THE PENETRATION OF STRONG ELECTROLYTES.

BY W. C. COOPER, JR., M. J. DORCAS, AND W. J. V. OSTERHOUT.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Accepted for publication, June 14, 1928.)

This paper deals primarily with the penetration of strong electrolytes. It touches incidentally on the problem of flotation since in some cases flotation was used as a criterion of penetration: as examples of this we may cite experiments in which NH₄Cl and CsCl were added to sea water.

Striking results were obtained by adding small amounts of 0.6 M NH₄Cl to sea water. The specific gravity of the sea water was very slightly decreased and at first the cells remained at the bottom but after a time they began to float because NH₄Cl penetrated and lowered the specific gravity of the sap so much that the cells rose to the surface: here they continued to live and grow indefinitely. (It is probable that NH₃ or NH₄OH penetrates and is subsequently changed to NH₄Cl).

When the concentration of NH₄Cl in the sea water was 0.005 M enough NH₄Cl penetrated in 10 days to cause the cells to float. Examination showed that the specific gravity of the sap was then lower than that of the sea water (see Table I); it was analyzed to determine the proportions of salts present and it was found that artificial solutions made up in these proportions had practically the same specific gravity as that of the samples of sap. Obviously therefore the penetration of NH₄Cl is sufficient to account for the change in specific gravity (see Table I).

2 This did not increase the osmotic pressure of the sea water more than 0.1 per cent.
3 These cells were placed in bottles and submerged in the ocean where growth continued.
4 The composition of these samples will be discussed in a later paper. The specific gravity of the sap in some cases fell (in the course of 22 days) to 1.0195 (taking water at 25°C. as 1.000).
We may surmise that if the ocean contained a little more NH₄Cl many organisms might float which do not now do so. Such a change has great biological importance since it profoundly alters the environment (and the distribution) of an organism to bring it from the bottom to the surface of the water. Some organisms such as Halicystis float by excluding certain substances, especially sulfates. It would be exceedingly interesting to know how many mechanisms are employed for flotation and how it can be experimentally produced or suppressed.

Experiments with flotation produced by increasing the specific gravity of the sea water by adding CsCl also gave striking results. When 1 volume of CsCl 0.6 M was added to 9 volumes of sea water the halide content and osmotic pressure remained about the same as

<table>
<thead>
<tr>
<th>TABLE I.</th>
<th>Specific gravity (at 25°C. taking water at 25°C. as 1.000)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea water.</td>
<td>............................................ 1.02250</td>
<td>Cells sink</td>
</tr>
<tr>
<td>9 volumes sea water + 1 volume CsCl 0.6 M</td>
<td>............................................ 1.02740</td>
<td>Cells float</td>
</tr>
<tr>
<td>Normal sap</td>
<td>............................................ 1.02250</td>
<td>Cells sink</td>
</tr>
<tr>
<td>Sea water containing 0.005 M NH₄Cl</td>
<td>............................................ 1.02425</td>
<td>Cells sink</td>
</tr>
<tr>
<td>Sap of cells which had been 22 days in sea water containing 0.005 M NH₄Cl</td>
<td>............................................ 1.01950</td>
<td>Cells sink</td>
</tr>
</tbody>
</table>

in ordinary sea water; the specific gravity rose from 1.0225 (ordinary sea water) to 1.0274. Cells sink in ordinary sea water but after the addition of CsCl they float (see Table I). It is evident that they would sink if sufficient CsCl should penetrate but this did not happen although the cells lived for more than a year: the only cells that sank during this time were a few that died. This experiment recalls that of Loeb on Fundulus but differs in that the external medium was not toxic nor of higher osmotic pressure than the sea water.

5 This was formerly called Valonia ventricosa: for an account of its flotation see Osterhout, W. J. V., and Dorcas, M. J., J. Gen. Physiol., 1924–25, vii, 633.
6 The specific gravity of the sap is normally about the same as that of sea water but the weight of the cellulose wall and of the protoplasm causes the cells to sink.
7 The cells were kept in finger bowls covered by plates of glass near a north window.
8 Loeb, J., Biochem. Z., 1912, xlvi, 127.
The experiment was discontinued after 13 months and the sap was extracted by piercing the cells with a sharp glass capillary (after rinsing the outside for a few seconds with distilled water and drying lightly with filter paper). It was found that the halide concentration of the medium (sea water containing CsCl) had risen, as the result of slow evaporation, from 0.564 M to 0.858 M: that of the sap\(^9\) had risen to 0.885 M. The specific gravity of the medium had risen from 1.0274 to 1.0473 and that of the sap\(^9\) to 1.0410.

We are indebted to Professor G. P. Baxter for making a spectroscopic examination of the sap: this showed a very small amount of Cs (probably less than 0.1 per cent); this might have been due to injury of some of the cells, or to contamination of the sap during its extraction. In any case it is evident that little or no Cs penetrated the normal cells during a period of over a year.

A similar experiment was made with RbCl by adding 1 volume of RbCl 0.6 M to 4 volumes of sea water, making the concentration of RbCl 0.12 M. This produced scarcely any change in freezing point depression but raised the specific gravity from 1.0225 to 1.0271. Some of the cells sank after a day and appeared normal but others floated for a few days, after which the experiment was discontinued.

It is therefore evident that there is little or no penetration of CsCl but it seems probable that there is more penetration of RbCl. We know that KCl and NaCl penetrate (either as such or perhaps as KOH and NaOH)\(^1\) since they are found in the vacuole, but our experiments show that such penetration is very slow.

It would seem that K penetrates most rapidly and Cs least rapidly, the probable order being K > Na > Rb > Cs. Li also penetrates but its position in the series is doubtful. It may be that the alkalis\(^9\) penetrate the protoplasm as undissociated molecules (e.g. as hydrates)\(^11\) whose solubility in the protoplasm may increase in the order given. Höber\(^12\) has recently suggested that the order of penetration is that

\(^9\) The normal halide content of the sap is about 0.028 M greater than that of the surrounding sea water and its specific gravity not far from that of sea water.

\(^10\) Nothing is said in this connection regarding NH\(_4^+\) since it probably penetrates to a considerable extent as NH\(_2\)\(_3\).


\(^12\) Höber, R., and Höber, J., *Arch. ges. Physiol.*, 1928, cxix, 290.
of the ionic radius but this would require that Cs\(^+\) should enter more readily than K\(^+\).

It should be borne in mind that these ideas cannot be applied generally since in Halicystis (formerly called Valonia ventricosa\(^{15}\)) the composition of the sap suggests that Na penetrates more rapidly than K.

It may be of interest to consider the absolute amounts taken up. The best data are those on the absorption of K, Na, and Cl under favorable circumstances. Here, as in other experiments on strong electrolytes, conditions which favor growth favor penetration (as is to be expected since the relation of the salt content of the sap to that of the sea water remains nearly constant). It might be expected that even when no growth occurs cations might penetrate as the result of exchange but our experiments (e.g. attempting to exchange K for Na by increasing the K content of the sea water) indicate that such processes must be very slow. Cells placed in bottles and suspended in the ocean (from July 1 to September 5) increased in weight at the rate of about 1 per cent per day.\(^{14}\) If we take as an average representative of these cells a prolate spheroid 1.3 cm. \(\times\) 0.78 cm. (this measurement does not include the cell wall) we should have for the surface area of each cell \(2\pi \left[ b^2 + \left( ab + e \right) \sin^{-1}e \right]\) where \(a = \) one half the major axis (= 0.65), \(b = \) one half the minor axis (= 0.39), and \(e\), the eccentricity, is 0.8 (from the equation \(e^2 = a^2 - b^2 \div a^2\)). This gives for the surface 2.8 sq. cm. The volume is \((4/3)\pi ab^2 = 0.41\) cc.

Since the concentration of Cl in the sap is about 0.6 \(M\) and the concentration of K\(^+\)\(^{16}\) is 86.2 per cent of this, 1 liter of sap contains \((0.6) \times (0.862) = 0.5172\) mol of K, and one cell has approximately \((0.41 \div 1000) 0.52 = 0.000213\) mol of K and takes up per day 1 per cent of this or 0.00000213 = \(213 \times 10^{-8}\). Since the area of the cell is 2.8 sq. cm. the amount taken up per day per sq. cm. is \((213 \times 10^{-8}) \div 2.8 = 76 \times 10^{-8}\), or \((76 \times 10^{-8}) \div 24 = 3.17 \times 10^{-8}\) mols of K per hour per sq. cm. The intake of Na is this amount multiplied\(^{16}\) by


\(^{14}\) For measurements of growth see Brooks, M. M., \textit{Am. J. Bot.}, 1925, xii, 617.

\(^{15}\) This was determined by the perchlorate method.

\(^{16}\) The molar proportion of Na to K in the sap is 15.1 \(+\) 86.2 = 0.17.
0.17 or \( 0.55 \times 10^{-8} \) mol of Na per hour per sq. cm.: that of Cl is equal to the sum of K and Na or \( 3.7 \times 10^{-8} \).

It would be interesting to compare these figures with those of Northrop\(^{17}\) for diffusion through a collodion membrane but it would be necessary to know the pressure which drives K and Na into the cell and we are not able to calculate this with certainty. It may be noted that K moves into the Valonia cell against the concentration gradient (the concentration of K is about 40 times as great inside the cell as outside) but Na moves with the concentration gradient (the concentration of Na is nearly 6 times as great outside as inside).

Some experiments with anions may be mentioned here. Cells were placed in a mixture of 3 volumes of sea water + 1 volume of NaI 0.6 \( \mu \) where they floated. After 24 days (under conditions favorable for growth) all the cells still floated except those that had died: the iodide in the sap of the living cells was 0.045 \( \mu \) and in the sea water 0.150 \( \mu \), but when the growth of the cell is taken into consideration it appears that little or no Cl came out of the cell during the absorption of I.

The case seems to be similar with the intake of Br, at least qualitatively. When 3 volumes 0.6 \( \mu \) NaBr are added to 1 volume of sea water so that the cells float, they sink much sooner when conditions are favorable for growth, indicating that Br is taken up during the process of growth.

A recent paper by Höber and Höber\(^{12}\) states that considerable bromide penetrates in 7 hours. This is more rapid penetration than occurs in our material unless the cells are injured (we determined Br by the method they employed).

We may conclude that in general the penetration of strong electrolytes into Valonia is very slow unless the cells are injured.\(^{18}\) There


\(^{18}\) In some cases there is visible temporary injury (as evidenced by the disarrangement of chloroplasts when viewed under the microscope) which is followed by recovery: such experiments are rejected. The best criteria of injury are the microscopic appearance, the turgidity, the absence of disturbance to the protoplasm when the cell is rolled between the fingers or bounced lightly on a table, and the presence of sulfate in the sap. In addition cells should be transferred to sea water and kept under observation but this may tell us nothing in regard to temporary injury during the experiment. Cf. Irwin, M., \textit{J. Gen. Physiol.}, 1928–29, xii, 147.
appears to be slow penetration with Li, BrO₃, IO₃, and selenite, but other substances show much less penetration, e.g. SCN, ferricyanide, ferrocyanide, formate, salicylate, tungstate, selenate, NO₂, SO₄, Sb-oxide, glycerophosphate, and many heavy metals and the alkaline earths.

On the other hand our experiments and those of others show that certain weak electrolytes enter Valonia more rapidly, e.g., NH₃,¹⁹ H₂S,²⁰ CO₂,²⁰ and others.

We might explain these facts on the ground that the protoplasmic surfaces consist of non-aqueous layers²¹ through which electrolytes pass for the most part in the form of undissociated molecules. We may imagine that ions striking the outer surface of the protoplasm unite to form molecules and as such pass through the non-aqueous surface layer only to dissociate again on reaching an aqueous phase.¹

If ions penetrate as such it may be by exchange of those of the same sign going in opposite directions²² or by the entrance of ion groups (e.g. K⁺ and OH⁻) formed at the surface by collision of ions of opposite sign which then penetrate together. It would seem that such pene-


tration must be small in view of the fact that Dr. Blinks finds the electrical resistance of the protoplasm in *Valonia* to be very high. This is so, for example, when the protoplasm is in contact with NH₄Cl although the rise in the pH value of the sap shows that either NH₃ or NH₄OH penetrates rapidly: presumably if NH₄OH penetrates it does so in the form of undissociated molecules.

In the case of strong electrolytes the penetration of undissociated molecules or of ion pairs would probably be much slower than exchange of ions since it could take place only when ions of opposite sign happened to collide at the surface.

**SUMMARY.**

The entrance of strong electrolytes into *Valonia* is very slow unless the cells are injured. This, together with the very high electrical resistance of the protoplasm, suggests that they may penetrate largely as undissociated molecules formed at the surface of the protoplasm by the collision of ions.

Under favorable circumstances KCl may be absorbed to the extent of $3 \times 10^{-5}$ mols per hour per sq. cm. of surface together with about 0.17 as much NaCl. Other substances which seem to penetrate to some extent are Li, Rb, Br, BrO₃, I, IO₃, and selenite.

Little or no penetration is shown by SCN, ferricyanide, ferrocyanide, formate, salicylate, seleniate, NO₂, SO₃, Sb, glycerophosphate, and many heavy metals and the alkaline earths.

In sea water whose specific gravity had been increased by CsCl cells of *Valonia* floated for over a year and there was little or no penetration of Cs except as the result of injury.

The penetration of NH₄Cl decreases the specific gravity of the sap and causes the cells to float: under these circumstances they live indefinitely. It is probable that NH₃ or NH₄OH penetrates and is subsequently changed to NH₄Cl. It would seem that if the sea contained a little more ammonia this would be a floating organism.

This statement is based on preliminary experiments only.