ON THE IMPORTANCE OF MAINTAINING CERTAIN DIFFERENCES BETWEEN CELL SAP AND EXTERNAL MEDIUM.*

By W. J. V. OSTERHOUT.

(From the Laboratory of Plant Physiology, Harvard University, Cambridge, and The Rockefeller Institute for Medical Research.)

(Accepted for publication, January 26, 1925.)

The sap of *Valonia macrophysa* (collected at Bermuda) differs greatly in composition from the sea water in which the cells live, as is shown by Table I.[1]

TABLE I.

Composition of Valonia Sap and of Bermuda Sea Water.

Molecular proportions expressed as per cent of halides (Cl + Br).

	Cl+Br	Na	K	Ca	Mg	SO_4
Valonia sap	100	15.08	86.24	0.288	0	Trace?
Sea water	100	85.87	2.15	2.05	9.74	6.26

The sap contains about 1.4 parts per thousand of "organic matter."

The pH value of the sap is in the neighborhood of 5.9, while that of the sea water is in the neighborhood of 8.2.

The halide content of the sea water is about 0.6 M, while that of the sap (and likewise its freezing point depression) is a little greater.

The conductivity of the sap is noticeably higher than that of the sea water (about 20 per cent) as is to be expected, since the specific conductivity of KCl is greater than that of NaCl.

The sap is contained in a large central vacuole surrounded by protoplasm, which is in turn surrounded by a cell wall permeated with sea water. As is evident from the table, the inner and outer surfaces of the protoplasm are in contact with quite different media. Is it

* Contributions from the Bermuda Biological Station for Research, No. 148.

[1] *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1922–23, v, 225. The identification of this species is somewhat uncertain.

necessary for the life of the cell that these media should be different? What will happen if they are made identical? To answer this question, sap was collected by pricking live cells and quickly squeezing out the contents of the vacuole. The sap comes out as a perfectly clear liquid free from protoplasm. Most cells immersed in this sap lived but a short time (as a rule less than a week) while in sea water under the same conditions the majority live for several months.[2]

In view of the fact that the sap contains a little organic matter, it might seem possible that the rapid death of the cells was due in part to bacterial action. It was found that in sap which had been boiled and filtered the cells died as soon as in unboiled sap. To test the matter further an artificial sap free from organic matter was made by mixing 0.6 M KCl 172 cc. and 0.6 M NaCl 30 cc.: the pH values were brought to 6.0, 7.1, and 9.1.[3] In these solutions the cells lived less than 3 days.

The cells died promptly whether the normal difference in hydrogen ion concentration between sap and external solution was maintained or not; hence it is not the primary factor involved. It is, however, not negligible, since cells in sea water at pH 6 do not live as long as in normal sea water.

If death is due to a lack of balance in the proportion of salts, the toxic action should disappear on the addition of the salts needed to produce a balanced solution. Artificial sea water was made up by mixing the following (all 0.6 M): NaCl 1,000, $MgCl_2$ 78, $MgSO_4$ 38, KCl 22, $CaCl_2$ 20 cc.; this was brought to pH 8.1. Practically all the cells were alive at the end of 3 months,[4] when the experiment was discontinued. It is therefore evident that death is due to lack of balance and that the salts themselves contain no deleterious impurities (*e.g.*, copper) the effects of which could not be counteracted by adding other salts.

In order to test this idea further, calcium was added in various

[2] The cells were placed in finger-bowls covered with glass and kept away from direct sunlight.

[3] The pH value of the solutions was adjusted in all cases by adding HCl or NaOH.

[4] At the start the average temperature was about 25°C. and at the end about 19°C.

proportions to the artificial sap and the mixtures were brought to the pH values stated below (all the solutions were 0.6 M). To 202 cc. artificial sap (KCl 172 cc. + NaCl 30 cc.) were added $CaCl_2$ (*a*) 0.6 cc. (pH 6, 6.9, 7.9): (*b*) 2 cc. (pH 6.2, 6.9, 8.0): (*c*) 4 cc. (pH 6.1, 6.9, 8.1): (*d*) 6 cc. (pH 5.8, 7.0, 7.9): (*e*) 8 cc. (pH 6.0, 7.1, 7.9): (*f*) 10 cc. (pH 6.0, 7.0, 8.0). In these solutions all the cells died within 2 weeks.

It is therefore evident that the sap is not a balanced solution in the ordinary sense of the word nor can it be made so by the addition of calcium. In order to preserve the life of the cells the external medium must contain a larger proportion of sodium[5] than was used in the experiments just described. It was found that after a month in 0.6 M NaCl 1,000 cc. plus 0.6 M $CaCl_2$ 20 cc. (pH 8.1) half the cells were alive and in good condition whereas they died in a few hours in 0.6 M NaCl (pH 8.0).

The object of these experiments was not to determine the antagonistic action of different ions, nor to discover the precise mechanism of death, but to ascertain whether certain differences between the internal and external medium are necessary for the life of the cell. This seems to be the case beyond question, at least as far as the experiments have gone. If we could in any other way make the external and internal solutions identical, as by drawing the sap out of the cell and putting sea water in its place, it can hardly be doubted that such a drastic change in the composition of the sap would be disastrous.

We must therefore, in this case, conceive of the living substance as a very thin membrane stretched between two solutions of very different composition, and it appears that certain of these differences are necessary for the normal life of the cell.

The reason for this is not yet apparent. It is quite conceivable that it might be connected with differences in potential, but this does not seem to be the case. When a fine capillary tube filled with sap is thrust into the cell the wound at once closes up and the cell may live for several days and present a normal appearance. If we lead off from this tube, and also from the outside solution, to a Compton

[5] *Cf.* Osterhout, W. J. V., *Bot. Gaz.*, 1912, liv, 532.

electrometer we are unable to detect any difference of potential[6] greater than 1 or 2 millivolts. This result is somewhat unexpected since the hydrogen ion concentration of the sap is about a hundred times as great as that of the sea water. The cause of this lack of potential difference is under investigation. It is probably connected with the high concentration of salts.

It should be borne in mind that as yet we know very little about the activities of ions in mixtures and it is possible that a study of these activities may throw some light upon this question.

To what extent the conditions here described are paralleled in other cases must be left for future investigation to decide.[7] It is probably true of both plant and animal cells that differences exist between the internal and external solutions and that these differences are highly important.

SUMMARY.

A striking difference exists between the internal and external solution in the case of *Valonia macrophysa*. If this difference is abolished by placing cells in their own sap most of them quickly die.

There is some ground for believing that the maintenance of differences between the sap and the external medium is of importance for vital processes.

The sap of *Valonia macrophysa* is not a balanced solution in the ordinary sense and the question may be raised whether in general the interior of the cell requires a balanced solution in order to maintain life: or it may be that we must distinguish between internal and external balanced solutions.

[6] Before the measurement the cell was lifted out of the sea water and the surface carefully dried with filter paper. In some cases the cell was rinsed for a few seconds in distilled water before the application of filter paper: this made no material difference in the result. During the measurement the cell was suspended in air with its lower end in contact with sea water to which one calomel electrode was connected by a string moistened with sea water. The capillary tube inserted in the upper end of the cell was connected with the other calomel electrode by a string moistened with saturated KCl. Both calomel electrodes were filled with saturated KCl (saturated with calomel). Cells 1 to 2 inches long were employed in these measurements.

[7] Investigations of this nature are in progress. In applying these conceptions to smaller cells it should be borne in mind that sap obtained by crushing cells may be altered or contaminated during its preparation.