THE ACCUMULATION OF ELECTROLYTES

X. ACCUMULATION OF IODINE BY HALICYSTIS AND VALONIA

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Owing to its rôle in the thyroid, the accumulation of iodine has aroused especial interest. Evidently we cannot understand how any sort of accumulation comes about unless we know what happens inside the living cell during the process. Such information can be obtained by using the very large multinucleate cells of certain marine algae (Halicystis and Valonia) containing a clear watery sap which can be extracted with little or no contamination. In both these the concentration of iodide in solution in the sap greatly exceeds that in the sea water.

In both cases the chemical potential of NaI, KI, HI, and CaI₂ is greater inside than outside. It would therefore seem that an expenditure of energy on the part of the cell is necessary to bring this about.

It is well known that the halide concentration of the sap of both Valonia macrophysa, Kütz., and Halicystis Osterhoutii, Blinks and Blinks, is greater than that of the sea water. In Bermuda sea water the total halide concentration is about 0.58 M; in the sap it is usually more than 0.60 M but seldom as much as 0.64 M. This accumulation of total halide amounts to 10 per cent at the most. The object of the present paper is to show that iodide accumulates to a greater extent, particularly in the case of Halicystis.

In the course of work on the rate of entrance of iodide into Valonia and into Halicystis the sap from untreated cells was analyzed, and it was found that Valonia contained no iodide detectable by the analytical procedure used at the time, but there was invariably an appreciable concentration in Halicystis sap. Subsequently a more sensitive method was used for Valonia sap which revealed that there is some accumulation in it also.

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TABLE I

<table>
<thead>
<tr>
<th>Description of the cells</th>
<th>Treatment prior to sap extraction</th>
<th>Concentration of iodide (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Attached cells from several sources</td>
<td>Kept in the laboratory in running sea water up to 12 mos.</td>
<td>0.000484</td>
</tr>
<tr>
<td>2. Attached cells from Tuckers Town. 0.5 to 1.5 ml. in volume</td>
<td>Sap extracted immediately after collection</td>
<td>0.000407</td>
</tr>
<tr>
<td>3. &quot; &quot; &quot; &quot;</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>0.000295</td>
</tr>
<tr>
<td>4. &quot; &quot; &quot; &quot;</td>
<td>Kept in laboratory in running water 4 hrs.</td>
<td>0.000253</td>
</tr>
<tr>
<td>5. Attached cells from Tuckers Town. 30 small cells. 0.5 ml. or less</td>
<td>Kept in laboratory 3 wks. in running sea water</td>
<td>0.000655</td>
</tr>
<tr>
<td>6. Attached cells from several sources</td>
<td>Kept in laboratory in running sea water 12 to 18 mos.</td>
<td>0.000642</td>
</tr>
<tr>
<td>7. Attached cells from Tuckers Town. 4 large cells. 1 ml. or more</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>0.000642</td>
</tr>
<tr>
<td>8. Unattached large pale cells stranded on beach. 1.5 ml. or more in volume</td>
<td>Kept 24 hrs. in laboratory in running sea water</td>
<td>0.000256</td>
</tr>
<tr>
<td>9. One large unattached cell floating at sea off Coopers Island. 3 ml.</td>
<td>Sap extracted immediately after collection</td>
<td>0.000204</td>
</tr>
<tr>
<td>10. 3 large unattached cells stranded on beach at Ft. St. Catherine. 2 to 4 ml. in volume</td>
<td>Kept in laboratory in running sea water 4 days</td>
<td>0.000387</td>
</tr>
<tr>
<td>11. Unattached cells floating in Ferry Reach. 0.75 to 2.5 ml. in volume</td>
<td>Kept in laboratory in running sea water</td>
<td>0.000453</td>
</tr>
<tr>
<td>12. Unattached cells floating at sea 1/4 mile west of Gurnet Rock, less than 1 ml. in volume</td>
<td>Kept in laboratory in running sea water 24 hrs.</td>
<td>0.000279</td>
</tr>
<tr>
<td>13. Unattached large cells, 2 to 4 ml. in volume</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>0.000289</td>
</tr>
</tbody>
</table>

Analysis

The method of analysis usually used for *Halicystis* was that described in a previous paper,¹ in which the iodide was oxidized to free iodine by potassium iodate.

in the presence of tartaric acid. But in some cases KMnO₄ was used to oxidize the iodide. Results agreeing within the limits of error of the method were obtained on the same sap samples. Before analysis the sap was centrifuged to remove insoluble organic matter.

For Valonia, a 2 ml. sample of centrifuged sap was first treated with 0.5 ml. of saturated bromine water and 0.2 ml. of 5 per cent acetic acid, whereby the iodide present was oxidized to iodate. Then the excess bromine was removed by boiling and an excess of KI was added. The iodine formed was extracted into chloroform and determined photometrically with the Zeiss-Pulfrich step-photometer.

The sea water was analyzed for total inorganic iodine by the method of Reith, and for iodide ion by the method of Winkler.

The analyses were made in Bermuda at various times in the winter of 1935-36, and of 1936-37 on the sap of Halicystis cells from various sources. Some of the cells were found growing attached, while others were stranded on beaches or floating at sea.

The Valonia sap analyzed was taken from cells of a composite collection made at various times in the winter of 1936-37.

Table I gives the results for Halicystis which was most extensively investigated.

The concentration of iodide in Valonia sap was found to be 1.1 × 10⁻⁵ M.

In the sample of Bermuda sea water analyzed the iodide was found to be 5 γ per liter and total inorganic iodine 30 γ per liter. This is a little lower than Reith has found for total inorganic iodine, his highest value being 69.5 γ for the very concentrated Red Sea, and his lowest, 43.4 γ, for the North Sea. His values are all somewhat higher than those obtained by other recent investigators. Several older determinations give much higher values, but these are undoubtedly unreliable owing to analytical errors. On the whole, therefore, we believe our value of 30 γ per liter to be a sufficiently reliable one.

DISCUSSION OF RESULTS

The average concentration of iodide for attached cells of Halicystis (1 to 7, Table I) is 0.000410 M, and for floating and stranded cells

(8 to 13, Table I) 0.000311 \( \pm \). But this difference is probably not significant. Greater differences occurred among the members of each group. The average for the whole series is 0.000361 \( \pm \).

The accumulation of iodine by some of the algae has, of course, been known for a long time, and several qualitative and quantitative analyses have been made. Most of these investigations deal with the brown algae which are the richest in iodine (especially Laminaria) and in most cases the results are expressed in terms of per cent of fresh or dry weight, without reference to the form in which iodine occurs or to its concentration.

Kylin, however, believes that iodine occurs in easily decomposed organic combinations and as iodides, but never as free iodine in normal plants, which is also the view of Chemin and Legendre, and Mangenot. But Dangeard and Sauvageau believe that free iodine is a normal constituent of some cells. Dillon suggests that iodine is stored by the union of free iodine in the plant with unsaturated organic compounds, while Freundler and his co-workers have stated that in Laminaria (which they have investigated thoroughly) at certain times of the year up to one half the total iodine is present in a form which cannot be demonstrated analytically even though the plant is ashed. Freundler has suggested that part of the iodine might at certain times be present as "latent iodine" which he regards as an isomer of iodine and a higher isotope of tin of atomic weight 127 and atomic number 50. This form is supposed to be transformable to ordinary iodine under certain conditions. Freundler and his co-workers have also suggested that in the case of Laminaria which, according to their analyses, contains tin and rubidium, complexes involving sodium, tin, and iodine, and rubidium, tin, and iodine, are concerned in the latent iodine problem.


These views have not met with much approval, most investigators believing that faulty analytical procedures are responsible for the curious results obtained. Kylin\(^4\) believes that most of the iodine in \textit{Laminaria} is in the form of iodide. But Lunde and Closs\(^*\) found that for different parts of the plant the percentage of iodine in the form of iodide varies from 89 to 36 per cent.

Only one investigator, Trofimov,\(^4\) has attempted to determine the actual concentrations of iodide in tissues of algae. His potentiometric method, involving a silver needle electrode, which is thrust into the tissue, is open to large errors, but gives approximate values for the activity of the iodide ion. But the needle traverses many cells and intercellular spaces, so that the potential observed is not due to cell constituents alone.

His results for \textit{Laminaria} indicate iodide concentrations between 0.00004 \( \text{M} \) and 0.0030 \( \text{M} \), depending on the species and on the kind of tissue. He states that in every tissue a considerable part of the iodine present was not in the form of iodide.

According to this view higher and lower iodide concentrations than those we have found for \textit{Halicystis} appear to exist in \textit{Laminaria} tissues. But owing to the complex structure of the plant and the smallness of the cells it is difficult to deal with it as a physicochemical system, since we have no way of knowing the iodide concentration in the sap of a single cell or of its environment.

In \textit{Halicystis} and \textit{Valonia} this difficulty disappears. There seems no reason to doubt that all of the iodine in the sap of \textit{Halicystis} is in the form of iodide ion. This is indicated by the ease with which it was oxidized to free iodine by potassium iodate and even by nitrite in the presence of a weak acid; \textit{viz.}, tartaric. The case for \textit{Valonia} is not quite so clear. In order to demonstrate the presence of iodine it was necessary to oxidize energetically with bromine water, so that organic compounds of iodine if present would also be included in this analysis. The possibility that the iodine is in the form of an easily decomposable iodo-compound cannot be excluded completely. But this seems unlikely in view of the small amount of organic matter in the sap (1.4 parts per 1000).\(^8\)

If all the iodine in Bermuda sea water is taken as iodide the concentration is 2.4 \( \times 10^{-5} \text{M} \), or if the iodide is taken as 5 \( \gamma \) per liter as

we have found, iodide $= 4 \times 10^{-8}$M. But it is generally believed that the greater part of it is present as iodate. Thus Winkler\textsuperscript{9} estimated that in the Adriatic Sea the iodide content was 11 $\gamma$ per liter and the iodate content 40 $\gamma$. And Cameron\textsuperscript{9} found for the Straits of Georgia, British Columbia, 2.5 $\gamma$ iodide and 22.5 $\gamma$ iodate.

Assuming that all the iodine in the sap is iodide, and taking our value for the iodide of the sea water, it appears that the iodide has been accumulated by Halicystis nearly 10,000-fold. Such a change even though the quantities involved are small may indicate a considerable energy change. Even if we take all the iodine of the sea water as iodide the accumulation is still more than 1000-fold.

In Valonia, the accumulation is either 250-fold or 40-fold on this basis. Experiments on the penetration of iodide into Valonia\textsuperscript{1} suggest that the iodide may pass through the protoplasm as sodium iodide. But in Valonia the concentration of sodium in the sea water is only about 5 times that of the sap and this is not enough to raise the concentration product $[Na][I]$ in the sea water to that in the sap.\textsuperscript{10} There is therefore no energy of diffusion available here for the accumulation of iodide. On the contrary the movement should be outward rather than inward.

The same argument, with greater force, applies to Halicystis, where the internal and external concentrations of sodium are about equal. What is here said of NaI applies to KI and HI. But in Valonia the chemical potential of MgI$_2$ is possibly greater in the sea water than in the sap.\textsuperscript{11} Hence it might be suggested that MgI$_2$ enters faster than NaI and KI come out, but this seems highly improbable.

It might be assumed that the iodine goes into the cell as iodate and is there reduced to iodide, which might be unable to pass through

\textsuperscript{9} Cameron, A. T., Contrib. Canad. Biol., 1922–24, 1, 73.

\textsuperscript{10} Assuming that the mean activity coefficients for NaI inside and outside are about equal.

\textsuperscript{11} Our analyses give a trace of magnesium in Valonia sap, but Steward and Martin (Steward, F. C., and Martin, J. C., Carnegie Institution of Washington, Pub. No. 475, 1937, 87) found in Tortugas the average magnesium concentration to be 0.0036 $\text{m}$ in which case, on the basis of 0.057 $\text{m}$ magnesium in the sea water, the gradient of chemical potential of MgI$_2$ is also directed outward.
the protoplasm. But in Valonia iodide appears to penetrate the protoplasm.¹

It might be suggested that in Valonia the iodine is accumulated in an undissociated organic combination in the sap. If this compound were to leave the cell much more slowly than iodide entered, accumulation might be observed.

It is certain that the cell has the energy available to bring about the effects observed, but the mode of application of this energy is not clear.

While the occurrence of iodine in the green algae is rare it is of interest that some of the algae related to Valonia and Halicystis, viz. Cladophora rupestris, Acrosiphonia pallida, Bryopsis plumosa, and Bryopsis hypnoides, are also stated to have iodine. In the case of the latter, according to Dangeard¹² the quantity is about the same as in Laminaria.

SUMMARY

Analyses of the sap of Halicystis Osterhoutii and of Valonia macrophysa for iodide indicate accumulations of the order of 1000 to 10,000-fold in the first case, and 40 to 250-fold in the second case. The chemical potential of KI, NaI, HI, and CaI₂ is greater inside than outside.