POTASSIUM-DEPENDENT SODIUM EXTRUSION BY CELLS OF PORPHYRA PERFORATA, A RED MARINE ALGA

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ABSTRACT

Potassium-free artificial sea water causes a loss of cell potassium and a gain of cell sodium in *Porphyra perforata*, which is not attributable to an inhibition of respiration.

On adding KCl or RbCl to such low potassium, high sodium tissues, net accumulation of potassium or rubidium takes place, accompanied by net extrusion of sodium. Rates of potassium or rubidium accumulation and sodium extrusion are proportional to the amount of KCl or RbCl added only at low concentrations. Saturation of rates is evident at KCl or RbCl concentrations above 20–30 mM, suggesting the role of an ion carrier mechanism of transport.

Evidence for and against mutually dependent sodium extrusion and potassium or rubidium accumulation is discussed.

In nerve fibers, kidney tubules, and other special secretory tissues the important role of active ion transport has been well demonstrated. In other cells, however, the significance of ion transport and the maintenance of a constant cellular ion constitution has not been fully appreciated. It is possible that the secretion of ions is of general physiological importance in cellular homeostasis, and with this in mind experiments on ion transport in *Porphyra* were undertaken with the hope that data of a comparative nature might be useful toward a general theory of active ion transport.

Recently Harris (10), Hodgkin and Keynes (11), and Huf *et al.* (12) have proposed sodium secretion mechanisms involving potassium. Coupled potassium accumulation and sodium extrusion are indicated in the first two cases, human erythrocytes and the squid giant axon, while potassium is not unidirectionally transported across the frog skin, as is sodium.

In frog muscles (19) and mammalian liver slices (1), as well as in human erythrocytes (10) and the squid giant axon (11) potassium-free media result in a loss of potassium and gain of sodium. Separate measurements of influx and

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efflux indicate that potassium lack reduces active sodium efflux, as well as blocking potassium influx in squid nerve (11).

Other workers have suggested independent mechanisms of sodium extrusion and potassium accumulation, however (3, 17). But in yeast, active sodium secretion independent of potassium accumulation was refuted by Foulkes (8).

In this paper evidence will be presented for potassium-dependent sodium extrusion and concurrent potassium or rubidium accumulation in *Porphyra*. Experiments indicating coupling of these processes will also be described. Cellular ion constituents, evidence for active transport of sodium and potassium, and the advantages of *Porphyra* in permeability studies were reported earlier (4).

Methods

Porphyra perforata was collected on rocks in the intertidal zone along the Monterey Peninsula, California, and was kept in the laboratory in running sea water before use. Sodium, potassium, and rubidium were determined by flame photometry in 5 per cent trichloracetic acid extracts of the sucrose-washed alga (4). Chloride was estimated by a modified colorimetric method (14) in hot water algal extracts. All experiments were performed in the dark and at room temperature (15–20°C.) unless otherwise indicated.

The potassium-free artificial sea water contained NaCl 0.48 M, $MgCl_2$ 0.027 M, $MgSO_4$ 0.027 M, and $CaCl_2$ 0.01 M.

Ion contents refer to total amounts in the tissue, not corrected for adsorption or apparent free space.

RESULTS AND DISCUSSION

Aerated potassium-free sea water induces a net loss of potassium in *Porphyra* (Fig. 1), although doubling the potassium content of sea water does not increase the potassium content (data not presented). The experimental points (Fig. 1) for loss of potassium best fit a first order reaction curve with rate constant approximately 0.029 hour^{-1}. Presumably the loss of potassium represents simple diffusion with the rate dependent upon the cellular potassium content. The half-time for the reaction, somewhat greater than that reported for frog muscle (19), is about 24 hours, suggesting a relatively low membrane permeability to potassium. Unlike myelinated nerves, it is not likely that potassium moving out of the cells is trapped in the extracellular material, thus allowing some potassium influx to continue. The potassium-free solutions were changed at 1 to 30 minutes, 4 hours, and 24 hours during the experiment, and a large volume of solution was used (about 200 ml. per gm. of tissue).

Tissue sodium increased during the potassium-free treatment, but followed a different time course from potassium loss (Fig. 1). Gain of sodium was essentially complete in 24 hours, while loss of potassium continued throughout the experiments.

The net gain of sodium is much less than the net potassium loss. A similar

unequal exchange was also observed during anoxia (4), in which case potassium efflux was accompanied by a small amount of chloride loss and sodium gain
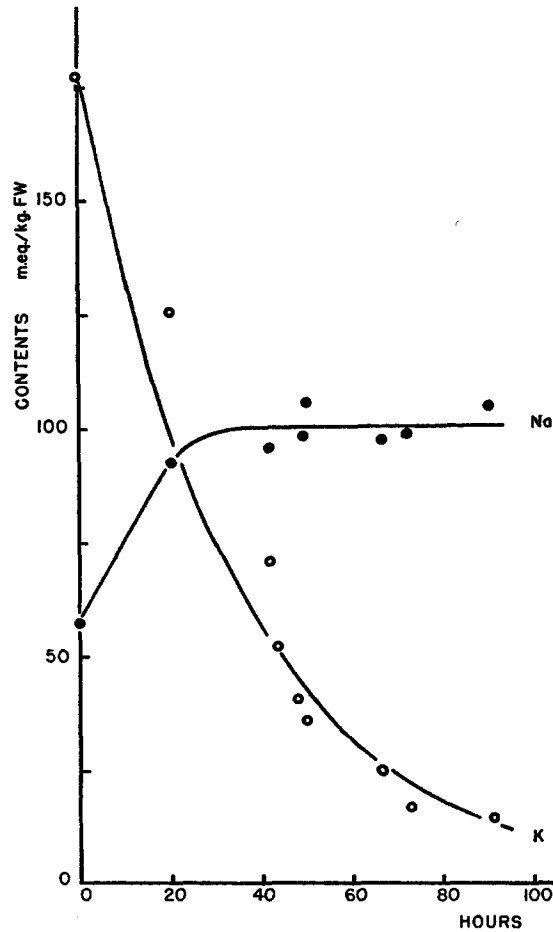

FIG. 1. Sodium and potassium contents of *Porphyra perforata* tissues exposed to K-free artificial sea water. Curve for potassium drawn from first order reaction with rate constant 0.029/hour. Curve for sodium drawn by eye.

(Table I), but chloride loss plus sodium gain remains less than potassium loss. Several explanations may possibly apply, but none has been experimentally tested. It is not considered likely that cell shrinkage would occur to the extent necessary to explain the net difference between sodium gain and potassium loss since the osmotic value of the K-free artificial sea water is similar to that of normal sea water. Although Shaw *et al.* (18) noted a lack of correlation between

the resting potential of *Bufo marinus* sartorii and potassium content, Hodgkin and Keynes (11) report that passive movements of ions in squid giant axons may vary with potential. Thus there seems to be a precedent for expecting excess potassium loss in *Porphyra* to be due to an alteration of potential, but the latter would be difficult to measure in this alga. It is also possible that another cation (Ca, Mg, or H) may replace cell potassium, or that the cells suffer a loss of organic anions during the potassium-free treatment without alteration of potential.

In any case, potassium lack partially blocks sodium extrusion in *Porphyra*.

When potassium-deficient tissues are exposed to artificial sea water containing KCl, sodium extrusion and potassium accumulation take place. Both processes are stimulated by light, inhibited by cyanide and dinitrophenol, and occur against concentration gradients. Sodium extrusion is nearly complete

TABLE I

Variation of Sodium, Chloride, and Potassium Contents of Porphyra perforata Tissues during Anoxia (m.eq./kg. FW)

Time under N₂	Na	Cl	K
hrs.			
0	60	30	160
4	62	26	159
24	—	—	121
48	87	17	102
95	97	9	34

in 2 to 4 hours (Fig. 2), while potassium accumulation may continue as long as 12 to 16 hours (Fig. 3). In each case the final levels attained are similar to the original untreated values.

If coupling occurred between sodium extrusion and potassium accumulation, on exposure to potassium, one might expect to find a constant ratio of sodium extruded to potassium accumulated during the initial period. This ratio averages 2.3 with standard deviation 1.7 for 18 determinations. Such variability of the ratio suggests a "loose coupling" such as appears to be the case in squid nerve sodium and potassium transport (11). However, one sodium ion per potassium is actively transported in that tissue, thus the mechanism transports no net charge and is independent of potential. Until separate efflux and influx values are obtained for *Porphyra* it will not be clear whether this condition is met.

Inhibition of sodium extrusion in frog skin (12) and in *Porphyra* is not due to a fall in respiratory rate. Oxygen consumption of tissues exposed to potassium-free sea water is slightly higher than that of controls in sea water with normal potassium content (Table II). Addition of KCl to potassium-deficient

tissues stimulates respiration, however (data not presented). Epstein (5), Oberholzer (15), and others have previously reported potassium-stimulated respiration in both plant and animal tissues. Quantitative measurements of the amount of respiratory stimulation are now in progress so that ratios of ions transported per molecule of oxygen consumed may be determined, as Leaf and Renshaw have done for frog skin (13). The values they obtained may require a modification of Conway's redox theory (2) as an explanation of sodium transport in that tissue (12).

When rubidium chloride, instead of potassium chloride, is added to potassium-deficient tissues, rubidium is accumulated (Table III). The amount ac-
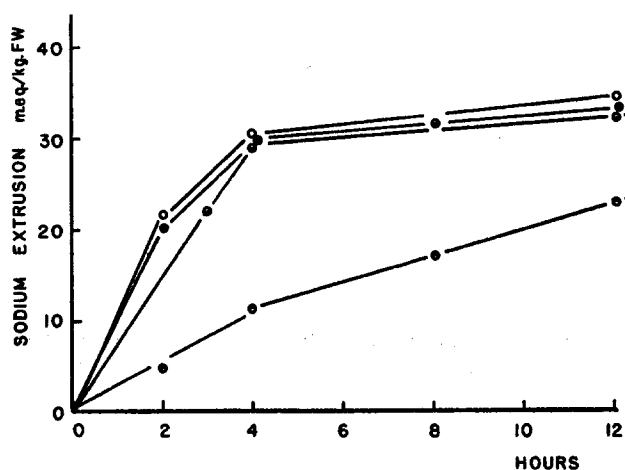

FIG. 2. Amounts of sodium extruded by potassium-deficient *Porphyra perforata* tissues on exposure to artificial sea water containing 5 mM ⊖——⊖, 10 mM ⊖——⊖, 20 to 30 mM ●——●, or 40 to 60 mM ○——○ KCl.

cumulated does not appear significantly different from the amount of potassium lost, thus an exchange of rubidium for potassium may be involved. Unfortunately the data are not of sufficient precision to determine whether all rubidium accumulation may be thus accounted for.

In spite of the loss of potassium, sodium extrusion proceeds when RbCl is added. Both the rate of sodium extrusion and the time course are similar to those observed on adding KCl. Apparently rubidium can substitute for potassium as a requirement for sodium extrusion.

Because the rate of sodium extrusion is dependent upon the concentration of potassium or rubidium in the medium (Fig. 2, Table III), it might be suspected that the rate of potassium or rubidium accumulation, and not their external concentration, determines the rate of sodium extrusion. However, Hodgkin and Keynes (11) found potassium influx to be largely passive when outside

potassium was 52 mM, while it was chiefly an active process when outside potassium was 10.4 mM. Sodium efflux was increased only about 40 per cent
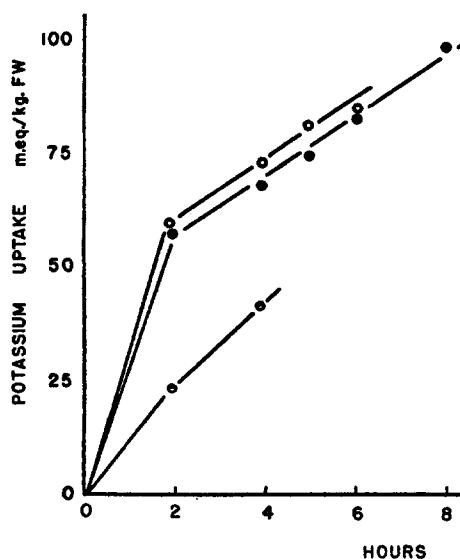

FIG. 3. Amounts of potassium taken up by K-deficient *Porphyra perforata* tissues on exposure to artificial sea water containing varying amounts of KCl. Symbols as in Fig. 2.

TABLE II

Rates of Oxygen Consumption of Porphyra perforata Tissues in Sea Water and after Long Exposure to K-Free Artificial Sea Water

Units Q_{O_2}:microliters/gm. fresh weight/hr. sodium and potassium contents in m.eq./kg. FW.

Experiment	K content	Na content	$Q_{O_2} \pm$ s.d.	No. samples
Sea water	180	60	177 ± 27	7
K-free sea water				
1	89	98	232 ± 3	2
2	60	134	179 ± 15	5
3	67	129	244 ± 29	5
4	54	96	191 ± 8	3

when external potassium was 52 mM as compared with 10.4 mM. Thus, if potassium or rubidium accumulation in *Porphyra* does not represent an active process at high external potassium or rubidium concentrations, then it seems more likely that variation in sodium extrusion with external potassium or rubidium

may be a surface effect. Hodgkin and Keynes (11) found addition of potassium to have an immediate effect on sodium efflux rate, consistent with this interpretation.

That rates of transport are linear with increasing potassium or rubidium concentration in the medium only at low concentrations, and appear saturated at high concentrations, may argue for the existence of an ion carrier mechanism of transport (6, 16). If such is the case potassium and rubidium are

TABLE III

Sodium, Potassium, and Rubidium Conterts of Porphyra perforata Tissues Rendered Low in Potassium and High in Sodium by Previous Treatment with K-Free Sea Water, Then Exposed to Artificial Sea Waters Containing Varying Amounts of Potassium and Rubidium Contents in m.eq./kg. FW.

Time	KCl	RbCl	K	Rb	Na
hrs.	m.eq./liter	m.eq./liter			
0	0	0	111	0	80
3.8	5	0	139	0	66
8.2	5	0	132	0	62
11.2	5	0	154	0	50
3.8	5	5	71	8	—
8.2	5	5	91	16	50
11.2	5	5	70	20	46
3.8	5	25	83	23	51
8.2	5	25	—	—	52
11.2	5	25	87	78	62
3.8	5	100	57	28	48
8.2	5	100	56	68	49
11.2	5	100	39	88	47

probably processed by one carrier site, while sodium is handled by another, as is the case in barley roots (7). However, the existence of separate carrier sites for different groups of ions does not necessarily imply independent transport mechanisms for these groups. A contractile protein (9) or other macromolecular carrier could be operating which would allow different binding sites on the same carrier.

Several experiments with Porphyra have given results inconsistent with the coupling concept. (1) Iodoacetate in the dark preferentially inhibits retention of potassium over and above the difference in magnitude of the concentration gradients of sodium and potassium (4). (2) Net potassium accumulation continues after net sodium extrusion has ceased, and net potassium loss in potas-

sium-free sea water continues after sodium gain is complete. The latter results may not be valid data for argument, however, since only net flux, not influx and efflux, was measured. Evidence from the iodoacetate experiment may also be invalid here, because there may be a potassium retention mechanism, independent of sodium extrusion, involving glycolysis as in yeast (8). As yet no data are available on the latter possibility.

At this point no complete picture of sodium extrusion and potassium accumulation in *Porphyra* may be drawn. The evidence for coupling, the dinitrophenol and cyanide sensitivity, the requirement for oxidative metabolism, and the temperature coefficient for transport (1.9 between 3 and 10.4°C. for both sodium extrusion and potassium accumulation in the dark after anoxia) suggest a cyclic mechanism similar to that proposed by Hodgkin and Keynes (11) for squid giant axon.

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