EVIDENCE FOR THE PRESENCE OF SEPARATE MECHANISMS REGULATING POTASSIUM AND SODIUM DISTRIBUTION IN ULVA LACTUCA*, ‡

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INTRODUCTION

A major problem of all living systems is the maintenance within the cell of a chemical composition which differs both quantitatively and qualitatively from that of the external environment. Such regularity of cellular composition is essential to life. No longer can a simple "permeability" explanation alone be invoked to account for this aspect of cellular homeostasis, since for a great variety of cellular components the cell membrane has been shown to be permeable. Rather a more active dynamic type of regulation must control cellular constancy. An important aspect of this regulation is cation control, and although a great number of studies have been concerned with the distribution of sodium and potassium between cells and environments, the precise mechanisms involved in these distributions remain to be defined.

In earlier papers (25–27) the authors presented the results of some investigations on this problem in the cells of the marine green alga *Ulva lactuca* var. *latissima*. At that time evidence was presented which indicated a close relationship between carbohydrate metabolism and cation integrity of the cell, and which further suggested partially independent mechanisms for regulating cellular sodium and potassium concentrations. The present paper is concerned with an extension and elaboration of the earlier studies on *Ulva* along two main lines: (1) further investigations of the energetic relationships between ion-transporting mechanisms and cellular metabolism; (2) a separation of the potassium and sodium-regulating mechanisms from each other in so far as they are separable.

To this end experiments were carried out to give the following types of information: (1) the influence of external sodium on the rate and extent of reaccumulation of potassium in cells depleted of both sodium and potassium;

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‡ A preliminary report of some of these experiments has been published (14).

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(2) the influence of arsenate on cation distribution in iodoacetate-treated alga; (3) the effects on cation regulation of inhibition of cellular metabolism by phenylurethane, and the extent of reversibility of this inhibition; (4) the combined actions of phenylurethane and iodoacetate; (5) the degree of protection afforded the cell by exogenous phosphoglycerate and pyruvate against the cation shifts caused by iodoacetate in the dark; (6) the influence of 4,6-dinitro-*o*-cresol on sodium and potassium; and (7) the action of exogenous ATP given with iodoacetate.

Methods

For the most part the methods employed here were those described earlier (25), but with the following modifications. In preparation of the material for analysis it was more convenient to extract the minerals simply by boiling the ground dried samples for 15 minutes in water. The complete extraction was facilitated by predigesting with about 1 ml. of concentrated nitric acid. This method of extraction gave sodium and potassium values essentially identical with those obtained using the wet ashing technic and the cold trichloroacetic acid method used previously (25). The extractions were carried out in 50 ml. volumetric flasks, and, after cooling, the contents were diluted to volume. Following filtration through Whatman No. 40 "ashless" filter paper, the filtrates were analyzed for sodium and potassium by flame photometry with the Beckman spectrophotometer.

Sea water contamination was removed from the samples by rinsing them for 30 seconds in isotonic sucrose. Evidence will be presented in the next section that the sucrose rinse removed essentially all the extracellular potassium and sodium and essentially none of the intracellular fractions of these cations.

Stock solutions of the inhibitors were brought to a pH of 7.5 before use in any experiment. The phenylurethane stock was prepared in 95 per cent ethanol; in experiments in which this inhibitor was used a corresponding concentration of ethanol was added to the controls, and was without effect on the sodium and potassium contents.

Inulin was determined by the method of Hubbard and Loomis (15), using a Klett-Summerson photoelectric colorimeter. This method was also applied to the analyses for sucrose, after preliminary experiments showed a linear relationship between color intensity and concentration of sucrose. Determinations of pH were made using the Beckman pH meter with glass electrode. In no experiment did the pH vary more than 0.5 pH unit.

As shown in the graphs samples were removed in triplicate; values shown in the tables are averages of three samples each, in which the variation is of the same order of magnitude as in the graphs. In the data to be presented potassium concentration is calculated in terms of m.eq./100 gm. cell water, while sodium values are expressed as m.eq./100 gm. dry weight, for reasons already discussed (25). All experiments were run at the temperature of running sea water (20–21°C.).

RESULTS

1. Extracellular Space.—In order to obtain valid values for the sodium and potassium concentrations in the cells it is essential to determine what fraction

of the electrolytes in the samples as they are analyzed is due to contamination by the sea water which might be in the interstitial spaces. To this end several experiments were carried out to determine the extracellular space in the tissue under the conditions employed in preparation of the samples for analysis.

In one set of experiments samples were placed in sea water containing 1 per cent inulin for several hours,[1] then transferred to exactly 100 ml. of fresh sea water. Immediately before transfer some of the samples were dipped very briefly (1 to 2 seconds) into fresh sea water to remove adhering inulin solution; the other samples were rinsed for 30 seconds in fresh sea water, then blotted three times in absorbent tissue (same procedure used in preparation for sodium and potassium analyses), and then transferred. "Inulin space" was measured

TABLE I

The Effects of Rinsing and Blotting on the Inulin and Sucrose "Spaces" in Ulva

See text for description of methods.

	1 to 3 sec. rinse No blot	30 sec. rinse "Triple blot"
Inulin space	19–21 per cent	0–1 per cent
Sucrose space	20–21 per cent	0–1 per cent

TABLE II

The Effect of Blotting Alone on the Inulin and Sucrose "Spaces" in Ulva

See text for description.

	1 to 2 sec. rinse No blot	1 to 2 sec. rinse "Triple blot"
Inulin space	20–22 per cent	2–3 per cent
Sucrose space	20–21 per cent	2–3 per cent

under these conditions by determination of the inulin in the final solution a few hours after transfer. At the end of an experiment the samples were removed, blotted, and weighed. Results of a typical experiment, indicating that the combination of the 30 second rinse with the triple blot is sufficient to reduce the inulin space to almost zero, are presented in Table I.

In the same manner sucrose was used as an indicator of extracellular space. Representative data, which agree very well with the inulin values, are also shown in Table I.

To verify these results other experiments were designed to determine the effects of rinsing procedure and the blotting technique alone on the extracellular space. Samples were soaked in inulin—sea water as before and rinsed for 1 to

[1] The time course for diffusion of inulin into the tissue indicates that equilibrium was established within 1 to 2 hours.

2 seconds in fresh sea water. One set was then transferred directly to fresh sea water, while the other was first blotted according to the standard procedure. Typical results are shown in Table II, in which it is seen that blotting alone reduces the extracellular space from about 20 per cent to about 2 to 3 per cent. A similar experiment was done using sucrose as the indicator of extracellular space.

Finally sodium and potassium determinations were run on samples removed from sea water under the following conditions: All samples were rinsed for 2 to 3 seconds in isotonic sucrose; one set was blotted three times according to the standard procedure while the surfaces of the other samples were brushed lightly with absorbent tissue to remove in the gross the adherent sucrose only from the surface. In this second group, then, the extracellular space has not been blotted out of the tissue. The samples were weighed, dried, extracted, and

TABLE III

Potassium and Sodium Values for Samples of Ulva Removed from Sea Water

All samples rinsed 1 to 2 seconds in isotonic sucrose. Some were brushed to remove sucrose from surface (*a*), while the rest were "triple blotted" (*b*). Values are expressed as m.eq./100 gm. cell water.

	K	Na
(*a*) Very light blot	21.8	13.5
(*b*) "Triple blot"	31.0	19.2
(*c*) *b-a*	9.2	5.7
(*d*) $\frac{c}{a} \times 100$	42.2 per cent	42.1 per cent

analyzed. Average sodium and potassium values are presented in Table III. It can be seen that the lightly blotted samples actually have less sodium than the triple blotted samples. Since as shown above the triple blotting almost completely removes the inulin space, these data clearly indicate that the smaller solutes in the extracellular space very rapidly diffuse from this space (1 to 3 seconds here) in isotonic sucrose. Further it can be seen that the relative difference in sodium concentration between the two sets of samples is exactly equal to the relative potassium difference (row d). Since the potassium concentration in sea water and in the isotonic sucrose is so small relative to the total amount of potassium in the cells, this difference in potassium content is probably almost exclusively due to weight differences; that is, the lightly blotted samples will be heavier because of the sucrose solution which was not blotted in these samples. Hence there will appear to be less potassium (and sodium) per unit weight in the lightly blotted samples. Since the per cent sodium difference is identical with the per cent potassium difference, it is presumably to be accounted for by the same explanation. Sodium and potassium ions, then, diffuse

very rapidly from the extracellular space of the samples after they are transferred from sea water to isotonic sucrose.

The possibility that the sucrose rinse washes out some of the electrolytes from within the cell is remote, since samples removed after 30 and 60 seconds in sucrose are not appreciably different in sodium and potassium content from those removed after only 1 to 3 seconds.

2. Effect of External Sodium on Potassium Accumulation.—It is of considerable importance to determine whether or not the potassium-accumulating mechanism is passive, dependent upon and secondary to the sodium-secreting mechanism, or whether it is active and independent of sodium secreton. To this end the following type of experiment was carried out. Samples were repeatedly suspended in isotonic sucrose for 2 to 3 hours, at which time the cells had lost about 85 per cent of their sodium and potassium (Figs. 1 and 2). At this time half the samples were transferred to complete natural sea water, while the others were placed in sodium-free Allen's artificial sea water, isotonicity maintained by the addition of sucrose. Potassium reaccumulation was followed in the two groups by removing samples at various times over a 24 hour period. Two features of the data are outstanding: (1) the potassium reaccumulation is, within experimental error, linear; and (2) the presence of sodium in the external fluid made no measurable difference in the rate of potassium reaccumulation. It is noteworthy that in other experiments the potassium reaccumulation was complete to the control level. The sodium concentration in those samples returned to complete sea water is seen to have reached the control level within the time at which the first samples of this series were removed; the curve for reentry of sodium, then, is probably steeper than the one shown here and need not be linear.

3. The Influence of Arsenate on Ion Shifts Caused by Iodoacetate.—Two types of experiments were done involving iodoacetate and arsenate. In the one the cells were exposed to the two agents simultaneously, in the dark, while in the other arsenate was added to alga which had been exposed to iodoacetate in the dark for 18 to 20 hours. The results are shown in Figs. 3 to 5. In Fig. 3 it is seen that arsenate alone under the conditions employed had no effect on either the potassium or sodium contents, while iodoacetate caused a marked fall in potassium and a smaller rise in sodium. The effect of arsenate given with iodoacetate was to prevent almost completely (95 per cent) the potassium loss with essentially no effect on the sodium increase.

In the experiment described by Figs. 4 and 5 the samples were maintained in 10^{-3} M iodoacetate for 20 hours. At this time 12 samples were transferred to fresh sea water (no iodoacetate) containing 5×10^{-3} M Na_2HAsO_4; six were maintained under illumination, the rest placed in the dark. As seen in Fig. 4 the arsenate in the dark, although some further loss of potassium continued, offered significant protection against the loss as compared to the samples

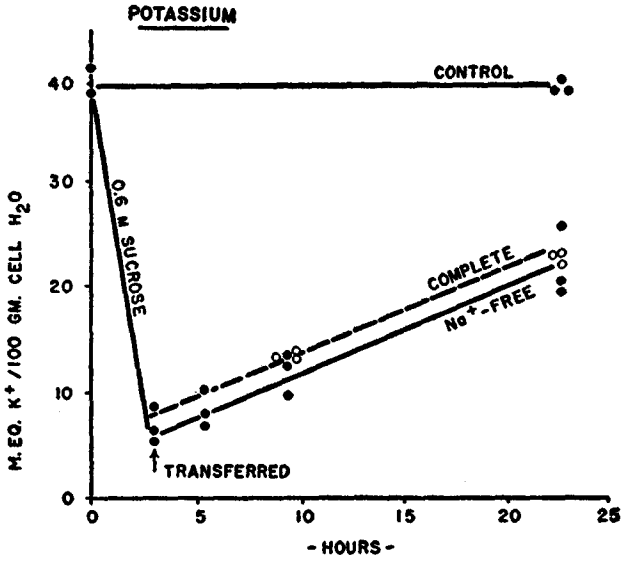


FIG. 1

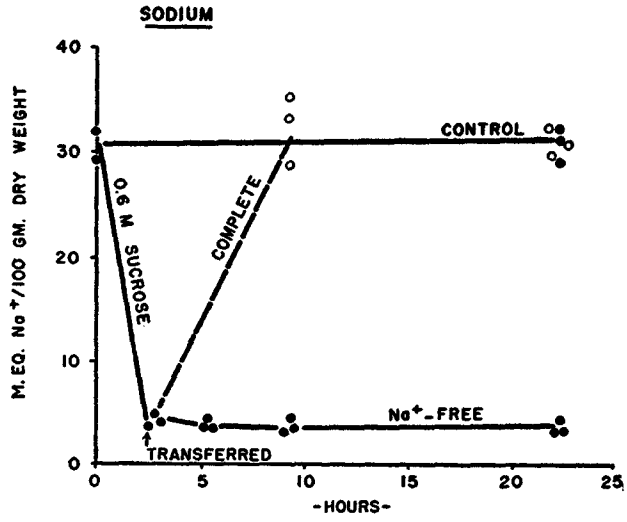


FIG. 2

FIGS. 1 and 2. The effect of external sodium on potassium accumulation. The minerals were "leached" from the samples by repeated washing in isotonic sucrose solution for 3 hours. At this time (arrow) some were transferred to complete natural sea water ("complete") and the rest to sodium-free Allen's artificial sea water ("Na-free").

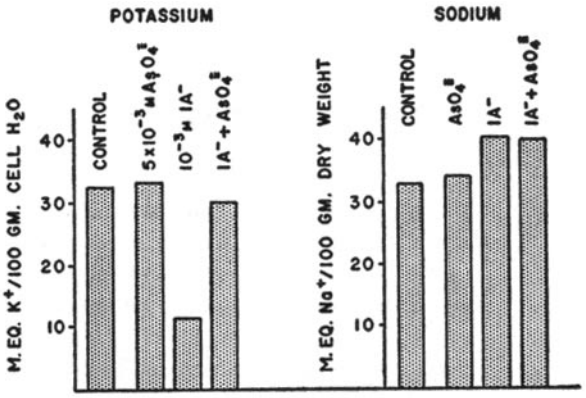


FIG. 3. Protection by arsenate against the potassium loss caused by iodoacetate in the dark. Triplicate samples were removed after 13 hours under the conditions described on the graph.

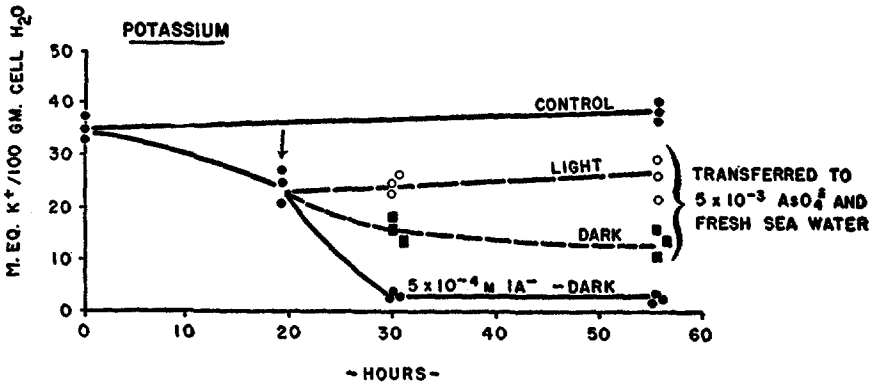


FIG. 4

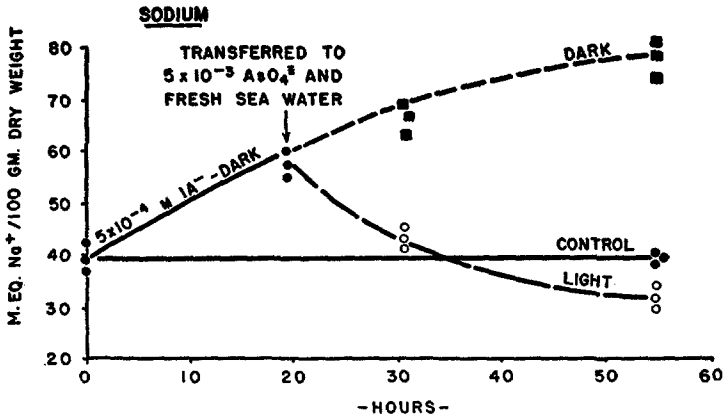


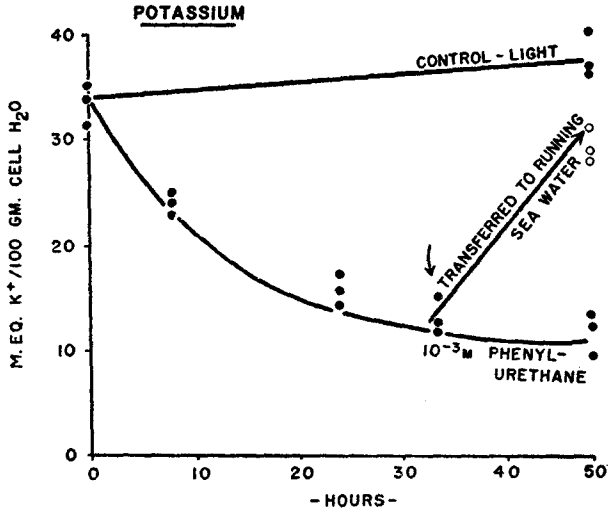
FIG. 5

FIGS. 4 and 5. The influence of arsenate after iodoacetate. Samples were maintained in the dark in 5×10^{-4} M iodoacetate. At 20 hours (arrow) some samples were transferred to 5×10^{-3} M Na_2HAsO_4 in sea water; some were illuminated (light), the others maintained in darkness.

maintained in iodoacetate in the dark throughout the experiment. In the light no further potassium loss occurred with arsenate, and a slight though significant reaccumulation of potassium took place.

It is to be noted that the sodium movements in these cells are of a distinctly different nature from those of potassium. Thus in the dark arsenate affords no protection against continued sodium influx resulting from the iodoacetate, while in the light a dramatic secretion of sodium to the control level occurs within several hours.

4. The Action of Phenylurethane and Its Reversibility.—The presence of 10^{-3} M phenylurethane in the sea water around the cells caused a marked pro-



FIGS. 6 and 7. Loss of potassium and gain of sodium resulting from 1×10^{-3} M phenylurethane. At the arrow 3 samples were transferred to running sea water.

gressive loss of potassium and gain of sodium over a 48 hour period (Figs. 6 and 7). At 36 hours, when the cellular sodium was 275 per cent of the original value and the potassium 38 per cent, some samples were transferred to running sea water to determine the reversibility of these ion shifts. At 48 hours, when these samples were removed, the cellular sodium concentration had been restored to the normal level, while 78 per cent of the potassium which had been lost was reaccumulated. In other experiments, in which potassium reaccumulation was followed over a longer period, it was observed to be complete (to the control level).

5. The Synergistic Effects of Iodoacetate and Phenylurethane.—Iodoacetate causes a loss of potassium and a gain of sodium in *Ulva* in the dark, but fails to do so in the light (25). This protection by light was interpreted as a result of

the photosynthetic production of phosphoglyceric acid (product of the reaction inhibited by iodoacetate) in the light, but not in the dark. To evaluate this interpretation iodoacetate and phenylurethane, a photosynthetic as well as a metabolic inhibitor, were added together to samples in the light. Results of a typical experiment are shown in Table IV. Thus 10^{-3} M iodoacetate has no

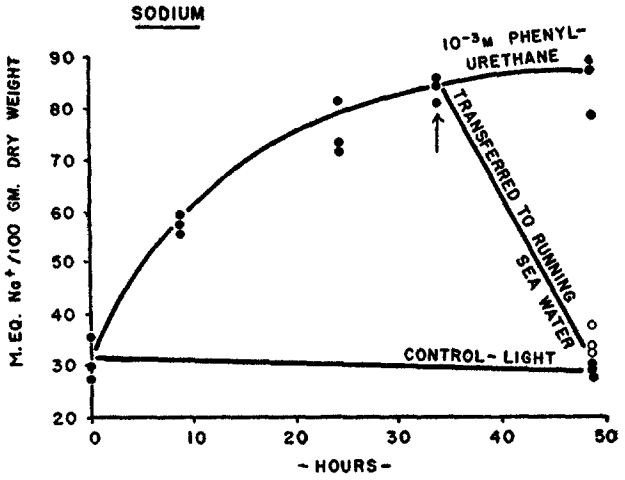


FIG. 7

TABLE IV

The Combined Effects of 10^{-3} M Iodoacetate and 10^{-3} M Phenylurethane on Potassium and Sodium Contents in Ulva

The experiment was run for 22 hours in the light.

	K	Na
	m.eq. per cent cell water	*m.eq. per cent dry weight*
Control	40.3	38.9
10^{-3} M iodoacetate	40.0	38.6
10^{-3} M phenylurethane	27.4	60.3
10^{-3} M iodoacetate plus 10^{-3} M phenylurethane	16.1	71.1

appreciable effect on cellular sodium and potassium concentrations. Phenylurethane in 10^{-3} M concentration alone causes some potassium loss and sodium gain, but iodoacetate and phenylurethane together cause considerably greater ion shifts. Figs. 8 and 9 show the acceleration in rate of potassium loss and sodium gain caused by the addition of 10^{-3} M iodoacetate at 11.5 hours to samples maintained in 10^{-3} M phenylurethane in the light. Attention is again

directed to the difference in the kinetics of potassium and sodium movements subsequent to the addition of iodoacetate: the greatest effect on potassium is

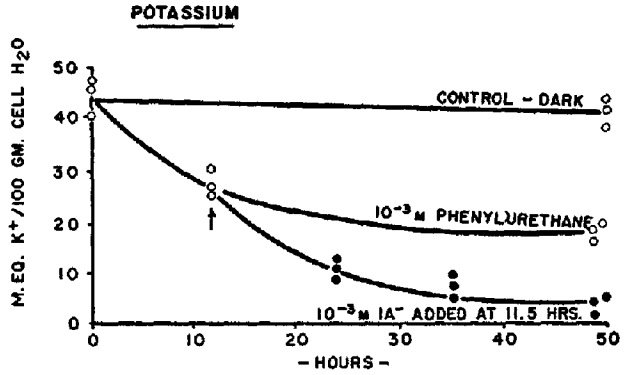


FIG. 8

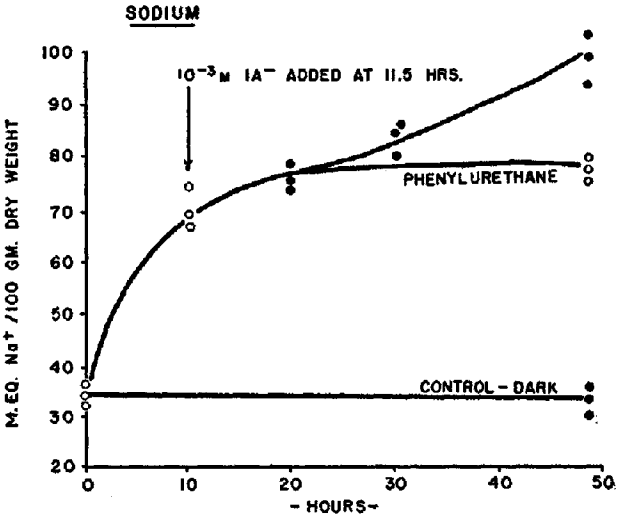


FIG. 9

FIGS. 8 and 9. The effect of 1×10^{-3} M iodoacetate on the potassium and sodium contents in *Ulva* treated with 1×10^{-3} M phenylurethane. The phenylurethane was added at zero time and the iodoacetate at 11.5 hours (arrow).

during the first 10 hours, while additional sodium increase is not marked until the last 12 hours of the time course after iodoacetate.

6. Exogenous Phosphoglycerate and Pyruvate Given with Iodoacetate.—To further evaluate the interpretation of the action of iodoacetate (in the dark) experiments were carried out in which either phosphoglycerate or pyruvate was

added to *Ulva* in the dark along with the inhibitor. These intermediates are, of course, normal components of the glycolytic cycle occurring below the level of 3-phosphoglyceraldehyde dehydrogenase, the site of action of iodoacetate. Representative data are presented in Tables V and VI.

Thus pyruvate affords the cell 100 per cent protection against the sodium increase caused by iodoacetate in the dark, while it protects against potassium loss only to a much smaller extent. Phosphoglycerate, on the other hand, offers essentially no protection against sodium increase but allows a very significant protection against potassium loss.

TABLE V

The Protective Influence of Pyruvate When Given with Iodoacetate

The experiment was run for 5 hours in the dark.

	K	Na
Control	38.6	35.6
2×10^{-3} M iodoacetate	28.9	44.7
2×10^{-3} M iodoacetate plus 50 mg. per cent pyruvate simultaneously, with 25 mg. per cent pyruvate at 4 hrs.	31.5	35.7

TABLE VI

The Protective Action of Phosphoglycerate When Given with Iodoacetate

The experiment was run for 6 hours in the dark.

	K	Na
Control	28.9	45.9
2×10^{-3} M iodoacetate	9.4	49.4
2×10^{-3} M iodoacetate plus 50 mg. per cent phosphoglycerate	19.0	49.3

7. The Action of 4-6-Dinitro-o-cresol.—Since apparently energy would be necessary to accomplish potassium accumulation and sodium secretion and since the high energy phosphate bond may be the principal type of energy currency the cell uses, the action of 4,6-dinitro-*o*-cresol (DNC) was investigated. This agent, like related substituted phenols, effectively dissociates aerobic respiration from the generation of high energy phosphate bonds (3, 18). Figs. 10 and 11 indicate the net movements of potassium and sodium in *Ulva* treated with 3.3×10^{-5} M DNC in the dark over a period of 48 hours. Again it is to be noted that the kinetics of potassium loss are distinctly different from those of sodium uptake in the presence of this "decoupling agent." It should be pointed out that these experiments were carried out in the dark not because DNC doesn't cause these ion shifts in the light (as is the case with iodoacetate) but rather because DNC is an effective inhibitor of photosynthesis. Thus the experiment is more easily controlled in the dark, since darkness alone causes some ion shifts (25).

8. Protection by ATP against Ion Shifts Caused by Iodoacetate.—Since DNC caused a loss of potassium and a gain of sodium by *Ulva*, presumably by dissociating respiration from the production of energy-rich phosphate bonds, the

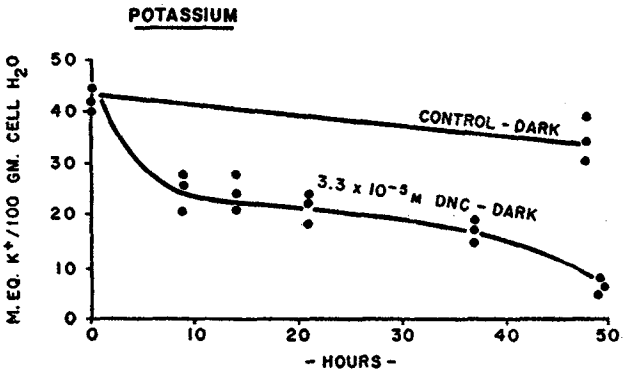


FIG. 10

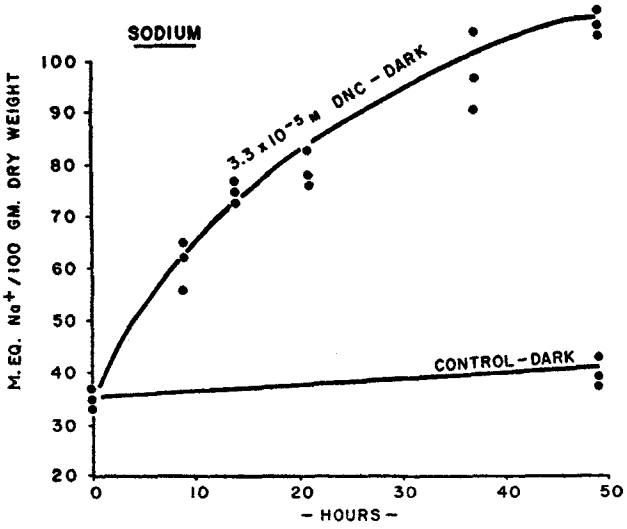


FIG. 11

FIGS. 10 and 11. The influence of 4,6-dinitro-*o*-cresol (DNC) on the potassium and sodium contents of *Ulva lactuca*.

possibility for some degree of protection by exogenous ATP against the ion shifts caused by iodoacetate was investigated. In these experiments ATP[2]

[2] The ATP used in these experiments was obtained from the Schwartz Laboratories, New York.

was added in small amounts at 2 to 3 hour intervals to samples maintained in iodoacetate in the dark. The data in Table VII represent one such experiment. Thus, over a 26 hour period the higher concentration of ATP afforded about 25 per cent protection against the potassium loss caused by iodoacetate. However, the ATP offered no protection against the sodium influx resulting from iodoacetate, but rather the iodoacetate-ATP samples showed a higher sodium content than the iodoacetate samples.

TABLE VII

The Protective Influence of ATP against Potassium Loss Observed with 10^{-3} M Iodoacetate in the Dark

The experimental period covered here is 26 hours. The ATP was added in the concentrations shown at 2 to 3 hour intervals throughout this period.

	K	Na
Control	37.9	38.7
10^{-3} M iodoacetate	17.6	48.3
10^{-3} M iodoacetate plus 3×10^{-5} M ATP	19.9	54.2
10^{-3} M iodoacetate plus 10^{-4} M ATP	23.5	58.7

DISCUSSION

It has been said for muscle (29) and red blood cells (9) that the primary activity in cation regulation is a sodium-pumping mechanism, and that potassium accumulation is passive and secondary to sodium extrusion. (Ponder (23), however, was unable to confirm the experiments of Flynn and Maizels (9) on red cells.) Ling (17), on the other hand, maintains that the distribution of sodium and potassium between muscle cells and plasma is to be accounted for on the basis of differences in coulombic forces between the cations and fixed negative binding sites within the cell; according to his hypothesis an active transport mechanism is not only unnecessary but indeed impossible.

The results of the present investigation on *Ulva*, however, can be reconciled with none of these views. Rather they demand that two independent mechanisms be postulated for regulating cellular sodium and potassium.

In the first place, as shown in Figs. 1 and 2, the presence or absence of sodium in the external medium has no effect on the rate or extent of uptake of potassium by low potassium cells. In the experiments of Steinbach (29) on muscles and of Flynn and Maizels (9) on human red cells intracellular sodium was replaced by choline and lithium ions, respectively. The ability or inability of such low potassium low sodium cells, (with choline or lithium replacing the sodium) to take up potassium was used as an index of the independence or dependence (on sodium secretion) of the potassium-accumulating mechanism. The physiological inertness of these two substances may be questioned, however, and

indeed Mudge (20) has found that substitution of lithium for extracellular sodium results in a 30 to 40 per cent depression of respiration in kidney slices. The results of our experiments, then, indicate a mechanism for potassium uptake which is independent of sodium secretion. Further, since the kinetics of potassium reaccumulation under these conditions are described by a straight line, the mechanism appears to have a limited capacity. A transport system for potassium, such as the carrier hypothesis first proposed by Osterhout (22) would seem to fit the data best. Mudge (21) has recently described experiments on rabbit kidney slices in which the turnover of potasssium as measured with K^{42} was not affected by variations in extracellular sodium concentration from 78 to 234 m. eq./liter. The interpretation of our data is consistent with his findings. Ponder (24) has suggested the possibility of two mechanisms for controlling red cell potassium and sodium.

The precise mechanism of action of the urethane series of narcotics has not yet been clearly defined. Cornman (6) has recently reviewed the effects of these narcotics with special reference to cell division inhibition. Lamanna and Campbell (16) have presented data which indicate that urethane inhibits yeast cell respiration by a surface action. But since these agents do inhibit cell respiration the action of phenylurethane was investigated here. The most striking feature of this disturbance of cation regularity is its *complete reversibility*. Heretofore, so far as the authors are aware, experiments with metabolic inhibitors have not been shown to be completely reversible as regards electrolyte disturbances; hence all such experiments have been potentially open to the criticism that the potassium loss and/or sodium gain observed with inhibitors might represent merely a general deterioration of the cell with a resulting non-specific movement of cations with their concentration gradients. The experiments presented here allow no such interpretation, for certainly some active process must be involved in the restoration of potassium and sodium to normal values after removal of the inhibitor.

Two criticisms have been suggested regarding our experiments with iodoacetate in the light and in the dark previously described (25, 26). The one is that perhaps the iodoacetate was unable to penetrate the cells in the light; the other is that perhaps light prevents iodoacetate from causing potassium loss and sodium gain by virtue of its reducing power; *i.e.*, by reducing disulfide groups to sulfhydryl groups which would then tie up the iodoacetate and prevent it from inactivating the enzyme.

We have already discussed some evidence that the inhibitor penetrates in the light (25). In addition, the experiments with iodoacetate and phenylurethane lend support to this interpretation. Phenylurethane was selected as the agent to inhibit photosynthesis in these experiments since the margin of safety between 50 per cent inhibition of photosynthesis and 50 per cent inhibition of respiration was shown to be largest of a series of urethanes studied by Warburg (30).

In actual practice, however, it was impossible to find a concentration which effectively blocked photosynthesis which did not at the same time cause some loss of potassium and gain of sodium, presumably from some inhibition of cell respiration. These ion movements cannot be the result of stopping photosynthesis, since, although darkness causes some net ion changes these occur only after a relatively long period of time and are not nearly so large as those with phenylurethane. At any rate, iodoacetate markedly enhances the potassium loss immediately after addition to samples in phenylurethane for 11.5 hours (Figs. 8 and 9) and, after several hours, increases the sodium uptake.

In these experiments an interesting feature of the potassium and sodium response to inhibitors—which has been observed repeatedly in the course of this investigation—is evident. This is that while marked potassium loss after iodoacetate begins almost immediately, appreciable net uptake of sodium is delayed several hours (25). On the other hand after phenylurethane sodium uptake begins at once. Similarly after DNC there is no delay before the sodium increase begins. These differences again indicate that separate mechanisms are operative in regulating cation distribution in this organism.

As regards the second criticism mentioned above, that light produces sulfhydryl groups which prevent iodoacetate from reacting with the enzyme, four points argue in favor of the original interpretation: (1) Illuminated iodoacetate samples always have a higher potassium and lower sodium than controls, as already discussed (25). (2) The experiments previously reported (25) in which potassium and sodium immediately began to move with their chemical gradients when samples were transferred to inhibitor-free sea water in the dark after 12 hours in the light with 2×10^{-3} M iodoacetate. Had the iodoacetate not been tied up by the enzyme but rather by other sulfhydryl groups within the cell, these ion shifts would not have been observed, since the reaction between iodoacetate and sulfhydryl groups (alkylation) is essentially irreversible (1, 8). (3) Experiments with para-chloromercuribenzoic acid, a sulfhydryl reagent which has a high affinity for succinic dehydrogenase (1), show a loss of potassium and gain of sodium occurring in the light as well as in the dark. (4) Finally, the experiments discussed above, in which iodoacetate is effective in the light in the presence of an inhibitor of photosynthesis, phenylurethane.

With regard to the arsenate experiments, it should be emphasized that in all cases these were aerobic. The action of arsenate in these experiments is presumably to be explained by a mechanism proposed by Warburg and Christian (31); that is, arsenolysis at the level of 3-phosphoglyceraldehyde dehydrogenase, with the formation of 1-arseno, 3-phosphoglyceric acid, and subsequent spontaneous decomposition of this to 3-phosphoglyceric acid. It has been observed in yeast (13) that arsenate offers complete protection against the loss of potassium and gain of sodium usually observed aerobically with iodoacetate. It was proposed, then, that arsenate allows the metabolic pattern to circum-

vent the site of iodoacetate inhibition and that the 3-phosphoglyceric acid formed is further metabolized *via* the usual Embden-Meyerhof scheme.

Several features of the experiment described by Fig. 3 should be pointed out. First, it is apparent that, so far as the potassium-regulating mechanism is concerned, 3-phosphoglyceraldehyde dehydrogenase is the only enzyme blocked by iodoacetate. Since arsenate is effective when given after iodoacetate (Figs. 4 and 5), the possibility that arsenate acts by preventing the iodoacetate from attacking the enzyme in the first place is not tenable. The most plausible explanation, then, is the one outlined above. The fact that arsenate does not protect against the sodium increase with iodoacetate suggests that the iodoacetate may be acting on another sulfhydryl enzyme—one which would be essential for an effective sodium pump in the dark, and not necessary for potassium accumulation—at a site where this special relief by arsenate is not possible.

Such an interpretation is consistent with the data shown in Figs. 4 and 5. Again the arsenate relieves the potassium loss resulting from iodoacetate. It is important to note that this relief is almost certainly in no part due to a washing out of the inhibitor since profound potassium loss occurs for many hours after transfer from iodoacetate to light and running sea water as already described (25). Furthermore, as mentioned above, iodoacetate inhibition is essentially irreversible. The protection in the dark after transfer to arsenate would be, according to the hypothesis, a measure of arsenolysis of cellular carbohydrate reserves. The additional protection in the light (to the extent of an actual slight reaccumulation) would be the result of additional phosphoglycerate formed in the cells during photosynthesis (2, 10, 25). It should be noted that these samples in the light were actively photosynthesizing, as judged by the formation of gas bubbles on the under surfaces of the samples.

In the complementary study of sodium movements in these same samples quite different behavior was observed. As in the experiment when arsenate and iodoacetate were given simultaneously, the arsenate added after iodoacetate (Fig. 5) offered no protection in the dark. In the light, on the other hand, a fairly rapid active resecretion of sodium occurred so that it reached the control level in several hours. A similar resecretion of sodium was observed when samples were transferred to light and running sea water, without arsenate (25). The presence of arsenate, then, as regards the sodium secretion in the light appears to be quite incidental. The fact that sodium is not resecreted in the dark is further evidence that the procedures employed involved no "washing out" of the inhibitor.

It is most probable in the minds of the authors that this effect of light on the sodium pump is quite apart from the generation of carbohydrate intermediates through photosynthesis (which appears to be the chief effect of light as regards the potassium-pumping mechanism), since under these con-

ditions light and darkness have a much greater effect on sodium than on potassium—the difference between no protection and complete resecretion. From the existing data it is not possible to postulate the exact nature of this effect, but it might be suggested, since the reducing properties of light are well known, that it is associated with a redox mechanism of some sort. In this connection it should be mentioned that Conway (4, 5) has postulated a redox pump for potassium in the yeast cell and Lundegardh (19) a redox mechanism for anion absorption in plant roots.

These experiments with arsenate serve to indicate that iodoacetate acts on the potassium mechanism in the same manner as already proposed (7, 11,

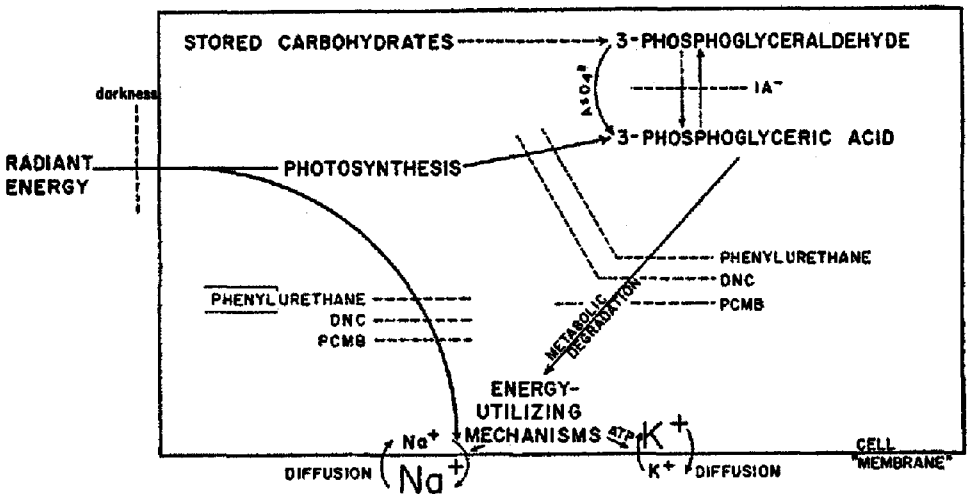


FIG. 12. Diagrammatic representation of the interpretation of the data presented above.

12, 25, 28) (*i.e.*, inhibition of 3-phosphoglyceraldehyde dehydrogenase with a resulting stoppage of energy-yielding reactions in the cell), that it acts on the sodium pump in a different manner, and that light, perhaps through its reducing power, has a primary action on the sodium-pumping mechanism (Fig. 12).

It might be suggested from the experiments with exogenous pyruvate and phosphoglycerate that the two pumps are energetically coupled to metabolism at different points in the glycolytic and/or respiratory cycles. Indeed, such a possibility is in line with some of the other experimental results presented here. But to define precisely where these points of coupling are (and there is no good reason at present to believe that there may not be more than one for each pump) is not possible on the basis of the existing data. We might say that energy for the potassium pump seems to come from below

the level of phosphoglycerate, while the sodium pump seems to be coupled to the metabolism of pyruvate or a product of its metabolic degradation.

The difficulties described below for experiments with exogenous ATP may well apply to experiments with any exogenous metabolite. Because of the metabolic complexities of all biological systems, any attempt to localize precisely the sites of coupling between ion transports and metabolism is beset with difficulties. Nonetheless, these experiments do show that while added phosphoglycerate is more effective in protecting against potassium loss with iodoacetate, pyruvate effectively maintains the sodium-pumping mechanism in the presence of the inhibitor.

The experiments with ATP, while they did not show a complete protection by ATP against the potassium disturbances caused by iodoacetate, did nevertheless demonstrate a significant protection for potassium. It would probably be an unjustified extrapolation to conclude on the basis of these few experiments that the sodium-pumping mechanism is not energized at one stage or another by ATP. Certainly the decoupling agent employed, DNC, caused a marked rapid increase in cellular sodium.

There are certain inherent difficulties in experiments with exogenous ATP. One problem is the penetration of this relatively large molecule into the cell, and to overcome this difficulty a high concentration in the external medium presumably would be advantageous. But on the other hand, too high an outside concentration of a normally occurring intracellular component might have detrimental effects on the cell. Thus the ideal situation probably represents a balance between these two opposing factors. In the experiments described here a compromise was attempted by adding the ATP in small amounts at short intervals. Since the potassium- and sodium-regulating mechanisms responded differently in the other experiments it should not be surprising that they behave differently here.

SUMMARY AND CONCLUSIONS

1. The methods employed in these and preceding (25–27) studies were shown to allow analysis of true cellular sodium and potassium concentrations.

2. The rate of reaccumulation of potassium by potassium-deficient cells is independent of the presence or absence of sodium in the external medium.

3. Phenylurethane (10^{-3} M), a photosynthetic and metabolic inhibitor, causes a marked progressive loss of potassium and gain of sodium, both of which changes are completely reversible on transferring the samples to running sea water.

4. Iodoacetate, while not effective in causing potassium and sodium shifts in the light, effects a loss of potassium and a gain of sodium in the light in the presence of phenylurethane.

5. Arsenate (5×10^{-3} M) completely protects *Ulva* against the potassium

loss usually observed with iodoacetate in the dark while it affords no protection against the sodium influx under the same conditions. Arsenate given after 18 to 20 hours in iodoacetate gives significant protection against potassium loss in the dark, and allows a slight net reaccumulation of potassium in the light. Arsenate in the dark after iodoacetate affords no protection against the sodium uptake caused by iodoacetate in the dark, while in the light under the same conditions sodium is rapidly secreted to the control level within a few hours. This resecretion of sodium is thought to be primarily an effect of light, the presence of arsenate being incidental.

6. The "decoupling agent" 4,6-dinitro-*o*-cresol causes a marked progressive increase in cellular sodium and a drop in cellular potassium, though the kinetics of these two movements are distinctly different from each other.

7. Pyruvate (50 mg. per cent) given with iodoacetate (2×10^{-3} M) for 5 hours in the dark completely prevents the sodium increase caused by iodoacetate, while affording less protection against the potassium loss. Phosphoglycerate, on the other hand, offers more protection against potassium loss, and essentially none against the sodium gain.

8. ATP added in small amounts at short intervals to samples maintained in 10^{-3} M iodoacetate in the dark affords significant protection against the potassium loss observed in iodoacetate. Cellular sodium is somewhat higher in the ATP-iodoacetate samples than in the iodoacetate samples.

9. In the discussion of the data presented two major points are emphasized: (1) the close correlation between cellular metabolism and normal cation control; (2) two mechanisms must be operative in cation regulation in this organism: one for moving potassium inwards and the other for transporting sodium outwards. These mechanisms are independent of each other.

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