SOME ASPECTS OF SELECTIVE ABSORPTION.*

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The problem of selective absorption presents certain difficulties which are well illustrated by the behavior of potassium. As is well known, potassium may be absorbed in larger quantities than sodium in spite of the fact that sodium greatly predominates in the solution which bathes the cell. Some investigators have attempted to account for this as the result of the formation of insoluble compounds or of selective adsorption, but it is obvious that this cannot furnish a satisfactory explanation for all cases. A much more plausible suggestion is that the potassium combines with some constituent of the cell to form a soluble compound which is unable to pass out through the semipermeable surface of the cell.[1] If sodium forms no such compound, we should expect salts of sodium to diffuse into the cell until they reached the same concentration within and without. This would also be true of potassium but in addition its concentration inside the cell would be increased by the formation of the compound in question.[2]

The writer has had an opportunity to test this suggestion by examining the sap of certain plant cells. Such cells offer especial advantages for the study of this question. It is particularly the potassium in solution which should be considered in this connection. In studying cell sap this presents no difficulty, but in the case of such cells as

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[1] *Cf.* Loeb, J., The dynamics of living matter, New York, 1906, 72. See also Höber, R., Physikalische Chemie der Zelle und der Gewebe, Leipsic and Berlin, 4th edition, 1914, 378, 388. Bayliss, W. M., Principles of general physiology, London, Bombay, Calcutta, and Madras, 1915, 120 ff.

[2] In case this compound dissociated the Donnan effect would play a part.

red blood corpuscles, or of muscle cells, it is impossible to determine how much of the potassium found in the cell is actually in solution.[3]

In the cells studied by the writer the sap is contained in a large central vacuole which is surrounded by a very thin layer of protoplasm, outside of which is the cell wall. In order to reach the vacuole a substance must pass through the outer and inner surfaces of the protoplasm, both of which are to be regarded as semipermeable.

In order to obtain cell sap free from contamination the cells should not be crushed since this sets up reactions by which the composition of the sap is altered, and also allows contamination by substances adhering to the cell surfaces or present in the intercellular spaces. For this reason the writer has studied the sap of certain large cells, which can be obtained without contamination.

The sap chiefly studied is that of the marine alga *Valonia*,[4] which forms large, balloon-shaped cells, each of which may contain several cubic centimeters of cell sap. The writer is under great obligation to Dr. W. J. Crozier, who generously collected the plants at the Bermuda Biological Station of Harvard University and employed the utmost care in extracting and preserving the sap. The plants were removed from the sea water, rinsed with distilled water, wiped dry, punctured with a glass needle and subjected to slight pressure, so that the sap squirted out. The sap was placed in bottles which had been very carefully cleaned; the stoppers were sealed with paraffin or vaseline. The total amount of sap collected was about 300 cc.

The sea water used for comparison was collected at the same time and preserved in the same manner.

The analysis was carried out by Mr. L. M. Van der Pyl under the direction of Professor G. P. Baxter. Preliminary trials showed that it was possible to analyze samples of 10 gm. of sea water with concordant results, which agreed fairly well with the published figures of other workers who have analyzed larger quantities. The analysis of

[3] The fact that an inorganic constituent may be abundantly present in the cell without being in solution is strikingly shown in the case of many plant cells which contain numerous crystals of calcium oxalate while little or no calcium is present in solution.

[4] According to Crozier this is *Valonia macrophysa*. *Cf.* Crozier, W. J., *J. Gen. Physiol.*, 1918–19, i, 581.

sap and of sea water was carried out in precisely the same manner (using samples of about 10 gm. in both cases). Cl was determined as AgCl; SO_4 as $BaSO_4$; Na as NaCl; K as perchlorate.[5] Ca was precipitated as oxalate,[6] ignited, and determined as CaO. Mg, after double precipitation as phosphate,[6] was ignited and determined as pyrophosphate.

It was found that, as stated by Wodehouse,[7] addition of $BaCl_2$ produced no visible precipitate. After addition of $BaCl_2$ the sap was filtered and the residue incinerated. From the resulting weight was subtracted the value obtained by the same procedure (using the same amount of sap) without the addition of $BaCl_2$. The difference was assumed to be $BaSO_4$. Since Wodehouse found that SO_4 penetrates injured cells it is possible that the small amount of SO_4 found in the analysis may be due to the presence of such cells.

Wodehouse, working at Bermuda, found that healthy cells, taken directly from the sea water, gave a test for NO_3 with nitron, while the sea water did not. Tests made by Mr. E. S. Harris on the preserved sap (by the phenoldisulphonic acid method) showed the concentration of NO_3 to be about 0.002 M. The concentration in fresh sap is probably higher than this. Sea water gave no test by this method.

Table I shows the results of the analysis. The value for chlorine in the *Valonia* sap is higher than in sea water. This may be due in part to evaporation during the extraction and subsequent preservation; the sap was contained for the most part in smaller bottles than the sea water and hence might be more affected by evaporation. In this connection it may be noted that the electrical conductivity of the sap was higher than that of the sea water even where both had been kept in glass stoppered bottles for the same length of time (about 3 weeks).

The results differ somewhat from the earlier analyses of Hansen[8] and Meyer.[9] The results of Hansen are only approximations. Those

[5] The potassium was determined according to the method of Baxter, G. P., and Kobayashi, M., *J. Am. Chem. Soc.*, 1917, xxxix, 249; 1920, xlii, 735. Baxter, G. P., and Rupert, F. E., *J. Am. Chem. Soc.*, 1920, xlii, 2046.

[6] *Cf.* Richards, T. W., McCaffrey, C. F., and Bisbee, H., *Proc. Am. Acad. Arts and Sc.*, 1901, xxxvi, 377.

[7] Wodehouse, R. P., *J. Biol. Chem.*, 1917, xxix, 453.

[8] Hansen, A., *Mitt. Zool. Station Neapel.*, 1893, xi, 255.

[9] Meyer, A., *Ber. deutsch. bot. Ges.*, 1891, ix, 77.

TABLE I.

Composition of Valonia Sap and of Bermuda Sea Water.

	Parts per thousand.		Molecular proportions (expressed as per cent of Cl).	
	Bermuda sea water.	Valonia sap.	Bermuda sea water.	Valonia sap.
Cl	19.585 19.600 19.629	21.180 21.186	100.00	100.00
Average..........	19.605	21.183		
Na	10.908 10.929	2.070 2.074	85.87	15.08
Average..........	10.919	2.072		
K	0.457 0.470	20.142 20.143	2.15	86.24
Average..........	0.464	20.143		
Ca	0.456 0.451 0.455 0.451	0.70 0.67	2.05	0.288
Average...........	0.453	0.69		
Mg	1.306 1.308 1.314	Faint tr.	9.74	
Average..........	1.309			
SO_4	3.325 3.328	0.004 0.006	6.26	0.0087
Average...........	3.327	0.005		
Total solids.	45.234 45.222			
Average...........	45.228			
Organic matter.	1.438 1.428			
Average...........	1.433			

of Meyer can not be regarded as accurate; this he recognized and explained on the ground that the amount of cell sap at his disposal was small. No details in regard to analytical methods are given by Hansen or Meyer. Both agree, however, that K is much more abundant than Na in the sap, and Meyer states that Mg, Ca, and SO_4 are less concentrated in the sap than in the sea water, while K is much more concentrated.

The amount of organic matter in the sap is small. It was determined by weighing out samples of approximately 25 cc. into platinum dishes and evaporating, without heat, in a vacuum desiccator. They were then weighed again. The amount of total solids was computed from this figure, which is undoubtedly high, due to the method of evaporation, which may leave as much as 0.5 per cent water included in the crystals. The dishes were then heated over a free flame to char organic matter; they were not allowed to become red hot. The residue was taken up in water, filtered, and evaporated on the steam bath until crystals appeared. The evaporation was completed in a vacuum desiccator and the dishes were weighed. The difference between this weight and that of total solids gives the amount of organic matter. The results are given in Table I.

The table makes it evident that we cannot accept the idea that potassium is held in the cell by entering into combination with an organic compound. It is evident that practically all of the potassium, sodium, and calcium in the cell exists in the form of chlorides.[10]

Evidently there is an excluding mechanism which prevents Na, Mg, Ca, and SO_4 from diffusing into the cell[11] to such an extent as to equalize the concentration within and without. There is also a trapping mechanism which causes KCl to accumulate in the cell until its concentration far exceeds that in the sea water.

[10] The high conductivity of the cell sap shows that these substances must be ionized to about the same extent as in sea water.

[11] Since this mechanism breaks down when the cells are killed it is probable that injured cells absorb more Na, Mg, Ca, and SO_4 than uninjured ones. If injured cells could be wholly excluded when sap is extracted the proportion of these substances might be lower. Wodehouse[7] found that SO_4 penetrates so little into normal cells that the sap gave no visible precipitate with $BaCl_2$; injured cells, on the other hand, gave a test for SO_4.

From a theoretical standpoint, it is clear that these two mechanisms may operate independently.

Discussion may be reserved until the completion of further experiments. It may be remarked, however, that no great stress should be laid on the fact that the conductivity of the sap does not differ greatly from that of sea water. In the case of *Nitella*, whose cell sap may also be obtained without contamination, it is found that the conductivity of the cell sap is very much greater than that of the fresh water in which it grows.

SUMMARY.

1. A mechanism exists in *Valonia* which prevents certain substances (Na, Mg, Ca, SO_4) from reaching as high a concentration inside the cell as in the sea water which surrounds it.

2. A trapping mechanism also exists which causes K to accumulate in the cell in a concentration far in excess of that found in sea water. Practically all the K in the cell exists in the form of KCl.

3. The concentration of Cl does not differ greatly within and without.

4. These facts are not in harmony with present theories regarding the accumulation of K in living cells.