THE ACCUMULATION OF ELECTROLYTES

XII. ACCUMULATION OF HALIDE AND NITRATE BY VALONIA IN HYPERTONIC SOLUTIONS

BY A. G. JACQUES

(From the Laboratories of The Rockefeller Institute for Medical Research and The Bermuda Biological Station for Research, Inc., Bermuda)

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The ratio of halide to nitrate in Bermuda sea water, as shown in a previous paper, is at least 80,000 to 1, and in the sap the average value is about 38 to 1. On this basis we might conclude that the nitrate penetrates much more rapidly than the halide unless the nitrate is formed in the cell from other nitrogen compounds or from atmospheric nitrogen.

If we decrease the ratio of halide to nitrate in the sea water, we might expect a corresponding change in the sap. This occurs but the change is smaller than was expected, and it is independent of the extent of the change of the ratio of halide to nitrate in the sea water.

By increasing the osmotic pressure of the sea water the cells can

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2 This value has been calculated from the analyses of Bermuda sea water samples collected in the localities where Valonia occurs. But, as pointed out in the previous paper (footnote 1), the figures obtained may be much too high, since the method of analysis by diphenylbenzidine is not specific for nitrates, but includes many other oxidants, which are possibly present in sea water along the shore. In this connection Rakestraw (Rakestraw, N. W., Biol. Bull., 1936, 71, 133) has stated that the surface water in the vicinity of Bermuda is exceptionally low in both nitrites and nitrates. In any case it is clear that there is a remarkable accumulation of nitrate in the sap.
3 Moore, Whitley, and Webster (Moore, B., Whitley, E., and Webster, T. A., Proc. Roy. Soc. London, Series B, 1921, 92, 51) believe that some marine green algae are able to synthesize soluble nitrogen compounds from atmospheric nitrogen. Others say that this process is due to nitrogen-fixing bacteria on the surface of the algae. If this process takes place the nitrate concentration of the sea water in contact with the cell surface may be much greater than that of the sea water as a whole.
be made to take in electrolytes even in dim light where the growth is negligible and this device has been resorted to in the present case. It has been found that the concentration of the sea water may be raised abruptly as much as 10 per cent without permanently injuring the cells or causing plasmolysis. The cells soften at first by losing water, but they rapidly regain their turgor.

**TABLE I**

Entrance of Halide and Nitrate Into Valonia macrophysa from Hypertonic Sea Water with Varying Halide Concentrations

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Halide concentration (g) in sap of cells in modified sea waters</th>
<th>Nitrate concentration (g) in sap of cells in modified sea waters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>0</td>
<td>0.6106</td>
<td>Av. 0.6096</td>
</tr>
<tr>
<td>84</td>
<td>0.6328</td>
<td>0.6321</td>
</tr>
<tr>
<td>156</td>
<td>0.6309</td>
<td>0.6378</td>
</tr>
<tr>
<td>180</td>
<td>0.6339</td>
<td>0.6481</td>
</tr>
<tr>
<td>276</td>
<td>0.6409</td>
<td>0.6500</td>
</tr>
</tbody>
</table>

Molar composition of sea water

<table>
<thead>
<tr>
<th></th>
<th>Halide</th>
<th>Nitrate</th>
<th>Halide + nitrate</th>
<th>Ratio halide/nitrate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.400</td>
<td>0.229</td>
<td>0.629</td>
<td>1.75</td>
</tr>
<tr>
<td>B</td>
<td>0.486</td>
<td>0.143</td>
<td>0.629</td>
<td>3.41</td>
</tr>
<tr>
<td>C</td>
<td>0.572</td>
<td>0.057</td>
<td>0.629</td>
<td>10.00</td>
</tr>
<tr>
<td>D</td>
<td>0.600</td>
<td>0.029</td>
<td>0.629</td>
<td>20.70</td>
</tr>
<tr>
<td>E</td>
<td>0.623</td>
<td>0.0057</td>
<td>0.6287</td>
<td>109.30</td>
</tr>
</tbody>
</table>

* The ratio in ordinary sea water is at least 80,000 to 1, the concentration of halide being 0.58 M and of nitrate 7.5 x 10^-3 M at most. Probably it is much less.

**EXPERIMENTAL**

The sea waters were modified by adding 1.2 M NaNO₃ solution, 1.2 M NaCl solution, and 0.6 M NaN₃ solution in such proportions as to produce the desired increase in the electrolyte concentration and decrease in the external ratio of Cl/NO₃.⁴ The cells were then placed in the modified sea water in deep rectangular
lar, covered Pyrex dishes, which were mechanically rocked very slowly. At
intervals cells were withdrawn for analysis for halide and nitrate.

Halide was determined by titration with AgNO₃, the end-point being found
electrometrically, using a silver electrode.

Nitrate was determined by Devarda's method as described in another paper.¹
Ammonia was determined in each sample and a suitable correction applied to the
nitrate determination. This correction was in general less than 2 per cent.

The results of a typical experiment are given in Table I. The ex-
periments were carried out in Bermuda in the winter of 1935-36.

DISCUSSION OF RESULTS

Because of natural variations in the halide and nitrate concentra-
tions of the sap, the results are too irregular to permit a mathematical
treatment of the kinetics. It is clear, however, that as a result of
raising the osmotic pressure of the sea water in the manner described
the concentrations of both halide and nitrate have increased in the
sap. At the end of the experiment the concentration of halide +
nitrate inside was greater than that outside by about 0.05 M which is
approximately the same difference as in the cells in normal sea water.

When the external halide is greatly decreased, as in sea water A,
we might expect halide to come out of the cell in exchange for incom-
ing nitrate. Instead of this we see that halide continued to enter.
Obviously then the ratio of halide to nitrate in the sap could decrease
only if nitrate entered relatively faster than in ordinary sea water.
This evidently took place. In ordinary sea water halide entered
38 times as fast as nitrate. In sea water A the increase in concentra-
tion of halide in the sap in 276 hours was 0.0313 M and of nitrate 0.0186
M, giving a ratio of 0.0313 ÷ 0.0186 = 1.68, not very different
from the ratio 1.75 found in the external solution. We cannot make
a more definite statement unless we know the actual concentration
gradients.⁶

¹ If we assume, for purposes of calculation, that chloride enters as NaCl and
nitrate as NaNO₃ we have the following concentration gradients at the start
in sea water A (we assume, as in previous papers, that the relative rates of entrance
are proportional to the inwardly directed concentration gradients in the non-
aqueous protoplasmic surface and that these in turn are proportional to the
difference in ionic products in the external solution and in the sap: the subscripts
o and i refer to outside and inside respectively), [Naᵢ] [Clᵢ] — [Naᵢ] [Clᵢ] = 0.5 (0.4)
It is not surprising that the decrease of the ratio of halide to nitrate in the sap was gradual for if no halide comes out the decrease in ratio must be gradual when electrolytes enter as slowly as in this experiment. In sea water A the ratio of halide to nitrate in the sap decreased from 38 to 18.5 in 276 hours.

The fact that the increase in nitrate concentration in the sap has not kept pace with that in the sea water has caused the ratio of nitrate in the sap to that in the sea water to fall off. This ratio decreases as the outside concentration of nitrate increases, and becomes less than unity in sea waters A, B, and C. The question whether there is a relative change in the rates of entrance of nitrate and halide as the ratio of halide to nitrate in the sea water changes from 100 to 1.75 cannot be satisfactorily settled. Since the change of ratio in the sea water from 80,000 to 109.30 merely resulted in doubling the concentration of nitrate in the sap a further change to 1.75 could scarcely affect the result very greatly during the time of the experiment. The ratio of halide in sap to halide in sea water increased in all cases except in solution E, the greatest increase being in solution A, from 1.05 to 1.59.

Thus we seem to have an induced halide accumulation greater than that found under normal conditions. Since it is by no means certain that the cell has settled down to a steady state a further increase of concentration may be possible.

We assume that the movement of substances through the non-aqueous surface layer of the protoplasm is chiefly in molecular form. The following molecules are available for the transfer of chloride, NaCl, CaCl$_2$, MgCl$_2$, KCl, and HCl. The gradient of chemical potentials of the first three is directed inward, and of the last two outward. The net movement of chloride will depend on the flux of all these molecules.

On this basis it might be suggested that chloride enters the cell chiefly as NaCl.\footnote{As the sap and sea water contain much less magnesium and calcium than sodium, their rôle may be unimportant and the discussion will therefore be confined to NaCl.} If all the chloride has entered as NaCl, we should

\[
- 0.1 (0.6) = 0.140 \text{ and } [Na_+] [NO_3^-] - [Na_+] [NO_3^-] = 0.5 (0.23) - 0.1 (0.016) = 0.113.
\]

Hence the concentration gradient of NaCl is about 25 per cent greater than that of NaNO$_3$.\footnote{As the sap and sea water contain much less magnesium and calcium than sodium, their rôle may be unimportant and the discussion will therefore be confined to NaCl.}
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expect to find as much sodium as chloride in the sap. But this is not necessary if sodium enters as NaCl and goes out in combination with another anion.  

In normal sea water the gradients of chemical potential of NaNO\textsubscript{3}, KNO\textsubscript{3}, and HNO\textsubscript{3} are directed outward. The status of Mg(NO\textsubscript{3})\textsubscript{2} and of Ca (NO\textsubscript{3})\textsubscript{2} is not so clear. If the average nitrate concentration of the sap is 0.016 M and that of the sea water is taken as 7.5 × 10\textsuperscript{-6} M the concentration of magnesium in the sap would have to be only 1.3 × 10\textsuperscript{-8} M to make the gradient of chemical potential for Mg(NO\textsubscript{3})\textsubscript{2} zero. To make the gradient of chemical potential of Ca(NO\textsubscript{3})\textsubscript{2} zero the concentration of calcium in the sap would have to be slightly less than 2.6 × 10\textsuperscript{-9} M.  

According to the results of Steward and Martin, far more than the required amounts of calcium and magnesium are present in cells at Tortugas.

It might be assumed that halide and nitrate enter by exchange with bicarbonate ion. It is possible that when the concentration of electrolytes in the sea water is increased, the entrance of both potassium and sodium increases. As a result the rate of exchange of bicarbonate ions for halide ions might increase, since the entrance of the bases would increase the pH of the sap (particularly in the layer next to the protoplasm) and therefore the concentration of the bicarbonate ion. Under these conditions it would be the concentration of the relatively scarce bicarbonate ion and not of the abundant halide ion which would determine the rate of entrance of halide. But if the surface of the protoplasm is a non-aqueous liquid of low dielectric constant, as potential measurements suggest, the rôle played by ionic transport is probably very small.

It is evident that the mechanism of accumulation of anions in these experiments needs further investigation.

7 This will be discussed in a later paper.

8 These results are based on the analyses of sea water as given by Osterhout (Osterhout, W. J. V., Ergebn. Physiol., 1933, 35, 967). The assumption is made that the activity coefficient for Ca is the same in the sap and sea water. The same is true for magnesium and for nitrate. Osterhout’s sap analyses show much more than the required amount of calcium, and a trace of magnesium which might easily be as much as 1.3 × 10\textsuperscript{-8} M.

When cells of *Valonia macrophysa* were placed in hypertonic sea water, the concentration of halide and of nitrate increased, and the sum of halide + nitrate became 0.05 M greater inside than outside, which is about the same difference as is found in cells in normal sea water.

In ordinary sea water the ratio of halide to nitrate is 80,000 to 1. When this was changed by substituting nitrate for halide so that the concentration of halide was 1.75 times that of nitrate the rate of entrance of halide was 1.68 times that of nitrate in 276 hours and the ratio of halide to nitrate in the sap decreased from 38 to 18.5.

No halide came out in exchange for entering nitrate. The retention of chloride may well be due to the fact that even when the halide concentration of the sea water is reduced as low as 0.4 M, there is still an inwardly directed activity gradient of sodium chloride.