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Kinetics and Energetics of Light-enhanced Potassium Absorption by Corn Leaf Tissue

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Abstract. **The effect of illumination on the absorption of K^+ by leaf tissue of *Zea mays* was investigated. The rate of K^+ absorption was enhanced by exposure of slices of corn leaf tissue to light, even of relatively low intensities. Potassium was transported inward, with virtually no efflux of previously accumulated K^+. The evidence indicates that the transport mechanism for absorption of K^+ is the same in the light as in the dark, but that the source of energy for absorption of K^+ is different in the light from that in the dark. Various antimetabolites were used to establish that the energy utilized for active ion transport in the light came partly from ATP supplied by cyclic photophosphorylation. Expenditure of ATP was required in the dark too, but this ATP was formed by oxidative phosphorylation. Establishing the ultimate source of energy for active ion uptake by higher plants might be facilitated by demonstration of an ion-transport process that is not linked directly with the transfer of electrons in the mitochondrial cytochrome chain.**

The principal organs which accumulate ions in higher plants are roots, but leaf cells also accumulate nutrient ions. These ions are absorbed from a dilute solution surrounding these cells. This solution is supplied by the xylem (17).

Investigations of cellular ion absorption by leaf tissue of terrestrial plants have not been feasible until recently. The ionic environments of leaf cells are variable because of differences in leaf temperatures, unequal ion distribution in leaf tissue due to the length of the diffusion path that the ions must traverse, and changes in transpiration rates. However, these variables have been eliminated by a technique developed by Smith and Epstein (35). Their studies with narrow leaf slices demonstrated that the absorption of ions proceeded through the cut edges and not the surface of the leaf lamina. This would seem to preclude the involvement of stomates in the absorption of ions when using the leaf slice technique. By the use of thin slices of leaves, leaf cells were exposed to the external solution with no diffusion limitations on ion movement. This technique made possible comprehensive studies of the kinetics of ion absorption in leaf tissue of corn, *Zea mays* (36), and the mangrove, *Avicennia marina* (31). Those studies form a basis for further investigations of the effect of light on ion absorption by green tissue. Some earlier work on the effect of light on ion transport by green tissue has been reviewed in a recent paper (29).

Green tissue contains 2 possible sources of energy for metabolic activities such as ion transport. One source is oxidative phosphorylation. Respiratory substrates are utilized in the mitochondria to form high-energy phosphate compounds. These compounds are then metabolized to transport ions against concentration gradients. The links between cellular ion transport and respiratory processes have been reviewed by Epstein (5) and Sutcliffe (38). The other site of energy production in green tissue is the chloroplasts. In chloroplasts ATP is produced by photosynthetic reactions (1, 40).

Understanding of the energetics involved in the absorption of ions in the light has grown in the last few years from detailed studies of the operation of chloroplasts, particularly their participation in ion transport (2, 14, 27, 28, 33, 37). Papers by Arnon *et al.* (1) and Vernon and Avron (40) give comprehensive discussions of the energetics of these photosynthetic processes.

MacRobbie (22, 23) investigated the effect of light on the absorption of K^+ and Cl^- by the freshwater alga *Nitella translucens*. She concluded that K^+ absorption utilized energy from ATP formed by cyclic photophosphorylation whereas Cl^- transport was linked to electron transfer during the operation of noncyclic photophosphorylation.

The dependence of anion absorption on electron transfer is not a general case. Smith (34) demonstrated that light-enhanced PO_4^{3-} absorption by *Nitella* is due to the expenditure of ATP formed by cyclic photophosphorylation. Also, Jeschke (15) has shown that absorption of Cl^- by *Elodea* in the light is not inhibited when noncyclic electron flow is eliminated by DCMU.[1]

[1] Abbreviations: DCMU, 3-(3,4 dichlorophenyl)-1, 1-dimethylurea; FCCP, carbonyl cyanide *p*-trifluoromethoxy-phenylhydrazone; mCl-CCP, carbonyl cyanide *m*-chlorophenylhydrazone; Fe-EDTA, ferrous sulfate in equal parts of NH_4-ethylenediaminetetraacetic acid; ATP, adenosine triphosphate.

Cyclic electron flow has been demonstrated in blue-green algae. When DCMU was added, pigment turnover was observed even though O_2 evolution was eliminated. The cyclic electron flow was enhanced when FCCP was added, indicating coupled ATP formation (39).

The evidence discussed above would indicate that the absorption of cations in the light is not a passive process controlled by electron-mediated anion transport. In general, cation and anion transport requires the expenditure of energy in the form of ATP.

The present investigation was made to study the effect of light on cellular K^+ absorption by corn leaf tissue and to determine the source of energy for active K^+ transport in the light.

Some preliminary experiments on light-enhanced K^+ absorption by corn leaf tissue have been reported elsewhere (29).

Materials and Methods

Corn (*Zea mays* 'DeKalb 805') was germinated in the dark and then grown in nutrient solutions in the greenhouse for approximately 2 weeks. The nutrient solutions contained one-fifth the normal concentration of nutrients supplied in Johnson's solution (16) except that K^+ was present at only 0.3 mM, about one-twentieth the concentration normally added. Iron was supplied as Fe-EDTA at a concentration of 0.2 mM.

At the end of 2 weeks the leaves were excised from the plants and brought into the laboratory. Leaf slices were prepared by the method of Smith and Epstein (35) except for minor differences in technique.

Ten pieces of corn leaves approximately 10 mm wide and 30 to 40 mm long were placed between 2 blocks of styrofoam of about the size of the leaf. This width of leaf represented the width of an entire leaf blade of a 2-week-old plant. The blocks and leaves were then placed in a hand microtome for slicing. Each sample yielded 60 slices, 400 μ wide. The slices were wrapped in cheesecloth, and the ends of the cloth were gathered and tied with thread (8).

The bags containing the samples were suspended for 1 hour in a solution containing 0.5 mM $CaSO_4$ at 30°. This pretreatment was done in the dark with samples to be subjected to a dark treatment, and in the light with samples to be exposed to light. The pH of all solutions was 5.7 ± 0.2 pH units.

At the end of the pretreatment period the samples were placed in a K^+ solution radioactively labeled with ^{86}Rb and containing $CaSO_4$ at a concentration of 0.5 mM. Experimental solutions of KCl were labeled with ^{86}Rb to the extent of approximately 0.02 μc/μmole K. This specific activity was held constant throughout any 1 experiment. The use of ^{86}Rb as a label for K^+ in investigations of ion absorption by leaf tissue has been shown to be a valid procedure (31). Also experiments reported by Smith and Epstein (36) indicated that K^+ and Rb^+ are transported by the same site in corn leaf slices and there is mutual competition between K^+ and Rb^+ in the absorption process. The validity of using ^{86}Rb to label K^+ was tested in my experiments. In 2 different experiments K^+ solutions were labeled with ^{42}K or ^{86}Rb. An experiment similar to the one reported in figure 2 in the section on results was carried out and there was no difference in the exchangeable fraction whether it was determined by using ^{86}Rb or ^{42}K. In another experiment using these 2 radioisotopes to label K^+ solutions there was no significant quantitative or qualitative difference in K^+ absorption in the light or dark. The possibility of isotopic exchange was further tested in the experiment described below. Samples were prepared as described in this section except each sample contained approximately 170 mg of leaf tissue. Triplicate samples were exposed to 3 different treatments. The first set of 3 samples was blotted, weighed, ashed and the K^+ content was determined by using flame spectrophotometry. Another set of samples was exposed for 4 hours to a solution containing K^+ at a concentration of 0.2 mM, radioactively labeled with ^{86}Rb, and $CaSO_4$ at a concentration of 0.5 mM. The third set of samples was exposed to a solution containing 0.5 mM $CaSO_4$ for 4 hours. At the end of this period the samples were rinsed for 30 minutes in 0.5 mM $CaSO_4$ to remove any freely exchangeable K^+. The samples were blotted, weighed, ashed and the K^+ content of the leaf tissue was determined by flame spectrophotometry. Also the K^+ absorbed was calculated on the basis of ^{86}Rb taken up by the tissue. The amount of K^+ absorbed from the solution containing K^+ was determined chemically by the difference in the K^+ content of the leaf tissue at the beginning and end of the 4-hour period and amounted to 6.5 μmoles per gram of fresh weight. The K^+ absorbed as determined by the uptake of ^{86}Rb was 6.1 μmoles per gram of fresh weight. The values presented are averages of the replicates. There was no significant loss of K^+ from the tissue exposed for 4 hours to the $CaSO_4$ solution. These observations gave confidence in the use of ^{86}Rb to label K^+ solutions. It is much more convenient to use this longer-lived isotope and it was therefore used for all the experiments reported in this paper. Calcium was included in all experimental solutions because of its essentiality for unimpaired ion transport (4, 13).

The samples were exposed to the radioactive solutions for various periods. Then they were placed for 30 minutes at room temperature (21°) in a desorbing solution containing 1 mM KCl and 0.5 mM $CaSO_4$. The desorption removed any freely diffusible or exchangeable radioactive ions (8). At the end of the desorbing period the samples were rinsed in distilled water and hung up to drain.

The cheesecloth was then removed, the slices were blotted to remove excess water, and each sample was weighed. The weights in the various experiments ranged from 50 to 70 mg, but in any 1 experiment the samples did not vary in weight by more than 2 mg.

The weighed samples were transferred to planchets and ashed for 1 hour at 500°. The ash was wetted, spread with a drop of detergent solution, and dried under heat lamps.

The samples were counted 3 times with a thin-window GM gas-flow counter, for a total of at least 2560 counts each time. The counts were compared with a standard, and the amounts of K^+ absorbed were calculated on the basis of a gram of fresh weight per hour.

Results

The rate of K^+ absorption was studied as a function of light intensity as measured with a General Electric Light Meter, Type 213. A piece of cheesecloth was placed over the receptor window so as to duplicate the light intensity the leaf slices were actually exposed to inside the cheesecloth bag. The results are presented in figure 1. The concentration of K^+ was 0.1 mM, and concentration of Ca^{2+} was 0.5 mM. The absorption period was 60 minutes. The rate of K^+ absorption increased 50% over that of the dark control, *i.e.*, from 1.4 to 2.2 μmole g^{-1} hr^{-1}, when the light intensity was increased to 500 lumen m^{-2}. This is 80% of the maximal response, reached at light intensities of less than 5000 lumen m^{-2}.

Since studies with algae indicate that illumination caused an efflux of various cations (3, 33), an experiment was designed to test the possible efflux of K^+. The leaf tissue was exposed for 1 hour in the light or dark to a radioactively labeled solution

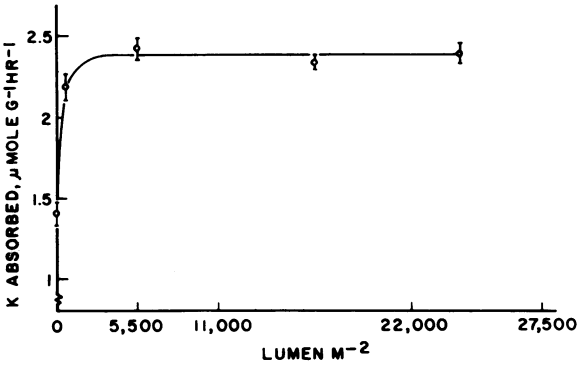

FIG. 1. Effect of increasing light intensity on the rate of K^+ absorption. Potassium: 0.1 mM, Ca^{2+} as $CaSO_4$: 0.5 mM. pH 5.7 and temperature 30°. Absorption period 60 minutes. All treatments are replicated; circles represent the means of 2 values indicated by the short horizontal lines. Horizontal lines not drawn where the distance between them would have been equal to or less than diameter of the circle.

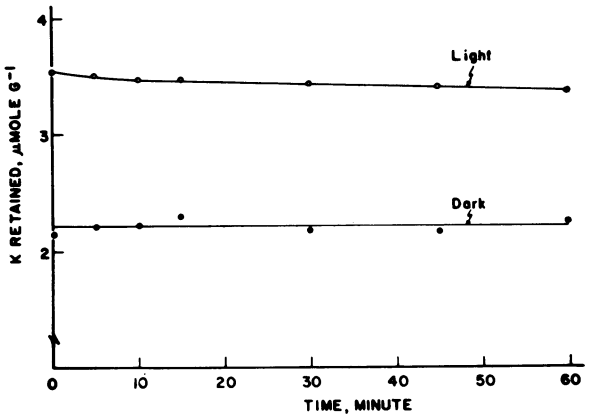

FIG. 2. Potassium retained by corn leaf tissue as a function of time. Potassium: 0.1 mM, Ca: 0.5 mM during absorption period. Potassium: 1.0 mM, Ca: 0.5 mM during desorption period. Points on zero time ordinate represent values for samples rinsed in water only. Other conditions and conventions as for figure 1 except samples were not replicated.

containing 0.1 mM K^+ and 0.5 mM Ca^{2+}. The values represented by open and closed circles on the zero time ordinate in figure 2 were obtained from samples that were rinsed in distilled water only and then analyzed for radioactivity. The other samples were placed in the light or dark for various periods in a nonradioactive solution containing 1.0 mM KCl and 0.5 mM $CaSO_4$. The results indicate little or no loss of previously accumulated K^+ in the light or dark.

To study the effect of increasing K^+ concentration on the rate of K^+ absorption in the light and dark, the K^+ concentration was varied from 0.01 mM to 0.2 mM, while Ca^{2+} was held constant at 0.5 mM. The tissues were exposed for 60 minutes to the solution indicated above. The curves in figure 3 indicate saturation-type kinetics, with the rate of K^+ absorption greater in the light than in the dark. The kinetics were analyzed essentially as described by Epstein *et al.* (7). The apparent Michaelis constant (Km), an approximate measure of the affinity of a site for an ion (6, 7, 30), was determined for the dark and light treatments. The Km's were similar, 0.035 mM in the light and 0.030 mM in the dark. The maximal velocity (V_{max}), the theoretical maximal rate of absorption at non-limiting substrate concentrations, varied by more than 60%. The maximal rates of K^+ absorption were 3.40 μmole g^{-1} hr^{-1} in the light and 2.08 μmole g^{-1} hr^{-1} in the dark.

The data indicate a light-enhanced K^+ absorption. The next question concerns the source of energy for this accelerated rate of uptake in the light. With this in mind, experiments were carried out with various antimetabolites. The results are presented in figure 4.

The concentration of K^+ was 0.1 mM, and that of Ca^{2+} was 0.5 mM. At the end of the 60-minute

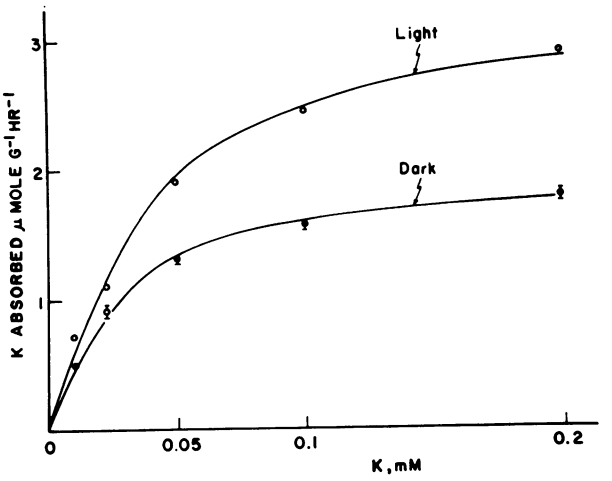

FIG. 3. Rate of K^+ absorption as a function of the concentration of K^+ in the light and dark. Potassium: 0.01 to 0.2 mM, Ca: 0.5 mM. Other conditions and conventions as for figure 1.

absorption period the samples were placed in solutions containing nonradioactive K^+ as described earlier. The concentrations of inhibitors added to the solutions in these experiments were chosen so as to give maximal inhibition without irreversible damage. These concentrations were such that after the sample was removed from the solution containing the inhibitor and rinsed free of the inhibitor, a rate of K^+ absorption was restored which was equivalent to that of the control.

Sodium cyanide was added at a concentration of 0.01 mM. This inhibitor depressed K^+ absorption in the dark but had little effect on uptake in the light.

The uncoupler of oxidative phosphorylation, DNP, at a concentration of 0.01 mM inhibited K^+ absorption in the dark to one-sixth of the rate in the absence of DNP. In the light, inhibition due to DNP was considerably less, to about half the uninhibited rate.

Sodium azide at 0.01 mM had approximately the same effect as DNP, but was somewhat less effective as an inhibitor in the dark.

In the dark the effect of mCl-CCP at a concentration of 0.001 mM was similar to that of DNP and NaN_3. In the light, however, mCl-CCP was twice as effective at inhibiting K^+ absorption as was NaN_3 or DNP.

Dichlorophenyl-1,1-dimethylurea is an inhibitor of photosynthetic reactions, particularly those involved in O_2 evolution (1, 15, 23, 39, 40). Figure 5

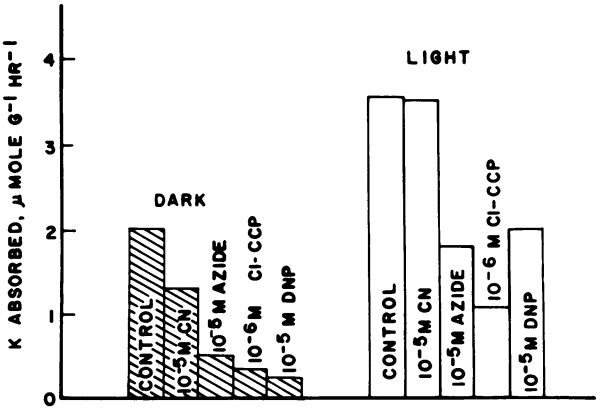

FIG. 4. Effect of antimetabolites on the rate of K^+ absorption in the light and dark. Potassium: 0.1 mM, Ca: 0.5 mM, inhibitor concentrations as indicated on figure. Other conditions and conventions as for figure 1.

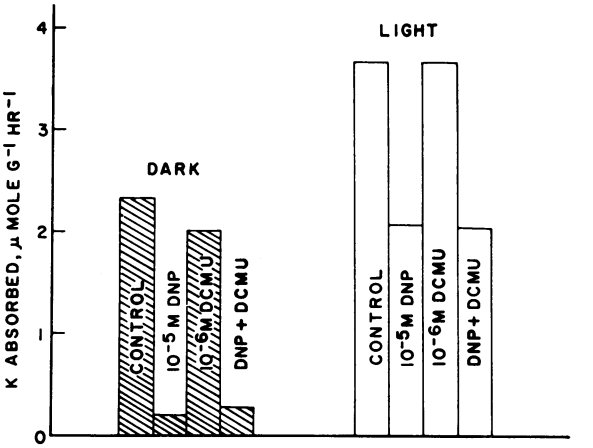

FIG. 5. Effect of antimetabolites on the rate of K^+ absorption in the light and dark. Potassium: 0.1 mM, Ca: 0.5 mM, inhibitor concentrations as indicated on figure. Other conditions and conventions as for figure 1.

gives the results of an experiment in which absorption of K^+ was studied as a function of light and dark in the presence of 0.01 mM DNP, 0.001 mM DCMU, and DNP + DCMU at the same concentrations. Dinitrophenol inhibited K^+ absorption to the same extent as shown in figure 4. There was no effect of DCMU on K^+ absorption. The 2 inhibitors combined had the same effect as DNP alone. Since green tissue evolves O_2 in the light, O_2 cannot be removed from the leaf cell environment simply by maintaining external anaerobic conditions. There is the possibility that O_2 evolved might be tightly coupled to the respiratory chain, allowing electrons to flow and oxidative phosphorylation to proceed.

An experiment was carried out to determine whether the cytochrome system is closely linked with ion absorption. The results are presented in figure 6. The concentrations were 0.1 mM K^+, 0.5 mM Ca^{2+}, and 0.001 mM DCMU. Nitrogen, which had been washed by a 0.2 M solution of KOH to remove any CO_2, was bubbled through the solutions. The absorption periods were 30 minutes. Thirty-minute periods were used because leaf tissue

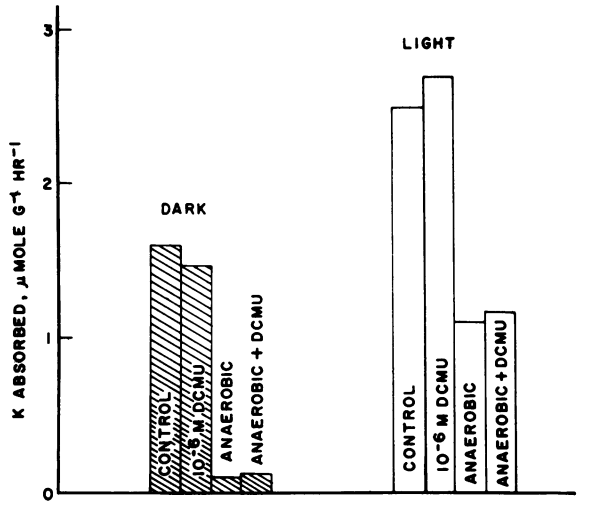

FIG. 6. Effect of anaerobiosis and DCMU on the rate of K^+ absorption in the light and dark. Potassium: 0.1 mM, Ca: 0.5 mM. Absorption period: 30 minutes, rate calculated for 1 hour. Other conditions and conventions as for figure 1.

exposed to anaerobic conditions for longer was irreversibly injured. Absorption of K^+ was eliminated almost completely when anaerobic conditions were maintained in the dark. The presence of DCMU made little difference. In the light there was considerable K^+ absorption when anaerobic conditions were maintained. This was true even when O_2 evolution by photosynthetic processes was presumably eliminated by the addition of DCMU.

Discussion

The absorption of K^+ by corn leaf tissue is enhanced when the tissue is illuminated. The light-enhanced rate is established after a 10-minute light exposure of tissue previously exposed to a 60-minute dark period (29). The light-enhanced K^+ absorption is obtained at relatively low light intensities, and light saturation is reached at a lower light intensity for the ion-transport process than for photosynthesis (20). The light-response curve is very similar in shape to the response curve observed by van Lookeren Campagne (21) for Cl^- absorption by *Vallisneria*.

The light-enhanced efflux of ions demonstrated by some investigators (2, 3, 33) was not observed under these experimental conditions. The ^{86}Rb labeled K^+ ions, once they were accumulated, remained in the tissue even in the presence of a large excess of nonradioactive K^+. The amount of K^+ in corn leaf tissue cultured by the procedure described in the section on Materials and Methods is approximately 30 μmoles K^+ per gram of fresh weight. If it is assumed that a gram of fresh tissue is nearly equivalent to 1 ml then the concentration of K^+ is approximately 30 mM. The ^{86}Rb taken up by the plant tissue should be isotopically diluted with the K^+ in the tissue. Ten samples with a total weight of approximately 0.5 g (about 0.5 ml in volume) are suspended in 2000 ml of a 1.0 mM K^+ solution during the desorption period. This means that this 0.5 ml volume of solution, containing 15 μmoles of K^+ labeled with ^{86}Rb is exposed to a solution containing 2000 μmoles of unlabeled K^+. If appreciable exchange took place ^{86}Rb in the tissue should be diluted by the better than 100-fold excess of K^+ in the external solution. As can be seen by the data shown in figure 2 there is no measurable movement out of the tissue. The same type of experiment was carried out with ^{42}K as a label for K^+ and the same results as shown in figure 2 were obtained. Previously absorbed label is not exchanged with non-labeled ions. The initial K^+ content of the leaf slices was not reduced even after 4 hours of exposure to a solution containing $CaSO_4$ at a concentration of 0.5 mM. This would seem to reduce the possibility of exchange in the presence or absence of K^+. On the contrary, the data would seem to indicate that there is some barrier to free movement of ions and unrestricted isotopic exchange does not take place in this system.

An investigation of the mechanisms controlling stomatal aperture was conducted by Fischer (9). He demonstrated that DNP and mCl-CCP, at concentrations used in my experiments, had the same effect on stomatal closing in the light and in the dark. This is quite different from results reported in this study. These inhibitors resulted in quite different amounts of inhibition of ion absorption in the light and dark. The present findings, together with those of Fischer (9), support the earlier conclusion that stomates are not a controlling factor in ion absorption by narrow leaf slices (35).

The demonstration of more than 1 ion-transport mechanism in higher plants (6, 7, 30, 31) suggests that an additional mechanism might become operational in the light. Since an analysis of the kinetic constants showed that the Km's in the light and dark were virtually identical, it is likely that the mechanism for K^+ absorption was the same in the light and the dark. Similar experiments on K^+ and Rb^+ absorption by corn leaf tissue were carried out by Smith and Epstein (36). They demonstrated that K^+ and Rb^+ compete in a common ion absorption process for which Na^+ has little affinity. In the present experiments, the rate of K^+ absorption was considerably greater in the light than in the dark, indicating a faster turnover of the mechanism. The greater rate of turnover of a K^+-absorbing mechanism in the light is possibly due to a more readily available supply of high-energy substances rather than to the activation of a second mechanism of K^+ transport.

The source for this energy could be ATP. The results illustrated in the last 3 figures (fig 4, 5, 6) lend support to the idea that K^+ absorption in the

light is closely linked to ATP formed by cyclic photophosphorylation (22, 23, 29). Cyanide and uncouplers of oxidative phosphorylation, DNP, mCl-CCP, or NaN_3 (1, 11, 12, 18, 19, 32), manifested their greatest effect in the dark when a large percentage of ATP is probably formed by respiratory processes. Absorption in the dark was less than 20 % of that of the control when DNP, mCl-CCP, or NaN_3 was present. When DNP was added in the light the absorption was 60 % of that of the control. With mCl-CCP present in the light the rate of absorption was only 30 % of that of the control. Although DNP at concentrations used has little or no effect on photophosphorylation (1, 26, 40), mCl-CCP is a potent uncoupler of oxidative and photophosphorylation (11, 34, 40). In the light the decrease in K^+ uptake with mCl-CCP present could possibly be attributed to a lower level of available ATP, resulting from a decreased rate of photophosphorylation

The possibility that enhanced ion transport in the light could be the result of increased electron flow due to 'photorespiration' does not appear likely in corn tissue (10, 24, 25). It seemed advisable, however, to consider the possibility. Electron transfer along the cytochrome chain can be prevented by removal of O_2, the terminal electron acceptor. However, in green tissue, O_2 is evolved during photosynthesis, and this O_2 might be tightly coupled to the cytochrome system, allowing electrons to flow. Respiration is severely restricted by maintaining external anaerobic conditions and inhibiting O_2 evolution by the addition of DCMU (39). This can be accomplished without interfering with cyclic photophosphorylation (1, 14, 22, 23, 34, 39, 40). The system was made anaerobic by bubbling CO_2-free nitrogen through the system, O_2 evolution and oxidative phosphorylation were assumed to be inhibited, and a considerable amount of K^+ was absorbed in the light. The possibility that DCMU might not be completely inhibiting O_2 evolution in this system was not entirely eliminated. However, the major source of energy for active K^+ absorption in the light under these conditions was assumed to be cyclic photophosphorylation. This process could supply ATP required in the energetic operation of the active ion-transport system. This conclusion is in agreement with work reported by Weigl (41). He concluded that ATP could be formed under anaerobic conditions when green tissue of *Limnophila gratioloides* was exposed to light. This ATP formed by photophosphorylation supplied energy for active ion transport. He did not, however, eliminate photosynthetic evolution of O_2 and some of the ATP formed could be the result of oxidative phosphorylation. In a more recent paper (42) Weigl studied the absorption of anions by *Elodea* and found that energy for active ion transport was available in the light even though anaerobiosis was maintained and DCMU was present to prevent O_2 evolution.

It is not likely that the uptake of K^+ is dependent upon the absorption of Cl^-. In experiments reported by Smith and Epstein (36) the absorption of K^+ by corn leaf slices was independent of the accompanying anion, whether it was the more slowly absorbed SO_4^{2-} or the more rapidly absorbed Cl^- anion. In unpublished data obtained in this laboratory similar results were produced in the light and dark treatments.

Inhibitors were used in this study on the assumption that the effect of these compounds would be specific and their mode of action would parallel that reported in the voluminous literature on the subject. The effect of these inhibitors is not limited to studies on cell particulates (chloroplasts and mitochondria). Algal cells have been used (22, 23, 34) and virtually identical results were reported when these inhibitors were employed in light-dark treatments. Also similar results have been reported for tissues of aquatic angiosperms, tissues more highly organized than those of algae (21, 41, 42).

Differential penetration of these inhibitors according to the light treatment is not indicated by this study. Anaerobic conditions gave virtually the same differential inhibition in the light and dark as did the chemical inhibitors. It is not likely that N_2 penetrates at different rates in the light and dark (*cf.* figs 5 and 6). The argument that the penetration of inhibitors varies with the light treatment would also apply to the experiments with chloroplasts, mitochondria and algae under various light regimes. All of these entities are also surrounded by limiting membranes. A study of the literature concerned with these particulates does not support the hypothesis of light-dependent differential penetration of commonly used inhibitors.

The present demonstration of an ion-transport system which is coupled to a process of ATP formation by a system other than respiratory electron flow may prove to be an important tool in determining sources of energy available for the accumulation of ions by higher plants.

Acknowledgments

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