AN OUTPUMP BALANCING PHOSPHATE-DEPENDENT SODIUM UPTAKE IN SCENEDESMUS.

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The ratio potassium/sodium is generally higher inside plants than in the outside medium. Among the mechanisms leading to this condition, considerable interest has lately been given to the active extrusion of sodium ions from the cells (Dainty 1962), or their subunits (Saltman et al. 1963). The best elucidated cases of such a sodium out-pump occur in algae from saltish or brackish water, whereas it is practically unknown in sweetwater algae and in higher plants as reviewed by Eppley (1962). An effect of small amounts of sodium on the uptake and utilization of phosphate in Ankistrodesmus was, however, noted by Simonis and Urbach (1963), but could not be directly related to a sodium pump of the ATP-ase type studied in animal tissues by Järnefelt (1962) and others. Information on metabolic activities of sodium also in other connections is currently appearing (cf. Josli et al. 1962).

In earlier work at Wageningen, The Netherlands, the author developed methods to grow the sweet-water alga Scenedesmus to an advanced stage of P deficiency (Kylin 1964). Such a material offers the theoretically interesting possibility to start with an inhibited system, and investigate to what extent an addition of phosphate will trigger a process under study. Applying this principle, it has been possible to demonstrate a sodium out-pump more or less balancing the intake of the ion, which intake depends upon the phosphate added.

The P deficient cells used were of the same strain and developed mainly according to the same methods as before (Kylin 1964), the chief change being a slightly lower light intensity (approximately 15000 ergs per second and cm^2). It should be noted that the culture solutions are low in sodium, containing only 0.625 milliequiv./l from sodium citrate and di-sodium-EDTA used to chelate the iron.

The experiments proper were performed at the light intensity mentioned, in 50 ml Erlenmeyer flasks open to the air and kept at 25°C in a shaking waterbath adjusted to about 90 cycles per minute. The cells for an experiment were centrifuged and rinsed twice by resuspension in glass-distilled water. From the light absorbancy at 525 mμ of the culture to be used, the volume of the final suspension could be calculated so as to contain approximately 2.5 mg dry weight per ml. 2 ml of this suspension was used for each experimental flask, and the exact dry weight determined separately (cf. Wedding and Black, 1960).

Each experimental flask finally held 10 ml solution with a concentration of 1m$\underline{M}$-NaCl labelled with 10 μC/l ^{22}Na, 25 m$\underline{M}$-KNO_3, 1 m$\underline{M}$-$MgSO_4$, 27.5 m$\underline{M}$-K-citrate buffer at a pH of 6.4-6.5, and algae as specified above. The solution also held a mixture of KCl and K-phosphates, giving a constant potassium supply corresponding to that of a 5 m$\underline{M}$-phosphate buffer at pH 6.5, and with the initial phosphate concentration varied from 0 to 5 m$\underline{M}$.

To avoid interference from sodium loosely adsorbed within the free space, the cells were twice centrifuged and resuspended in 2 ml of the P-deficient solution, with inactive NaCl, at the end of an experiment. They were then filtered off on a glass fiber filter in a Tracerlab E-8B precipitation apparatus, washed once more with the solution mentioned and, finally, with distilled water. The preparations were glued to discardable aluminum planchets, dried at 60°C, and brought to the counter. The radioactivity was determined at presets of 3000 to 10000 counts, and recalculated as

μmoles/g dry weight by reference to standards from the original solution. Each part of an experiment was made in duplicate, the single values differing less than 10 per cent from the averages given in the table. The lowest quantity of sodium determinable with any accuracy under the conditions described was in the order of 0.1 μmoles/g dry weight. The dry matter contents of the cells is about 30 per cent of the fresh weight (Kylin 1964).

Table 1

μmoles sodium per g dry weight of P deficient Scenedesmus cells after 30 and 240 minutes. Phosphate added to the initial concentration stated.

mM-phosphate	0	0.005	0.05	0.15	0.5	5
30 minutes	<0.1	< 0.1	0.6	2.9	2.9	2.8
240 minutes	< 0.1	< 0.1	< 0.1	1.1	9.0	10.6

If sodium is to be detected within the cells, phosphate additions are necessary (Table I). However, after 30 minutes there are determinable quantities of Na already with an initial concentration of 0.05 mM-phosphate, whereas 0.15 mM-phosphate at the start is necessary if the cells are to hold appreciable amounts of sodium at 240 minutes. 0.15-5 mM-phosphate gives about the same sodium contents of the cells at 30 minutes, and the same is true for the range 0.5-5 mM-phosphate at 240 minutes.

Table II

Variation with time of the sodium contents of P deficient Scenedesmus cells. Phosphate added at the beginning of the experiment to an initial concentration of 0.15 mM

Time in minutes	30	65	125	180	230
Na in μmoles/g dry weight	4.3	2.9	1.8	1.0	0.8

With regard to the results discussed, a closer study was made of the time curve of the sodium contents at an initial phosphate concentration of 0.15 mM (Table II). The sodium is pumped out again after the initial period of uptake, although the cells are still surrounded by the same radioactive solution.

In conclusion, we have demonstrated:

(A) In cells of Scenedesmus deficient in phosphate practically no sodium is taken up without additions of phosphate. This may be due to an active pump stimulated by phosphate or phosphate metabolism, but it may also be due to a passive influx as a consequence of an increased number of negative charges inside the cell membranes when phosphate is taken up and starts to act. No decision between these possibilities can be made for the moment.

(B) Sodium influx is counteracted by an out-pump, which in regard of the general set-up and the results of the experiments must be regarded as active.

A full description and discussion of our results, which comprise also other ions, will be presented elsewhere.

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