

METABOLIC FACTORS INFLUENCING THE SODIUM AND POTASSIUM DISTRIBUTION IN *ULVA LACTUCA**

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INTRODUCTION

The uneven distribution of sodium and potassium between living cells and their environments has long attracted the interest of a great number of physiologists. Within the past 10 to 15 years particularly, a wealth of data has been accumulated in an attempt to elucidate the mechanisms by which the cell is able to accumulate potassium and at least partially exclude sodium. The notion that permeability *per se* to these cations alone could account for this uneven distribution has been shown to be inadequate through the use of Na^{24} and K^{42} as tracers. Such investigations have revealed that a large fraction if not all the potassium and sodium in those cells studied is constantly exchanging with environmental cations (14-16, 21). Thus potassium and sodium ions are constantly diffusing across the cell membrane with their concentration gradients, outward and inward respectively. But the ionic composition of a particular type of cell is relatively constant; hence there must be cellular mechanisms which compensate for this continual flux of ions across the cell surface. Carbohydrate metabolism has been found to be of great importance in the normal maintenance of sodium and potassium balance in a large variety of cells (3, 4, 8, 12, 13, 20). The studies described below are directed toward a further investigation of the metabolic factors involved in cation regulation in the cells of the green alga *Ulva lactuca*.

This marine organism, like most cells living in a high sodium low potassium environment, normally accumulates potassium and partially excludes sodium. Consisting of large membranous fronds two cell layers in thickness, thus presenting a large surface area for interchange with the environment, this alga is particularly well suited for investigations of this nature. The green plant cell offers a further advantage in studying metabolic factors in cation regulation: the ease of turning on or shutting off a normal metabolic process, photosynthesis, by merely illuminating the cells or eliminating light from their environment. Thus, important carbohydrate intermediates are made available

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to the cell by photosynthetic reduction of carbon dioxide, either for assimilation into stored carbohydrates or for metabolic degradation through the glycolytic cycle.

In the present paper, the effects of illumination and of the glycolytic inhibitor, monoiodoacetate, on the sodium and potassium balance in this alga are reported, as well as the effects of light and temperature on the rate of potassium ion exchange between cell and environment.

Materials and Methods

The material used in these experiments was collected from the Eel Pond at Woods Hole and was conditioned in the laboratory before use under incandescent illumination in running sea water. Samples 4 to 5 inches square were cut from the same frond and placed in large finger bowls containing usually 1 liter of sea water. Illumination was supplied by a 100 watt incandescent bulb placed at a distance of 1 foot, while darkness was accomplished by covering the vessels with a few layers of black cloth. Except when indicated the experiments were performed at the temperature of running sea water (20–21°C.). In all experiments in which the inhibitor was used it was added as the sodium salt, the pH being adjusted to 7.3 with sodium hydroxide before addition to the sea water.

Samples, usually removed in triplicate, were rinsed for 1 minute in isotonic sucrose solution to remove the adhering sea water and consistently blotted in absorbent tissue to remove the sucrose. Wet weights were taken immediately, the material dried from 12 hours at 110°C., and dry weights determined. Tissue water was calculated by difference. The dried material was ground in a mortar and extracted for a few hours in 50.0 ml. of 10 per cent trichloroacetic acid. This method of extraction was compared with wet ashing technics, and in all cases found to liberate all the sodium and potassium from the alga. After filtration, the extract was analyzed for sodium and potassium by flame photometry using the Beckman spectrophotometer. The extract was also analyzed for inorganic phosphate by the method of Fiske and Subbarow (5). Methods for the experiments involving radioactive potassium have been described elsewhere (19). In the treatment of the data potassium is expressed on a tissue water basis and sodium in terms of dry weight.

RESULTS

The Influence of Light and Dark.—In these experiments two groups of algae were used: the one was illuminated throughout the experiment, while the other was illuminated after a period in the dark. Samples were removed at intervals and analyzed for sodium and potassium; representative data are presented in Figs. 1 and 2. After a latency of approximately 30 hours a progressive loss of potassium occurs in the dark, amounting to 20 per cent of the original potassium content of the plant in 82 hours. Upon illumination there occurs an abrupt accumulation of potassium which, for a short time, remains considerably above the level of the illuminated controls, then returns to this concentration level (Fig. 1).

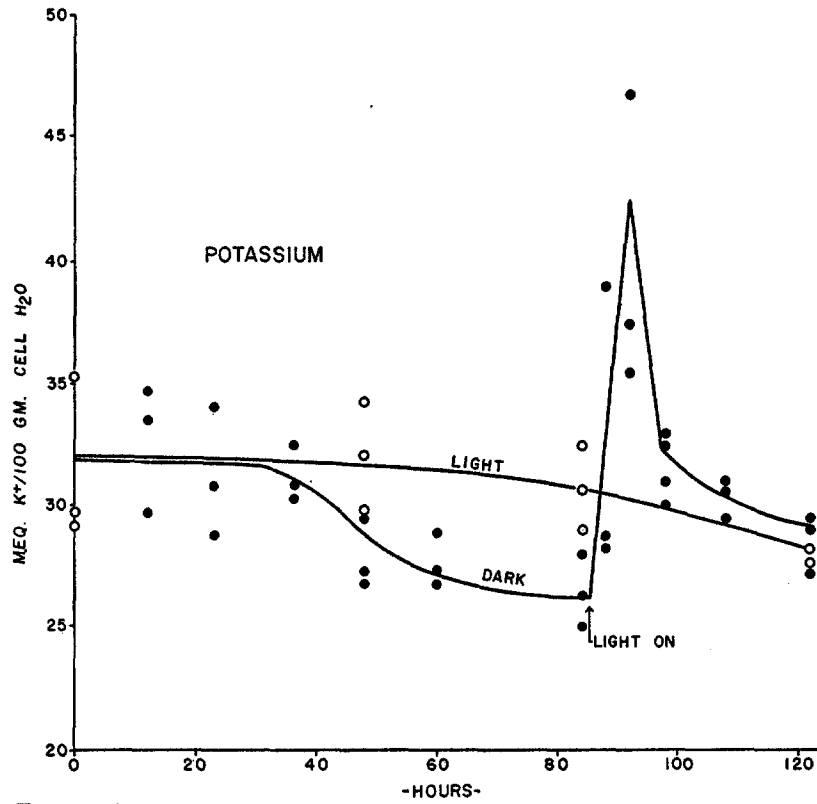


FIG. 1. The influence of illumination and darkness on the potassium content of *Ulva lactuca*.

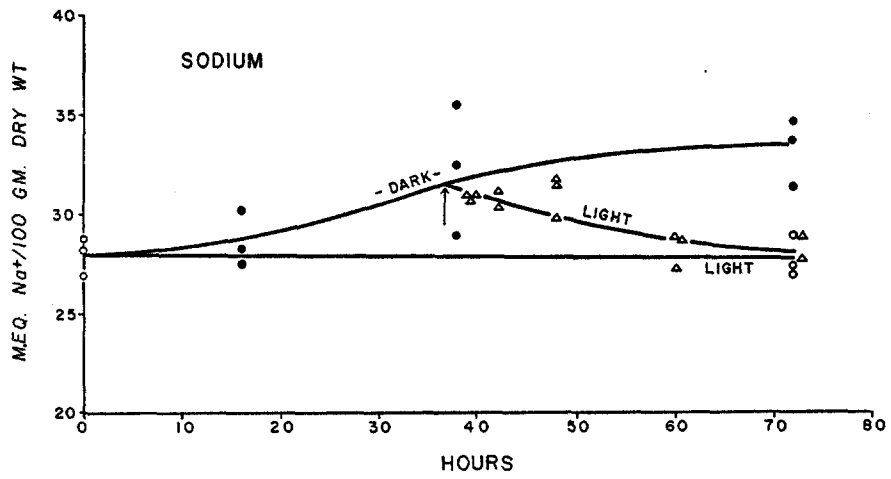


FIG. 2. The influence of illumination and darkness on the sodium content of *Ulva lactuca*.

The sodium content of the alga gradually increases over the course of the experiment, 72 hours, in the dark. On illumination the sodium content is reduced in a period of 20 hours to the level of the controls, while that of the illuminated controls remains essentially constant (Fig. 2).

The Influence of Iodoacetate in the Light and Dark.—The presence of the inhibitor in a concentration of 0.001 M results in a marked loss of potassium from the plants over a period of 24 hours in the dark, beginning shortly after addition of the inhibitor. Control samples taken at the beginning and end of this period were essentially constant in potassium content (Fig. 3). In the presence

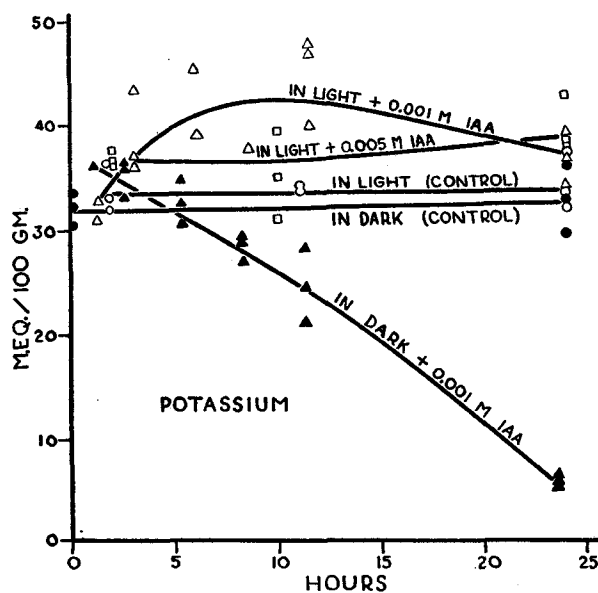


FIG. 3. The influence of iodoacetate on the potassium content of *Ulva lactuca* in the light and in the dark. Potassium is expressed in terms of tissue water.

of light the inhibitor is completely ineffective in causing the loss of potassium. Rather, the potassium content of the experimental plants temporarily increases over that of the controls.

To evaluate further the influence of light on the prevention of the iodoacetate effect, the concentration of the inhibitor was raised to 0.005 M. Again light prevented the loss of potassium.

Concomitant with the potassium loss caused by the 0.001 M iodoacetate in the dark the sodium increases over that of the controls, although the condition of darkness alone is sufficient to cause some sodium increase. Illumination again prevents this action of the inhibitor, and sodium is actually somewhat reduced compared to the controls (Fig. 4). Light also prevents an increase in sodium when the inhibitor concentration is raised to 0.005 M.

In the presence of 0.001 M iodoacetate in the dark there is a marked loss of inorganic phosphate, more or less paralleling the loss of potassium, over a period of 24 hours. The phosphate of the controls in both light and dark remained constant throughout the experiments and the inhibitor was ineffective as regards phosphate loss in the illuminated samples, even at a concentration of 0.005 M (Fig. 5).

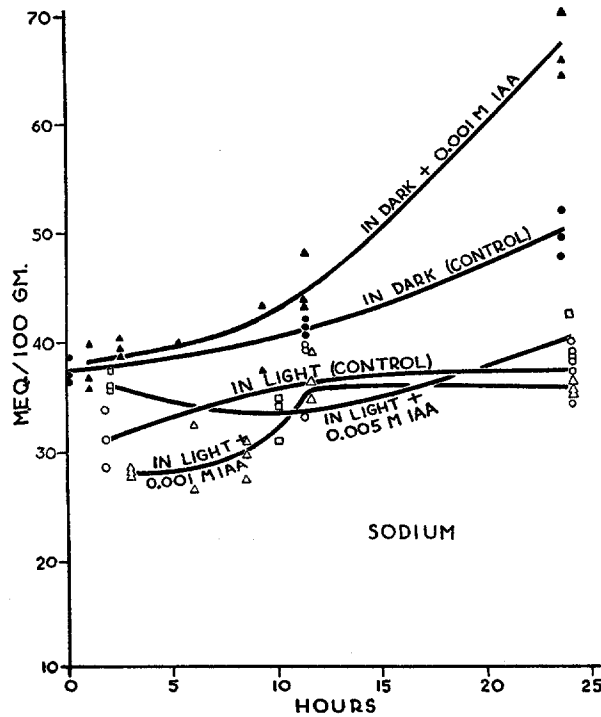


FIG. 4. The influence of iodoacetate on the sodium content of *Ulva lactuca* in the light and in the dark. Sodium is expressed in terms of dry weight.

In the interpretation of the prevention by light of the iodoacetate effect it is essential to know whether the cell when illuminated is permeable to the inhibitor. In order to examine this problem the inhibitor was added to samples in the light and maintained for 12 hours. At this time samples were transferred to sea water without the inhibitor and placed in the dark. Typical results are presented in Figs. 6 and 7, indicating a marked loss of potassium and gain of sodium immediately after transfer to the inhibitor-free medium in the dark.

Washing-Out of the Iodoacetate Effect.—It is of interest to determine whether or not the ion shifts caused by iodoacetate in the dark are permanent or whether, after removal of the inhibitor from the environment, the cell can tend to restore the normal levels of sodium and potassium. To elucidate this point, samples of

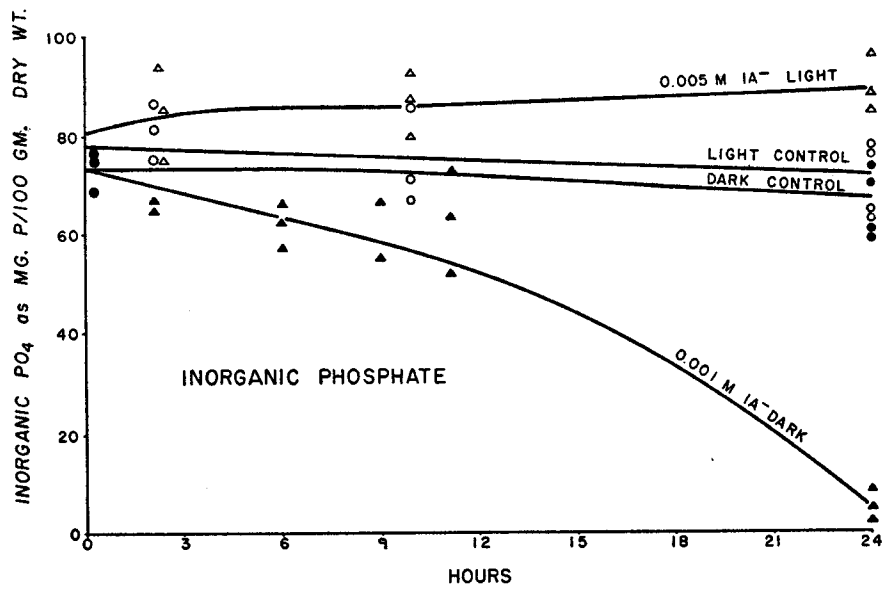


FIG. 5. The influence of iodoacetate on the inorganic phosphate content of *Ulva lactuca* in the light and in the dark.

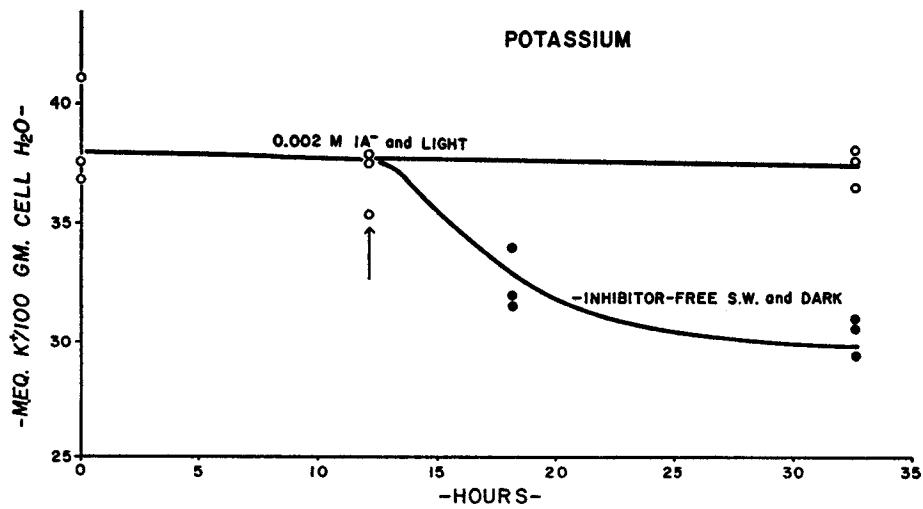


FIG. 6. The loss of potassium in the dark from *Ulva* previously treated with 0.002 M iodoacetate in the light. The arrow indicates time of transfer of six samples to inhibitor-free sea water and darkness.

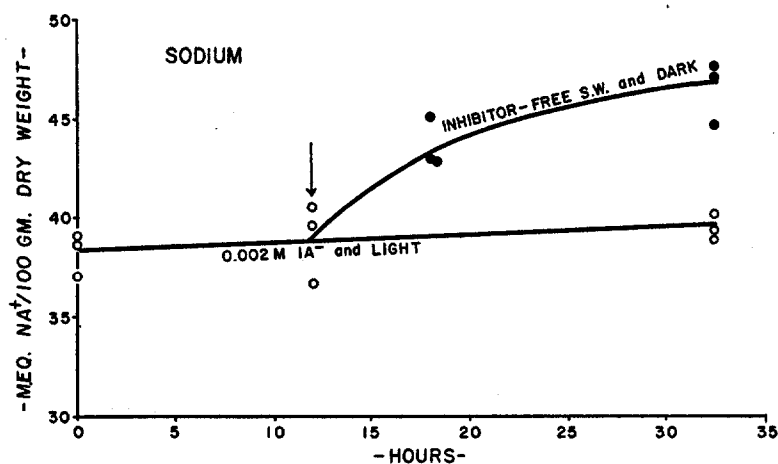


FIG. 7. The gain of sodium in the dark from *Ulva* previously treated with 0.002 M iodoacetate in the light. The arrow indicates the time of transfer of six samples to inhibitor-free sea water and darkness.

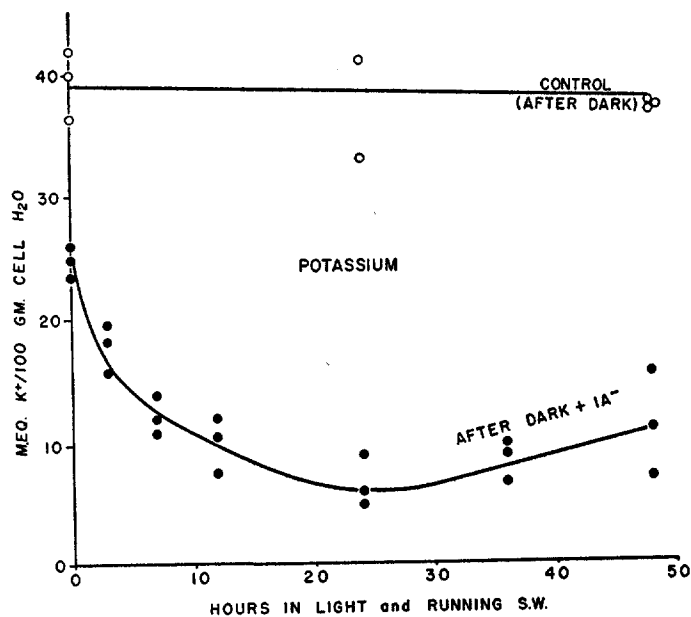


FIG. 8. The influence of light and running sea water on the potassium content of *Ulva* previously maintained for 16 hours in sea water containing 0.001 M iodoacetate in the dark. Zero time on the graph represents time of transfer to running sea water and light. Control samples were illuminated after 16 hours in the dark.

Ulva were maintained in 0.001 M iodoacetate in the dark for 16 hours, then placed in running sea water (no inhibitor) in the light.

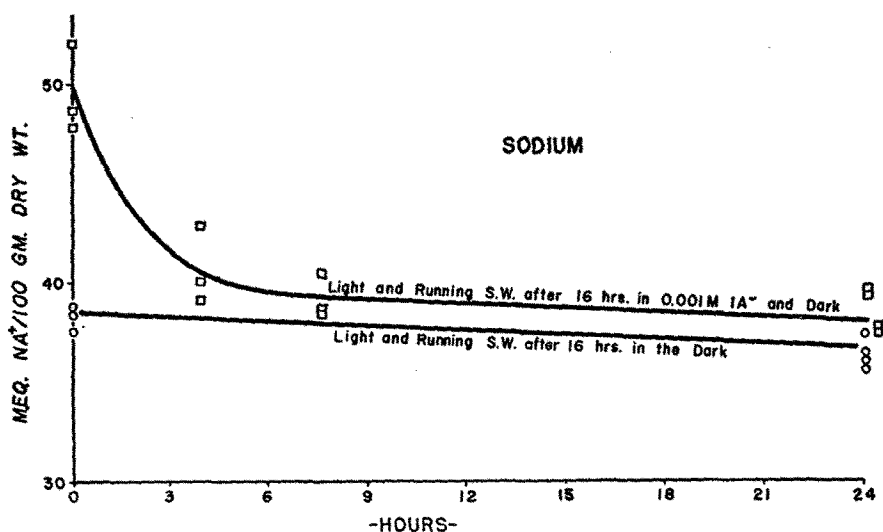


FIG. 9. The influence of light and running sea water on the sodium content of *Ulva* previously maintained for 16 hours in sea water containing 0.001 M iodoacetate in the dark. Zero time on the graph represents time of transfer to running sea water and light. Control samples were illuminated after 16 hours in the dark.

TABLE I

The Effect of Illumination and Temperature on the Rate of Potassium Ion Exchange in *Ulva lactuca*

Conditions	Time for 75 per cent exchange
	hrs.
Light 30°C.	0.4
Dark 30°C.	1.8
Light 20°C.	2.2
Dark 20°C.	3.2

After 16 hours in the iodoacetate, approximately 30 per cent of the potassium is lost, and a progressive loss amounting to an additional 50 per cent of the original concentration, continues in running sea water for about 25 hours. At this point a gradual but definite and reproducible reaccumulation begins, the full extent of which could not be measured because of termination of the experiment (Fig. 8).

The sodium concentration after 16 hours in 0.001 M iodoacetate in the dark is

increased to 30 per cent above the control level. In contrast to the potassium, the net movement of sodium against its concentration gradient begins immediately on placing the cells in light and running sea water, and restoration of the normal level reaches completion in about 4 hours (Fig. 9).

These experiments indicate that although the iodoacetate causes a marked loss of potassium and gain of sodium, this effect of iodoacetate can be "washed out" of the cell in running sea water in the presence of light. A net transport of the ions against their concentration gradients takes place. Attention is directed to the differential behavior of the alga as regards sodium and potassium movements in these experiments; *i.e.*, although potassium is progressively lost for 25 hours sodium is excreted to the normal level within 4 hours.

The Influence of Light and Temperature on the Exchange Rate of Potassium Ion.—In these experiments the rate of exchange of tissue with environmental potassium ion was measured using K^{42} as a tracer; samples were maintained in the light and dark at temperatures of 20 and 30°C. The rate of exchange, as indicated by the time for the specific activities of the tissue to reach 75 per cent of that of the sea water (75 per cent exchange), was markedly increased in the light and at the higher temperature (Table I).

DISCUSSION

An examination of a large body of unpublished data indicates a close correlation between potassium content and calculated tissue water in *Ulva lactuca*, while sodium content is most consistent on a dry weight basis. For these reasons the potassium and sodium data are expressed as indicated, although essentially the same results appear when the data for each ion are expressed on either basis. These observations may reflect a largely ionized cellular potassium and a partially bound sodium. Confirmatory evidence for the ionized state of potassium has recently been obtained in our laboratory by experiments with K^{42} which indicate that all the potassium in the cell is readily exchangeable (19).

It should be pointed out that the sucrose rinse probably does not remove all the sea water from the intercellular spaces in this organism. Therefore the true cellular potassium concentration is probably higher and the sodium concentration lower than the figures reported.

The variation in ion contents in these experiments cannot be a reflection of changing cell volume, since although the per cent dry weight varied between the limits of 25 and 33 there was no trend correlating with any variation in ion content. The normal variation in potassium content encountered in samples from different fronds or in different samples from the same frond, probably a reflection of varying physiological conditions within the cells, is usually accompanied by a reciprocal sodium variation.

The data presented in this paper indicate an essential role of cellular metabolism, and here particularly photosynthesis and carbohydrate degradation, in

the maintenance of the normal distribution of potassium and sodium in the cells of *Ulva lactuca*. The condition of darkness alone is sufficient to cause a loss of potassium and gain of sodium, presumably because the cell is using up its carbohydrate reserves or intermediates, normally generated by photosynthesis. The reexcretion of sodium and the reaccumulation of potassium when light is admitted to material previously maintained in the dark lend further support to this interpretation, for under conditions of illumination the important glycolytic intermediate, phosphoglyceric acid, is made available to cellular metabolism by photosynthesis (2, 6). The influence of light on the uptake of electrolytes in algae has been observed by Hoagland and Davis (9) and Jacques and Osterhout (10). The latter authors interpret the uptake of potassium on more intense illumination in terms of an increase in pH in the medium caused by photosynthesis.

The selective inhibitory action of iodoacetate on the glycolytic enzyme phosphoglyceraldehyde dehydrogenase, has been studied by Green, Needham, and Dewan (7). In causing the cation shifts described here the inhibitor may act by preventing the normal release of energy required for ion transports, which under normal conditions would compensate for the continual flow of the ions with their concentration gradients across the cell surface. Similar observations have been made on the potassium and sodium content of other cells to which this agent was added (3, 8, 20). The results of the experiments in which the iodoacetate effect is prevented by illumination may be interpreted within the same hypothesis, for apparently photosynthesis is supplying such intermediates as phosphoglyceric acid, the degradation of which may be associated with the energetics of ion transport. Such an interpretation of these experiments is predicated on the assumption that the inhibitor penetrates the cell in the presence of light. That such is the case is demonstrated above, since *Ulva*, treated with iodoacetate in the light, when placed in the dark with the inhibitor absent from the external medium, promptly shows the characteristic loss of potassium and gain of sodium, while those samples maintained in the light with the inhibitor maintain sodium and potassium balance (Figs. 6 and 7). It should be pointed out that the magnitudes of these ion shifts are probably reduced here by some washing out of the inhibitor from the cells into the inhibitor-free medium.

The observation has been made repeatedly throughout these investigations that iodoacetate in the presence of light serves to temporarily increase the potassium content above the level in the control samples and to decrease the normal sodium content. A greater than normal glycolytic degradation of the phosphoglycerate formed in photosynthesis might well be expected when iodoacetate is present, since apparently iodoacetate prevents the synthesis of carbohydrate reserves from this intermediate (1) (Fig. 10). The increased potassium and decreased sodium under these conditions may be interpreted as

indicating increased activity of transport mechanisms; the potassium increase might also be explained on the basis of an increased organic anion production (18). Consistent with these explanations is the marked influence of light in causing an abrupt increase in potassium, temporarily rising above the normal level, in *Ulva* previously maintained in the dark (Fig. 1). This may be due to an increased efficiency of photosynthesis with a resulting high level of phosphoglycerate, which would be utilized as described above.

Data have been presented (Figs. 8 and 9) indicating that the influence of iodoacetate on sodium and potassium in the tissue in the dark may be removed under conditions of light and running sea water. It would be impossible to assume any situation in which a selective permeability of the cell membrane could in itself account for the cation movements observed in these experiments. The extrusion of the sodium can be explained only on the basis of an active

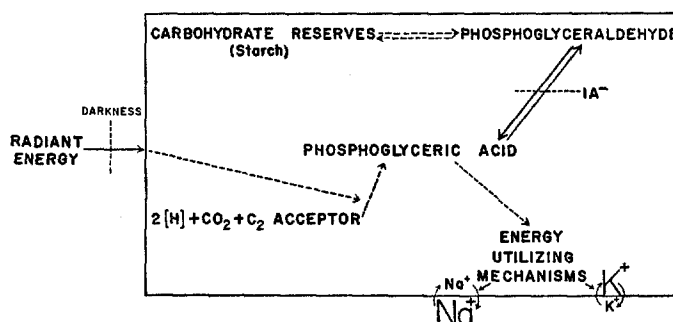


Fig. 10. Diagrammatic representation of the interpretation of the data presented above.

secretion of sodium ion out of the cell. The gradual reaccumulation of potassium might be explained either on the basis of a separate inward transport mechanism for potassium; or perhaps a passive absorption as the result of metabolic production of organic anions within the cell. An active transport would require metabolic coupling with energy sources; these data further suggest that the source of the energy for the ion transport(s) is the degradation of phosphoglyceric acid or a product of its metabolism. In the dark in the presence of the inhibitor these cells are deprived of their normal sources of phosphoglyceric acid, and according to the hypothesis this would account for the ion shifts observed. When the cells are illuminated and transferred to running sea water without inhibitor, the observed ion transport(s) are presumably due to the degradation of phosphoglyceric acid formed from either one or both of two sources. On the one hand the inhibitor could diffuse out of the cell into the running sea water, thus relieving glycolytic inhibition and allowing the formation of the intermediate. On the other hand, in the presence of light phospho-

glyceric acid could be formed, since, according to Calvin and Benson (2) and Gaffron, Fager, and Rosenberg (6), this is an important intermediate in photosynthesis. Thus it would appear that the degradation of phosphoglyceric acid (from whatever source) or of one of its breakdown products is essential for ion transport(s). Preliminary experiments carried on in this laboratory indicate that the addition of phosphoglycerate to the medium affords the cell some protection from the ion shifts caused by iodoacetate.

The disparity in the kinetics of the sodium extrusion and the potassium loss in these experiments (Figs. 8 and 9) suggests at least partially independent mechanisms for the movements of these cations. Since potassium continues to be lost for 25 hours while sodium is extruded to the normal level within 4 hours, the hypothesis proposed by Ling (11) for the selective distribution of sodium and potassium between muscle cells and plasma cannot possibly apply to this organism. Such an hypothesis implies a passive elimination of sodium from the cell secondary to potassium accumulation. Further it is highly improbable that the potassium reaccumulation here could be secondary to active sodium extrusion, as proposed by Maizels (12) for the red blood cell and Steinbach (17) for muscle, since a period of 20 hours intervenes between the termination of net sodium secretion and the beginning of net potassium reaccumulation.

The increased exchange rate of potassium ion at the higher temperature or in the light (Table I) may be due to a more rapid uptake of potassium by the cell, correlated with increased metabolic activity, and secondarily a more rapid loss of potassium. This would maintain a steady state with no net change in the potassium concentration of the cell. An alternative hypothesis might be a primary increase in the outward permeability of potassium ion, compensated for by a more rapid inward transport of potassium ion. In either case a more active metabolism resulting from either illumination or the higher temperature would appear to be involved in the increased exchange rate and in the maintenance of the dynamic equilibrium.

SUMMARY AND CONCLUSIONS

1. Methods for the use of the marine green alga, *Ulva lactuca*, in studies on electrolyte metabolism are described.
2. The effect of illumination and iodoacetate on the potassium and sodium content, as well as the influence of light and running sea water on the iodoacetate effect was investigated. The rate of exchange of cellular potassium ion for K^{42} under conditions of light and dark at 20 and 30°C. was studied.
3. *Ulva* maintained in the dark for long periods loses some potassium and gains sodium, both effects being reversed upon illumination. The presence of 0.001 M iodoacetate in the dark causes a marked progressive loss of potassium and gain of sodium, phenomena which do not occur when the alga is illuminated. Evidence for the penetration of the inhibitor into the cell in the presence

of light is presented. The iodoacetate effect on potassium and sodium content, once established, can be "washed out" of the alga when the plant is placed in light and running sea water without the inhibitor. Illumination and increased temperature each favor a more rapid exchange of tissue for environmental potassium ion.

4. In the interpretation of these findings it is emphasized that metabolic work, perhaps in the form of ion transports, must be done by the cell to compensate for the continual flow of potassium ion and sodium ion with their respective concentration gradients and thus maintain homeostasis within the cell. Evidence is presented which indicates separate mechanisms for the distribution of sodium and potassium in this organism. It is further suggested that the degradation of phosphoglyceric acid, an important glycolytic and photosynthetic intermediate, or one of the products of its metabolism supplied the energy for these ion transports(s). The role of permeability *per se* is considered.

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