not changed by guaiacol. The partition coefficients of the alkali metals increase as the ionic radius increases until we come to cesium which is exceptional. This increase is in line with the suggestions of Shedlovsky and Uhlig.

The application of guaiacol raises all the partition coefficients except those of KCl and RbCl (Table I, last column). Too little is known about the theory of partition coefficients to make it desirable to comment on this.

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15 To calculate the partition coefficient in the presence of guaiacol we employ the mobilities found in the presence of guaiacol, e.g., $u_{Na} = 7.30$ instead of 2.33. The value of $u_K$ is not changed by guaiacol (see footnote 12).
but it may be stated that the value of $S$ for guaiacol in contact with aqueous solutions of NaCl and KCl is very low\textsuperscript{17} (about 0.001).

According to the calculations the partition coefficient of MgCl$_2$ is very low. This might be expected on chemical as well as on biological grounds. We might expect that of CaCl$_2$ to be equally low: this is not the case.

### Table I

**P.D.'s and Partition Coefficients of Chlorides (MCl or MCl$^+$) with and without Guaiacol**

<table>
<thead>
<tr>
<th>MCl or MCl$^+$</th>
<th>Concentration effect (0.01 \text{ M} \text{ vs.} 0.001 \text{ M})</th>
<th>Chemical effect (0.01 \text{ M} \text{ vs.} 0.001 \text{ M})</th>
<th>Value with guaiacol</th>
<th>Value without guaiacol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No guaiacol</td>
<td>With 0.01 M guaiacol</td>
<td>No guaiacol</td>
<td>With 0.01 M guaiacol</td>
</tr>
<tr>
<td></td>
<td>P.D.</td>
<td>$S_M$</td>
<td>P.D.</td>
<td>$S_M$</td>
</tr>
<tr>
<td>LiCl</td>
<td>19.8</td>
<td>2.04</td>
<td>41.8</td>
<td>5.83</td>
</tr>
<tr>
<td>NaCl</td>
<td>23.2</td>
<td>2.33</td>
<td>44.0</td>
<td>7.30</td>
</tr>
<tr>
<td>KCl$^+$</td>
<td>46.1</td>
<td>8.76</td>
<td>46.1</td>
<td>8.76</td>
</tr>
<tr>
<td>RbCl$^+$</td>
<td>46.0</td>
<td>8.76</td>
<td>46.0</td>
<td>8.76</td>
</tr>
<tr>
<td>CsCl</td>
<td>15.3</td>
<td>1.72</td>
<td>31.5</td>
<td>3.38</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>35.0</td>
<td>4.05</td>
<td>38.4</td>
<td>4.91</td>
</tr>
<tr>
<td>MgCl$_2$**</td>
<td>25.0</td>
<td>20.7</td>
<td>25.0</td>
<td>20.7</td>
</tr>
<tr>
<td>CaCl$_2$**</td>
<td>18.8</td>
<td>7.52</td>
<td>23.4</td>
<td>14.46</td>
</tr>
</tbody>
</table>

* The number of observations was usually 8 or 10 and the probable error of the mean less than 7 per cent of the mean.
† The dilute solution is in all cases positive in the external circuit.
‡ KCl is in all cases negative in the external circuit.
§ $S_M$ is the same with and without guaiacol.
¶ The P.D.'s for the concentration effect were the same within experimental error.
** Here $S_M$ refers to $\frac{1}{2}$ Mg or $\frac{1}{2}$ Ca.

To what extent are these calculations valid? One way of testing this is to try to predict the P.D.'s of various dilutions, e.g. of KCl and NaCl, from the calculated values of $S_\text{Na}$, $S_\text{K}$, $S_\text{NaCl}$, and $S_\text{KCl}$. This has been done with satisfactory results\textsuperscript{18} with NaCl, KCl, and NH$_4$Cl.

We may also try to predict chemical effects. For example, in the present paper, using the values (in absence of guaiacol) for the mobilities calculated from the concentration effects and for the partition coefficients calculated

\textsuperscript{17} For guaiacolates it is higher (cf. Osterhout, W. J. V., Kamerling, S. E., and Stanley, W. M., *J. Gen. Physiol.*, 1933–34, 17, 469). It is higher for salicylates than for chlorides.

from the observed p.d.'s of 0.01 M NaCl vs. 0.01 M KCl and of 0.01 M LiCl vs. 0.01 M KCl we may predict the p.d. of 0.01 M LiCl vs. 0.01 M NaCl. The prediction is 8.7 mv. and the observed value is 8.0 mv. (NaCl negative to LiCl in the external circuit). For 0.01 M RbCl vs. 0.01 M NaCl the prediction is 94 mv. and the observed value 102 (RbCl negative). For 0.01 M CsCl vs. 0.01 M NaCl the prediction is 11.0 mv. and the observed value is 12.6 mv. (NaCl negative). When we are not dealing with alkali metals the prediction is somewhat less satisfactory.

The success of these predictions for the alkali metals and the fact that the mobilities and partition coefficients of the alkali metals as calculated in the present paper are reasonably in line with expectation indicate that the method of calculation may be trusted to a certain extent. We must be on our guard, however, against secondary effects. 19

If concentrations are too high or exposures too long such secondary effects may appear: such effects are, of course, not predicted by these equations. These secondary effects may be reversible up to a certain point beyond which they become irreversible. They doubtless involve structural alterations, 20 and may be brought about by non-electrolytes as well as by electrolytes.

We may therefore speak of primary effects and secondary effects. By primary effects we mean those which involve no structural alteration of the protoplasmic surface. If, for example, the potential of the protoplasm is largely due to an outwardly directed concentration gradient of potassium 21 we abolish the potential when we abolish the concentration gradient: this we do by placing the proper concentration of potassium outside. 22 Here there is no need to assume any structural alteration of the surface.

A change in the chemical composition of the surface might conceivably occur without any structural change. Possibly guaiacol produces this kind of alteration.

19 Such secondary effects are much more apt to occur with alkaline earths than with alkali metals. For a striking effect of calcium see Blinks, L. R., J. Gen. Physiol., 1929–30, 13, 223; Blinks, L. R., Rhodes, R. D., and McCallum, G. A., Proc. Nat. Acad. Sci., 1935, 21, 123.

It may be mentioned in this connection that the concentration effect of 0.01 N KCl + 0.01 N CaCl2 vs. 0.001 N KCl + 0.001 N CaCl2 is decreased by guaiacol, contrary to the result obtained with either salt singly.


The fact that both primary and secondary effects may be inhibited to a certain extent by alkaline earths does not, of course, mean that they are identical. It may signify that alkaline earths decrease the solubility in the protoplasmic surface of the various substances which depress the potential. This view is favored by the fact that it requires a very high concentration of alkaline earths to be effective and in Nitella they fail to inhibit the depressing action of potassium when its concentration is raised to 0.1 M.

**SUMMARY**

Values have been calculated for apparent mobilities and partition coefficients in the outer non-aqueous layer of the protoplasm of Nitella. Among the alkali metals (with the exception of cesium) the order of mobilities resembles that in water and the partition coefficients (except for cesium) follow the rule of Shedlovsky and Uhlig, according to which the partition coefficient increases with the ionic radius.

Taking the mobility of the chloride ion as unity, we obtain the following: lithium 2.04, sodium 2.33, potassium 8.76, rubidium 8.76, cesium 1.72, ammonium 4.05, Mg$^2+$ magnesium 20.7, and Ca$^{2+}$ calcium 7.52.

After exposure to guaiacol these values become: lithium 5.83, sodium 7.30, potassium 8.76, rubidium 8.76, cesium 3.38, ammonium 4.91, Mg$^2+$ magnesium 20.7, and Ca$^{2+}$ calcium 14.46.

The partition coefficients of the chlorides are as follows, when that of potassium chloride is taken as unity: lithium 0.0133, sodium 0.0263, rubidium 1.0, cesium 0.0152, ammonium 0.0182, magnesium 0.0017, and calcium 0.02.

These are raised by guaiacol to the following: lithium 0.149, sodium 0.426, rubidium 1.0, cesium 0.82, ammonium 0.935, magnesium 0.0263, and calcium 0.323 (that of potassium is not changed).

The effect of guaiacol on the mobilities of the sodium and potassium ions resembles that seen in Halicystis but differs from that found in Valonia where guaiacol increases the mobility of the sodium ion but decreases that of the potassium ion.

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