CONTRASTS IN THE CELL SAP OF VALONIAS AND THE PROBLEM OF FLOTATION.*

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It has been shown1 that the marine alga Valonia macrophysa, Kütz. (collected at Bermuda), has the power to absorb K and to store it up so that its concentration becomes much greater in the cell sap than in the sea water. This is also the case with Valonia utricularis (collected at Naples) according to Meyer and Hansen.2 A similar behavior might be expected in other Valonias but the authors find that this is apparently not the case with the large species found at Bermuda which is commonly referred to Valonia ventricosa, J. G. Agardh.3

Table I gives a comparison of the analyses of Bermuda sea water, and of the saps of V. macrophysa and V. ventricosa.

It will be noted that the proportion of K to Na in V. macrophysa is about 5.72:1 while in V. ventricosa it is about 0.0278:1.

* Contributions from The Bermuda Biological Station for Research, No. 149.

1 Osterhout, W. J. V., J. Gen. Physiol., 1922–23, v, 225. There is some doubt regarding the identity of this species.

2 Meyer, A., Ber. bot. Ges., 1891, ix, 77. Hansen, A., Mitt. zool. Station Neapel, 1893, xi, 254. Czapek (Czapek, F., Biochemie der Pflanzen, Jena, 2. Auflage, 1920, ii, 353) mentions (without quoting the source) an analysis of V. utricularis in which Na predominates over K but the analysis was presumably made on whole plants and there may have been a good deal of contamination by sea water, as is suggested by the high values for SO4.

In the latter the concentrations of Na and K are nearly the same as in sea water.

The table shows that Mg, which is absent from *V. macrophysa*, occurs in *V. ventricosa*, though in lower concentration than in sea water; Ca is present in both cases but in much lower concentration than in sea water.

In both cases NO₃ is present although the sea water gives no indication of it by the ring test. In both species the halide content, and osmotic pressure are higher than in sea water.¹ The pH of the sap is about the same in both, approximately 5.9, while that of the sea water is about 8.2.

There is practically no Br, PO₄, or SO₄ in the sap of intact cells of either species, but SO₄ begins to diffuse in as soon as injury occurs and this serves as a useful indicator of normal condition.

In order to gain an approximate idea of the amount of organic matter the loss on ignition was determined. The procedure employed in the case of *V. macrophysa*¹ was followed in the main except that the filtration was omitted on the ground that some Ca and Mg might be retained on the filter as carbonate. The sap was dried to constant weight in an oven at 105°C and ignited just below red heat. Water was added (to replace any water held between the crystals which may have been driven off by ignition) and the residue was again dried to constant weight at 105°C. The difference in weight before and after ignition amounted to 2.09 parts per thousand: the corresponding figure obtained by Van der Pyl for *V. macrophysa* is 1.433 parts per thousand.

These striking differences between two species of *Valonia* raise the question whether it is justifiable to assume, as is often done, that close relationship involves similarity in chemical composition and

¹ Determinations of conductivity were made by Mr. L. R. Blinks who found that the conductivity of the sap of *V. ventricosa* to be about the same as that of sea water. In *V. macrophysa*, however, the conductivity is about 20 per cent higher than that of sea water, as is to be expected in view of the larger proportion of KCl, which has a higher specific conductivity than NaCl.

² Freshly collected normal cells show only a trace of turbidity when BaCl₂ is added to the acidified sap: this very slight turbidity may be due to the precipitation of organic matter.
metabolic processes. The implications of this question are far reaching. If it is possible for nearly allied forms to differ so profoundly it is evident that we have as yet no satisfactory conception of the nature of the variables involved in differentiation or in the development of specific characters.

These differences suggest another question of considerable im-

TABLE 1.*

Molecular Composition Expressed as Per Cent of Halide (Cl + Br).

<table>
<thead>
<tr>
<th></th>
<th>Bermuda sea water.</th>
<th>Sap of Valonia macrophysa</th>
<th>Sap of Valonia ventricosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl + Br</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Na</td>
<td>85.87</td>
<td>15.08</td>
<td>92.80</td>
</tr>
<tr>
<td>K</td>
<td>2.15</td>
<td>86.24</td>
<td>2.58</td>
</tr>
<tr>
<td>Ca</td>
<td>2.05</td>
<td>0.283</td>
<td>1.35</td>
</tr>
<tr>
<td>Mg</td>
<td>9.74</td>
<td>Trace ?</td>
<td>2.49</td>
</tr>
<tr>
<td>SO₄</td>
<td>6.26</td>
<td>Trace ?</td>
<td>Trace ?</td>
</tr>
</tbody>
</table>

* The figures for sea water and for the sap of *V. macrophysa* are based upon the results of L. M. Van der Pyl (cf. Osterhout, W. J. V., *J. Gen. Physiol.*, 1922-23, v, 225). The methods described in that paper were followed in the work on *V. ventricosa* except that the halide was determined volumetrically and the Ca, after separation as oxalate, was determined by titrating against KMnO₄, instead of gravimetrically.

In the table the sea water appears to have an excess of anions over cations of 0.825 per cent (each bivalent ion being counted as two univalent ions): this is due in part to the fact that the halide was determined gravimetrically and called Cl, no account being taken of the Br present, which in molar terms is approximately 0.14 per cent of the Cl. This procedure would make the Cl appear too great (owing to the greater atomic weight of Br). On the other hand no account was taken of carbonates.

In the sap of *V. macrophysa* the table shows an excess of cations amounting to 1.9 per cent; in the case of *V. ventricosa* the apparent excess of cations is 3.08 per cent. This is undoubtedly due in part to the fact that no account was taken of carbonates, nitrates, or organic anions.

The extraction and analysis of the sap of *V. ventricosa* were carried out by the junior author.

† Br is present in sea water but absent from the sap of both species of *Valonia*. 
portance, whether Na or K predominates in the sap\(^6\) of plant and animal cells. For the study of this question, it is desirable to employ cells whose sap can be obtained without alteration, as in the case of *Valonia*. The fresh water plant *Nitella* fulfills this condition. Analysis of the sap of *Nitella*\(^7\) from Woods Hole (*Nitella "A"") shows approximately equal amounts of Na and K: in a species collected near Cambridge (*Nitella "C"") there is a little more K than Na. Hoagland and Davis\(^8\) state that in *Nitella clavata*, taken from pond water, the proportion of K to Na in the sap is as 5.43 to 1. In the case of large cells in tap water, however, they report that Na and K were more nearly equal and in small cells in culture solution Na predominated over K.

In these cases the sap could be extracted without contamination or alteration. Where this is not possible we cannot be sure of its composition. Ordinary ash analyses, made without extracting sap, do not tell us whether substances are present in soluble or insoluble form, whether they are located within the cell or in intercellular spaces. But such analyses may nevertheless give us some idea of the general situation.

Ash analyses show a decided predominance of K over Na throughout the flowering plants and mosses.\(^9\) In the case of marine algae\(^10\) they often indicate a preponderance of Na over K, but this might be due to sea water held in the cell walls or adhering to the surfaces of the plant.

The analyses of animals are less consistent than those of flowering

\(^6\) The word sap is here used to designate the fluid within the cell whether imbibed in the protoplasm without formation of vacuoles, as is commonly the case in animals, or forming vacuoles, as in plants.

\(^7\) The cells were carefully washed, rinsed with distilled water, and quickly crushed. The crushed mass was subjected to pressure and the liquid obtained by this procedure was filtered. In this case there is opportunity for alteration of the sap but Hoagland and Davis\(^8\) found that sap extracted in this way did not differ in respect to Na and K from sap collected by squeezing it out from individual cells in such a manner as to prevent alteration.


plants but K seems to predominate over Na in the majority of cases. Erythrocytes are of special interest. In some cases they contain little or no Na and there is reason to believe\textsuperscript{11} that the large amount of K which is present exists in solution as an inorganic salt. On the other hand there are species whose erythrocytes contain mostly Na with very little K.

It is therefore apparent that we are not warranted in concluding that K invariably predominates over Na in the sap of living cells.

Another interesting problem is suggested by the fact that the dissimilarity in composition of the two Valonias brings about a marked difference in their behavior in that \textit{V. macrophysa} sinks in sea water while \textit{V. ventricosa} floats.

The specific gravities at 25°C. are as follows: sea water 1.0277, sap of \textit{V. macrophysa} 1.0290, sap of \textit{V. ventricosa} 1.0250 (distilled water being taken as 1.0000). In order to see whether the salt composition as ascertained by analysis could account for these differences artificial sea water and saps were made up according to the analyses given in Table I and their concentrations were adjusted until the halide content of the artificial sea water was 0.59 M and that of the saps 0.626 M, which represents a typical condition in respect to sea water and the two saps. The specific gravities are as follows: artificial sea water 1.0285, artificial sap of \textit{V. macrophysa} 1.0285, artificial sap of \textit{V. ventricosa} 1.0252. These figures are in fair agreement with those given above.

Since the cell walls and the protoplasm of both species sink in sea water it is evident that living cells of \textit{V. macrophysa} must sink in sea water as their sap is heavier. The sap of \textit{V. ventricosa} is so much lighter that the cells float despite the weight of the cell wall and protoplasm.

To test roughly whether the differences mentioned above would suffice to account for the behavior of the cells a solution was prepared containing NaCl and KCl in the same molecular proportions and concentration as the sap of \textit{Valonia ventricosa}. Dead cells of \textit{Valonia macrophysa}, placed in this and left until it had diffused in, were then transferred to sea water where they floated for a few minutes, until

the specific gravities of the internal and external solutions became so nearly equal by diffusion that the weight of the cellulose wall and protoplasm caused the cells to sink.

We may ask whether other cells are able to float by means of the mechanism found in *Valonia ventricosa*. Aside from cells which float because of the presence of gas vacuoles or of oil, there are cases like that of *Noctiluca*. From the work of Mrs. Harvey and of Lund and Logan we may conclude that the numerous large vacuoles of *Noctiluca* contain an aqueous solution whose specific gravity is less than that of sea water. Lund and Logan observed that after treatment with an electric current the vacuoles coalesce at the surface and there seems to be a destruction of the plasma membrane at the points where this coalescence occurs; in consequence the acid contained in the vacuoles diffuses out of the cell. After this the cell is no longer able to float.

It is evident that the destruction of the plasma membrane, together with accompanying changes, might allow an outward diffusion of substances (whether contained in the vacuoles or not) and at the same time substances might diffuse in from the sea water. The differences in the specific gravity between the cell sap (whether collected in vacuoles or imbibed in the protoplasm) and the sea water would thus disappear and the cell would sink.

If the cell sap had a composition similar to that of *Valonia ventricosa* this might explain the observation of Mrs. Harvey that when *Noctiluca* is placed in sufficiently dilute sea water it sinks, but after a time rises to the surface. If we assume that *Noctiluca* floats in the same manner as *Valonia* it would sink in dilute sea water and then take up water until its normal osmotic balance was restored (with an

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15 The degree of dilution necessary to cause the cell to sink would depend not only on the cell sap but on other constituents of the cell, including those lighter than sea water (such as oil, etc.).
osmotic pressure equal to or greater than that of the dilute sea water): it would then float because of the smaller specific gravity of its cell sap.

If the dilution is so great as to injure the cell and increase its permeability it sinks, as is to be expected, since, for the reason given above, dead cells of Noctiluca and Valonia ventricosa are unable to float.

The question may now be raised whether the cells of Valonia ventricosa, as described above, are to be regarded as normal. It might be suggested that while they are growing their sap resembles that of Valonia macrophysa, but that as they begin to die their permeability changes, allowing KCl to diffuse out and NaCl to diffuse in, without, however, admitting SO₄ until a later state in the death process. As a result they would float until they were so nearly dead as to admit SO₄ and would then sink. As a matter of fact when cells of Valonia ventricosa which seem perfectly normal are brought into the laboratory they may remain so for a long time but as soon as marked signs of injury appear (e.g. disturbance of the chloroplasts) they sink. Sinking, therefore, appears to be a sure sign of death and it is accompanied by the entrance of SO₄.

It should be remembered that these cells not only appear normal but that they differ from dead cells in the content of NO₃, Mg, Ca, Cl, H ion, and organic matter in the sap, and in their osmotic pressure.

The writers have made every effort to obtain young, growing cells. Many of the cells found floating are very small, but this does not constitute proof of normal condition. During the last 2 years a few cells apparently of this species have been found attached. These were very small and floated when detached; they appeared normal in all respects. Unfortunately there seems to be no prospect of obtaining such cells in sufficient quantity for analysis. It therefore seems desirable to publish the facts already obtained, since they suggest important problems, some of which may be attacked by investigation of other material.

**SUMMARY.**

The marine alga Valonia macrophysa contains in its cell sap K and Na in the proportion of 5.72 to 1. In a form regarded as closely
related, *Valonia ventricosa*, growing in similar environment, the ratio of K to Na is as 0.0278 to 1. The former contains no Mg but in the latter it is present. There are other differences in the composition of the sap.

These differences bring about a remarkable contrast in behavior in that *Valonia ventricosa* floats in sea water while *Valonia macrophysa* sinks.