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Coupling of Ion Transport in Green Cells of *Atriplex spongiosa* Leaves to Energy Sources in the Light and in the Dark

ULRICH LÜTTGE*, CHARLES K. PALLAGHY, and C. B. OSMOND

Research School of Biological Sciences, Australian National University, Canberra, Australia

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Summary. The coupling of ion transport to energy sources in the light and in the dark in green cells of *Atriplex spongiosa* leaves was investigated using light of different qualities, an inhibitor of electron transport (dichlorophenyl dimethyl urea), and an uncoupler (p-CF_3O-carbonyl cyanide phenylhydrazone). Two different mechanisms of ion uptake were distinguished. (1) A light-dependent Cl^- pump which is linked to light-dependent K^+ uptake. The energy for this pump is probably derived from photosynthetic electron transport or from nicotinamide adenine dinucleotide phosphate, reduced form. This mechanism is dichlorophenyl dimethyl urea-sensitive and enhanced by uncouplers. (2) A mechanism independent of light, which operates at the same rate in the light and in the dark. This mechanism is sensitive to uncouplers. It is probably a K—Na exchange mechanism since K^+ and Cl^- uptake and a small net uptake of H^+ are balanced by Na^+ loss.

The coupling of active ion uptake to energy sources in the light and dark has been well documented in the cells of green algae (MacRobbie, 1965, 1966*a*, *b*; Hope, 1965; Raven, 1967, 1968, 1969; Coster & Hope, 1968; Smith & West, 1969). Fewer data have been reported for the green cells of the leaves of aquatic higher plants (Arisz & Sol, 1956; van Lookern-Campagne, 1957; Winter, 1961), but these suggest a different form of coupling to energy sources in the light (Weigl, 1964*b*, 1967; Jeschke, 1967; Jeschke & Simonis, 1967).

The leaf cells of terrestial plants also accumulate ions by active light-stimulated processes (Osmond, Lüttge, West, Pallaghy & Shacher-Hill, 1969; Rains, 1968). Leaf cells of higher plants are bathed by the transpiration stream which delivers electrolytes in solution via the tracheidal endings of the xylem system. Earlier studies have dealt with K^+, Na^+ and Cl^- fluxes in *Atriplex* leaf mesophyll cells (Osmond, 1968), with membrane

* Permanent address: Botanisches Institut der Technischen Hochschule, Darmstadt, Germany.

electrical potentials in the light and dark in these cells (Osmond *et al.*, 1969; Lüttge & Pallaghy, 1969) and with ion secretion from the leaf (Osmond *et al.*, 1969; Lüttge & Osmond, 1970). This communication examines the coupling between ion uptake and energy sources in the light and dark in the mesophyll cells of higher plant leaves. The form of coupling found in these cells is compared with that already described for algal cells and for the leaves of aquatic plants.

Material and Methods

Atriplex spongiosa plants were grown in water culture as reported previously (Osmond *et al.*, 1969). A number of leaves were stacked between thick (approximately 5 mm) discs of fresh carrots, and sectioned with a sledge microtome to 0.5-mm-wide strips as described earlier (Osmond, 1968). The leaf slices were separated from the carrot discs, washed several times with 0.5 mM $CaSO_4$ and then left overnight in aerated $CaSO_4$ solution (approximately 2 liters/25 g of tissue) in the dark at room temperature.

Experimental solutions used for ion uptake studies (40 ml) contained 0.5 mM $CaSO_4$ and the desired concentration of KCl and inhibitors (pH 5.5 to 6.0). Isotopes used (^{42}K and ^{36}Cl) were supplied by the Australian Atomic Energy Commission, Lucas Heights, N.S.W. Flasks containing the tissues in the experimental solution were maintained at 24 °C in a shaking waterbath with illumination provided by a Philips HPLR lamp [250 Wm^{-2}; intensity (400 to 700 nm) $= 3.5 \times 10^6$ ergs $\times$ $cm^{-2} \times min^{-1}$].

Samples of 50 to 150 mg of tissue were removed from the flasks after 1, 2.5 and 5 hr. Isotope in the free space was removed by washing two times for 15 min in an unlabelled KCl solution at 0 °C (Osmond, 1968). The tissue was blotted dry, weighed and dried on a planchet after addition of propanol and gelatine. Each treatment was triplicated. Samples were counted with a Nuclear Chicago gas flow counter. All counts were standardized to the same time zero, with allowance for ^{42}K decay. The uptake of potassium and chloride was usually studied simultaneously in the same tissue using a medium labelled with both ^{42}K and ^{36}Cl. The samples were counted immediately and then again after about 10 days, by which time the ^{42}K activity was negligible. Other experiments were performed with ^{36}Cl only. Ion uptake is expressed as $\mu moles \times g^{-1} \times hr^{-1}$ of fresh weight, calculated from the external specific activity.

Net flux experiments were carried out using similar plant material. Cl^- was determined by electrochemical titration with an Aminco-Cotlove chloride titrator. K^+ and Na^+ were measured spectrophotometrically. The pH changes of the external solution were followed with a glass electrode. Carbon dioxide fixation was determined by placing small samples of tissue (approximately 120 mg) in 10 ml of solution containing 0.25 mM $KH^{14}CO_3$ (specific activity 0.61 $\mu c/\mu mole$) in 20 mM MES [2-(N-morpholino) ethane sulfonic acid] buffer (Good *et al.*, 1966) at pH 5.7 with 5 mM KCl and 0.5 mM $CaSO_4$. After 30 min, duplicate samples were blotted dry, weighed and dried on planchets with the addition of gelatine and 5 N formic acid to release unfixed $^{14}CO_2$. CO_2 fixation is expressed as $\mu moles \times g^{-1} \times hr^{-1}$, on the basis of the external specific activity of $H^{14}CO_3^-$.

The cutoff filters used were constructed as described by MacRobbie (1965), and by Lüttge and Pallaghy (1969). Filter I allows both photosystems 1 and 2 to operate. Filter II excites principally photosystem 1, whereas with filter III neither of the photosystems is operative. Kodak neutral density gelatine filters (N.F.) were arranged to give approximately equal transmittance of total intensity by the filters (approximately 20 %

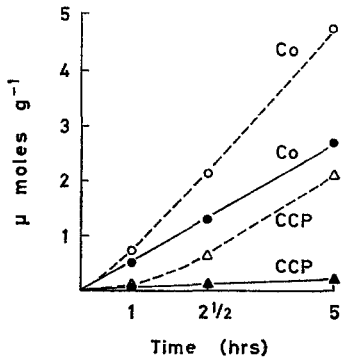

Fig. 1. Time course of Cl^- uptake by leaf slices in the light (o---o, ▵---▵) and in the dark (•——•, ▲——▲) in controls (o, •) and in the presence of 10^{-6} M FCCP (▵, ▲)

of the intensity of the Philips lamp; *see* Table 1). No attempt was made to correct for the declining absorption spectrum of chlorophyll in the far-red region so that percentage absorption under filter II may be less than for filter I. However, this absorption difference is unlikely to explain the inhibition to dark level by filter II (Table 1). The uncoupler and inhibitor used were p-CF_3O-carbonyl cyanide phenylhydrazone (FCCP) and dichlorophenyl dimethyl urea (DCMU), respectively.

The time course of $^{42}K^+$ uptake, both in the light and the dark, and $^{36}Cl^-$ uptake in the dark was linear over 5 hr. The rate of $^{36}Cl^-$ uptake in the light, and especially in the presence of FCCP, increased with time over the first 5 hr of labelling (Fig. 1). This influence of light was also observed during pretreatment of the tissue in unlabelled 5 mM KCl and was most marked when 0.5 mM $CaSO_4$ alone was used as the pretreatment solution ($^{36}Cl^-$ uptake was enhanced by a factor of 2 to 3). The mechanism of this adaption of Cl^- uptake to light is unclear. All rates of ion uptake reported in this paper were obtained from the slope of the time course between 2.5 and 5 hr. Each point represents the mean of triplicate samples which was usually within ± 5 %. Most experiments were done at least three times, with 10 to 30 % variation in a given rate between batches of tissue.

Results

Uptake of Labelled K^+ and Cl^-, and CO_2 Fixation

Effects of Light Quality in the Far-Red End of the Spectrum. Table 1 shows the effect of cutoff filters in the far-red end of the spectrum on $^{42}K^+$ and $^{36}Cl^-$ uptake and on $^{14}CO_2$ fixation. The light intensity was altered in some instances by using the filters with and without the neutral density filters (N.F.). It can be seen that both light-stimulated K^+ and Cl^- uptake, as well as CO_2 fixation, depend on the operation of photosystems 1 and 2 or on noncyclic electron flow (full light and filter I). Rates of ion uptake and CO_2 fixation in light which excites principally photosystem 1 (filter II), and in light in which neither of the systems is operative (filter III), are similar to the rates observed in the dark. Dependence of light-stimulated

Table 1. *Effects of wavelength and intensity (ergs $\times 10^{-6} \times cm^{-2} \times min^{-1}$) in the far-red region on ion uptake and CO_2 fixation in* A. spongiosa *leaf slices ($\mu moles \times g^{-1} \times hr^{-1}$)*

	Light conditions and filter						
	Full light + N.F.	Filter I	Filter I + N.F.	Filter II	Filter II + N.F.	Filter III	Dark
Photosystems active	1 + 2	1 + 2	1 + 2	1	1	none	none
Incident intensity	0.83	0.94	0.62	0.74	0.67	0.62	0.00
Chloride uptake							
5 mM KCl	0.93	0.71	0.71	0.59	0.53	0.53	0.56
0.2 mM KCl	0.43	0.47	0.41	0.36	0.33		0.35
Potassium uptake							
0.36 mM KCl	0.27[a]	0.20	0.14	0.12	0.12	0.13	0.12
CO_2 fixation	2.19 (0.24)[b]		0.35 (0.20)[b]		0.23	0.24	0.24

[a] Cl^- uptake by this tissue in full light was 0.12 μmoles $\times g^{-1} \times hr^{-1}$.
[b] Values in parentheses indicate rate of $^{14}CO_2$ fixation with 2×10^{-6} M DCMU added.

Table 2. *Effects of DCMU on K^+ and Cl^- uptake from 5 mM ^{42}K ^{36}Cl solution ($\mu moles \times g^{-1} \times hr^{-1}$)*

Treatment	Total uptake		Light-dependent uptake		DCMU-inhibited uptake	
	Cl^-	K^+	Cl^-	K^+	Cl^-	K^+
Light, control	1.75	3.88	1.18	2.45		
Light + 5×10^{-7} M DCMU	0.96	3.33	0.39	1.90	0.78	0.55
Light + 10^{-6} M DCMU	0.95	3.22	0.38	1.79	0.80	0.66
Light + 2×10^{-6} M DCMU	0.85	3.45	0.28	2.02	0.90	0.43
Dark, control	0.57	1.43				
Dark + 2×10^{-6} M DCMU	0.53	1.34				

Cl^- uptake on the activity of both photosystems is observed irrespective of the external Cl^- concentration. Light stimulation of Cl^- uptake appears to be less dependent on light intensity than K^+ uptake. That filter I plus N.F. supports normal photosynthesis (at a reduced rate) is shown by the DCMU sensitivity of $^{14}CO_2$ fixation.

Effects of DCMU. Table 2 shows the effect of DCMU on $^{42}K^+$ and $^{36}Cl^-$ uptake. In the light, DCMU inhibits both K^+ and Cl^- uptake, but K^+ is affected to a much lesser extent. In the dark, relatively high concentrations of DCMU show only slight inhibitory effects.

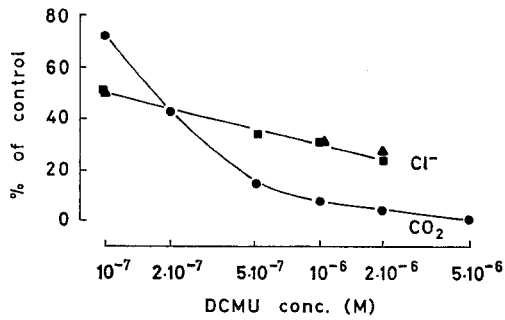

Fig. 2. Effect of DCMU on light-dependent Cl^- uptake from 0.2 mM KCl (▲) and 5 mM KCl (■) and on light-dependent CO_2 fixation (pH 7.5; identical results obtained at pH 5.7)

Light-dependent ion uptake was determined by subtraction of the rates observed in the dark from the rates found in the light. Justification of this procedure is given below (*see* "Effects of DCMU and FCCP" and Discussion). It can be seen that light-dependent Cl^- uptake is very sensitive to DCMU. In other experiments, Cl^- transport in the light was reduced to the rates of dark uptake by 2×10^{-6} M DCMU. In the experiment shown in Table 2, only about 20% of the light-dependent K^+ uptake was DCMU-sensitive. The last two columns of the table show that the DCMU-sensitive part of K^+ and Cl^- uptake is quantitatively similar. In two other experiments, the amount of light-dependent K^+ uptake inhibited by 2×10^{-6} M DCMU was significantly greater than that found for Cl^- ($Cl^- = 0.39$ and $K^+ = 1.04$ μmoles $\times g^{-1} \times hr^{-1}$ in one experiment; $Cl^- = 0.23$ and $K^+ = 2.04$ μmoles $\times g^{-1} \times hr^{-1}$ in another).

Fig. 2 shows the effects of DCMU on light-dependent Cl^- uptake from solutions of different Cl^- concentration and on light-dependent $^{14}CO_2$ fixation. DCMU effects on uptake are similar at low and high Cl^- concentration. CO_2 fixation seems to be more sensitive to DCMU than does Cl^- uptake.

Effects of DCMU and FCCP. Table 3 shows two experiments comparing the effect of DCMU, FCCP, and a combination of the two inhibitors on $^{42}K^+$ and $^{36}Cl^-$ uptake. DCMU reduced Cl^- uptake in the light almost to the level of dark uptake. In the experiment shown here, unlike that of Table 2, light-dependent K^+ uptake is also almost entirely DCMU-sensitive.

FCCP is a more effective inhibitor of ion uptake in the dark than in the light, as has been shown by other workers using different plant materials (Jeschke, 1968; Smith & West, 1969). However, when DCMU is present in concentrations which inhibit ion uptake in the light almost to the level of

Table 3. *Effects of DCMU, FCCP, and DCMU+FCCP treatment on K^+ and Cl^- uptake from 5 mM KCl ($\mu moles \times g^{-1} \times hr^{-1}$)*

Exp.	Treatment		Uptake[a]					
			K^+	Cl^-	K^+	Cl^-	K^+	Cl^-
			(light)		(dark)		(light-dark)	
1	Control			1.40		0.65		0.75
	FCCP	2×10^{-7} M		1.02		0.11		0.91
		10^{-6} M		0.76		0.05		0.71
	DCMU[b]	5×10^{-7} M		0.76		—		0.11[c]
		10^{-6} M		0.76		—		0.11
		2×10^{-6} M		0.76		—		0.11
	DCMU	2×10^{-6} M+						
	FCCP	2×10^{-7} M		0.13		—		0.08
		10^{-6} M		0.05		—		0.00
2	Control		2.78	0.79	1.47	0.28	1.31	0.51
	FCCP	5×10^{-7} M	1.90	0.45	0.64	0.04	1.26	0.41
	DCMU	2×10^{-6} M	1.74	0.40	—	—	0.27	0.12
	DCMU	2×10^{-6} M+	0.64	0.04	—	—	0.00	0.01
	FCCP	5×10^{-7} M						

[a] Only chloride uptake was measured in Exp. 1.
[b] All DCMU concentrations were equally effective.
[c] Since DCMU has no effect in the dark (Table 2), the dark values used in these subtractions were those at the appropriate FCCP concentration.

dark uptake, subsequent FCCP inhibition in the light is similar to that in the dark. In other words, the uptake of K^+ and Cl^- in the light-plus-DCMU responds to FCCP in the same manner as in the dark. When light-dependent Cl^- and K^+ uptake is determined by subtracting uptake in the dark from uptake in the light, it becomes evident that FCCP, which inhibits uptake drastically in the dark, shows only slight inhibition, and even enhances light-dependent uptake under some conditions.

Detailed Analysis of the Effects of FCCP. Fig. 3 shows the effect of FCCP on $^{42}K^+$ and $^{34}Cl^-$ uptake in the dark and on light-dependent $^{42}K^+$ and $^{36}Cl^-$ uptake (i.e., on uptake in the light minus uptake in the dark) over a wide range of FCCP concentrations. It is evident that uptake in the dark is highly sensitive to the uncoupler. Low FCCP concentrations (5×10^{-8} to 2×10^{-7} M) which clearly inhibit uptake in the dark have no clear-cut effect on light-dependent K^+ and Cl^- uptake. In-between concentrations of the uncoupler (approximately 5×10^{-7} M) enhance light-dependent uptake while uptake in the dark is inhibited dramatically. It is only at high

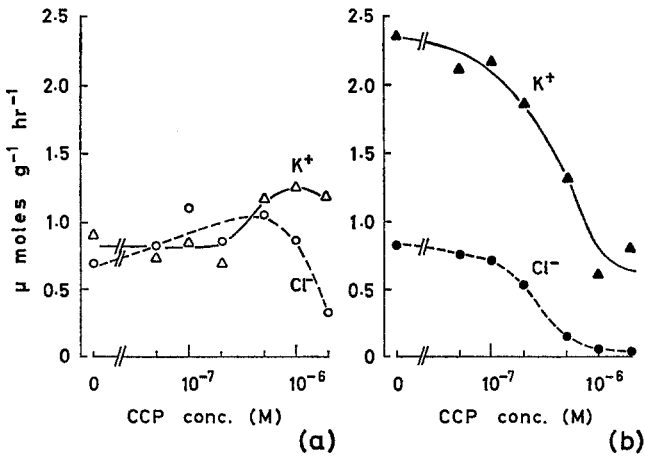

Fig. 3. a) Effect of FCCP on light-dependent Cl^- (○) and K^+ (△) uptake. b) Effect of FCCP on Cl^- (●) and K^+ (▲) uptake in the dark

Table 4. *Comparison of effects of 10^{-6} M FCCP on light-dependent K^+ and Cl^- uptake, light-dependent CO_2 fixation, and K^+ and Cl^- uptake in the dark (% of control values in the absence of FCCP)*

Light-dependent uptake		Light-dependent CO_2 fixation	Uptake in the dark	
K^+	Cl^-		K^+	Cl^-
162	114	23	27	8

FCCP concentrations that FCCP eventually reduced light-dependent ion uptake to rates below the control levels.

Light-dependent CO_2 fixation is inhibited by the uncoupler in a manner similar to the inhibition of K^+ and Cl^- uptake in the dark (Table 4). It seems that Cl^- uptake in the dark is more sensitive to FCCP than K^+ uptake and light-dependent CO_2 fixation. The most noteworthy point made in Table 4 is, however, that 10^{-6} M FCCP inhibits both light-dependent CO_2 fixation and ion uptake in the dark to less than 50% of the control, while light-dependent K^+ and Cl^- uptake are still proceeding at rates above their control values.

Effects of Ouabain. Ouabain at concentrations of 5×10^{-5} and 5×10^{-4} M in the uptake solution does not significantly effect Cl^- or K^+ uptake, either in the light or in the dark.

Net Flux Experiments

Fig. 4a, b and c shows the ion content of leaf slices, measured by direct analysis of the tissue, when placed in 5 mM KCl – 0.5 mM $CaSO_4$ solution after pretreatment in 0.5 mM $CaSO_4$ as described in Methods. Variation in the H^+ and Na^+ content was followed by analysis of the external solution (Fig. 4d, e). It can be seen that the Na^+ contents in Fig. 4c, after subtraction, correlate reasonably well with those determined independently in Fig. 4e. Each point was obtained from triplicate samples using tissues from the same batch. These results were reproducible in two other similar experiments.

It should be noted, however, that these results were obtained from a different batch of plants than those used for the isotope-labelling experiments (*see* first section of Results) and should therefore not be compared quantitatively because of batch variation.

Since the net flux of Na^+ is the same in the light and in the dark, light-dependent net uptake of K^+ and Cl^- is entirely independent of Na^+ fluxes

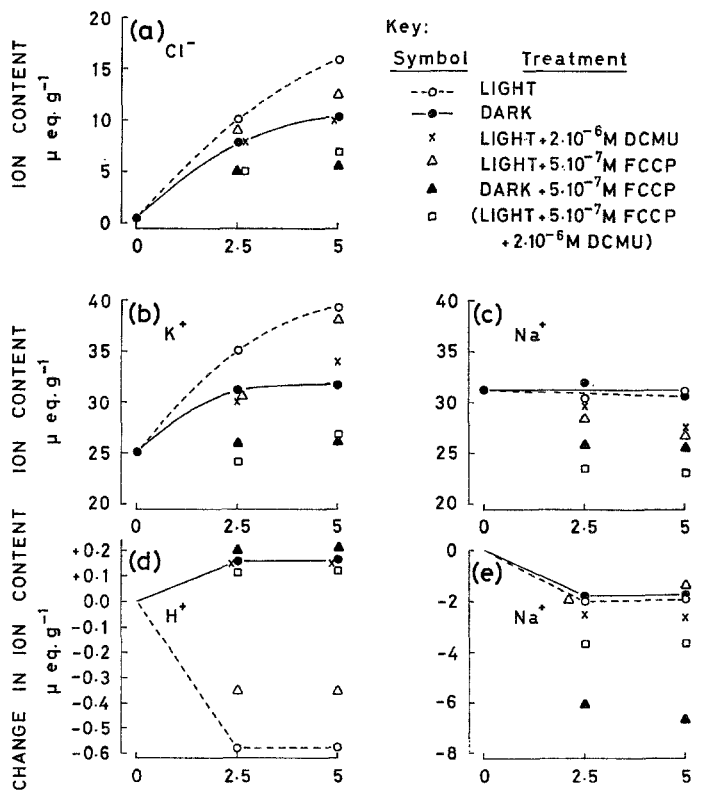

Fig. 4. a–c) Change of ion content with time after transfer of leaf slices to 5 mM KCl – 0.5 mM $CaSO_4$ solution at 25 °C. d) Amount of H^+ released (–), or taken up (+) with time in leaf slices in 5 mM KCl – 0.5 mM $CaSO_4$. e) Amount of Na^+ released by leaf slices with time in 5 mM KCl – 0.5 mM $CaSO_4$

in the tissue. Within experimental error, the data indicate that the uptake of K^+ is similar to that of Cl^- in both the light and the dark, and is always associated with a loss of Na^+ (approximately 2 μequiv $\times g^{-1}$) from the tissue. This may represent exchange of cations in the free space of the tissue (Osmond, 1968).

The effects of DCMU and FCCP on the net fluxes of K^+ and Cl^- correlate with those observed on ^{42}K and ^{36}Cl uptake. As expected from the labelling experiments, light-dependent net uptake of K^+ and Cl^- is inhibited by 2×10^{-6} M DCMU and also by 2×10^{-6} M FCCP (not shown in Fig. 4), but it is enhanced by 5×10^{-7} M FCCP. In the dark, 5×10^{-7} M FCCP is a powerful inhibitor of KCl uptake and stimulates Na^+ efflux from the tissue.

It is interesting to note that in the light H^+ is excreted from the tissue (approximately 0.8 μequiv $\times g^{-1}$), whereas in the dark the tissue absorbs H^+ from the external solution. As shown in Fig. 4d, H^+ excretion in the light is inhibited by 2×10^{-6} M DCMU, resulting in net uptake of H^+ by the tissue as observed for dark-treated samples. In the light, 5×10^{-7} M FCCP only partially inhibited H^+ excretion. In the dark, FCCP increased the alkalinity of the medium by 0.4 to 0.5 pH units, indicating enhanced H^+ uptake by the tissue.

Ouabain (10^{-3} M) had no effect on any of the above fluxes.

Discussion

Determination of Light-Dependent K^+ and Cl^- Uptake

The determination of light-dependent K^+ and Cl^- uptake in *A. spongiosa* leaves is based on the data presented in Table 3. These results suggest that light has no effect on ion transport other than driving the mechanism responsible for the stimulation of transport observed in the light. The response to wavelength and to DCMU suggests that this mechanism is closely linked to photosynthesis. When photosynthesis is specifically inhibited by DCMU, ion transport in the light proceeds at rates similar to those in the dark and is driven by the same mechanism, as indicated by the identical sensitivity of ion transport in the dark and in the light-plus-DCMU to the uncoupler FCCP. Since FCCP treatment in the light has a small effect on ion transport (compared with its effect in the dark) but drastically reduces CO_2 fixation, it seems likely that ion uptake in the light represents additive light and dark processes. It therefore appears that the principal effect of light on ion transport in this tissue is to bring an additional energy-providing mechanism (i.e., photosynthesis) into operation,

and that the energy source responsible for ion transport in the dark remains unaffected in the light. This energy source may, however, be a single pool of ATP generated by respiration in the dark or by cyclic photophosphorylation in the light, or light-plus-DCMU.

On this basis, it seems justifiable to determine light-dependent ion uptake as the algebraic difference between total uptake in the light and total uptake in the dark. We have emphasized this approach because there is uncertainty as to the operation of dark respiratory events in green cells in the light. This analysis of data from uptake experiments also leads to a better understanding of the nature of light-stimulated ion uptake than does a direct consideration of uncorrected rates in the light.

The Linkage of Light-Dependent K^+ and Cl^- Uptake to Photosynthesis

Linkage of light-dependent ion transport in green plant cells to photosynthesis was first demonstrated by van Lookeren-Campagne (1957) who compared the action spectra of ion transport and photosynthesis. MacRobbie (1965) attempted to relate light-stimulated ion transport to specific photosynthetic processes. On the basis of the wavelength dependence of K^+ and Cl^- uptake by cells of *Nitella translucens* and the effects of inhibitors such as DCMU, she concluded that light-dependent K^+ uptake is powered by photophosphorylation, whereas Cl^- uptake in the light is linked directly to noncyclic electron flow. The experiments reported above suggest that Cl^- uptake in *A. spongiosa* mesophyll cells in the light is also linked to photosynthetic electron flow.

This concept of the linkage of ion uptake to photosynthetic electron flow has been challenged on two principal grounds:

(1) The response of ion uptake to wavelength in the far red end of the spectrum, as determined by the cutoff filters used by MacRobbie (1965) and in this investigation, would also be compatible with a phytochrome mechanism. In the light transmitted by filter I, which allows both photosystems to operate, phytochrome is largely in its active P_{FR} configuration, whereas in the light transmitted by filter II, which blocks photosystem 2, phytochrome is converted to the reactive P_R configuration. However, the sensitivity of light-dependent ion uptake to DCMU, which at the concentrations used is a specific inhibitor of photosynthesis, makes it rather unlikely that phytochrome plays a significant role in light-dependent ion uptake in *A. spongiosa* leaves. Furthermore, by combining light of different wavelengths, Raven (1969) has conclusively shown that phytochrome does not control light-dependent ion pumps in *Hydrodictyon.*

The main arguments in favor of the direct action of phytochrome on ion transport across membranes of plant cells are based on the observation that phytochrome regulates nyctinastic movements of legume leaves (Fondeville, Borthwick & Hendricks, 1966; Jaffe & Galson, 1967; Hillman & Koukkari, 1967; Koukkari & Hillman, 1968) and on phytochrome effects on bioelectric phenomena (Tanada, 1968; Jaffe, 1968). The nyctinastic movements are mediated by turgor mechanisms (*see* Bünning, 1953) involving sudden changes in membrane permeabilities.

It appears, therefore, that phytochrome affects ion transport by controlling membrane permeability, whereas photosynthesis provides the energy for light-dependent ion pumps.

(2) Jeschke and Simonis (1967) demonstrated that inhibition of photosystem 2 either by DCMU or by the use of specific wavelengths (Jeschke, 1967) is not sufficient evidence for a direct linkage of ion uptake to electron flow. They showed that Cl^- uptake by *Elodea* leaves was less sensitive to DCMU in the absence of CO_2 rather than in its presence, and they emphasized the role of CO_2 as an electron acceptor. They suggest that when photosystem 2 is inhibited by moderate concentrations of DCMU, the reduced electron flow through the system and the ATP produced are preferentially used for CO_2 fixation. Hence Cl^- uptake is dramatically inhibited. In the absence of CO_2, these electrons cannot be consumed by CO_2 fixation. Electron flow to pyridine nucleotides decreases as NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) accumulates. Electrons then "overflow" through the cyclic pathway, producing ATP which drives Cl^- uptake. The inhibition of Cl^- uptake in the absence of CO_2 at higher concentrations of DCMU is thought to be due to inhibition of cyclic electron flow by these DCMU levels.

If CO_2, in the form of HCO_3^-, competes with Cl^- transport for electrons at the site of ion transport (Hope, 1965) rather than at the site of fixation (an alternative not discussed by Jeschke and Simonis, 1967, but considered to be unlikely in *Elodea* by Jeschke and Simonis, 1969), the effect of CO_2 on light-dependent Cl^- transport could be explained. Since either of these explanations is equally plausible, inhibition of noncyclic electron transport *per se* does not allow one to distinguish between ATP and electron flow as energy sources for light-dependent ion uptake. Analysis of ion uptake isotherms in *Elodea* leaf tissue in the light and dark also led Weigl (1967) to conclude that the availability of ATP was largely responsible for increased anion uptake in the light.

A mechanism depending on phosphorylation should be inhibited by uncouplers. Our results show, however, that CO_2 fixation, which requires

both electron transport and ATP, is inhibited by FCCP concentrations which have no effect on light-dependent ion uptake or may even enhance it. This is conclusive evidence for a mechanism independent of phosphorylation (Smith & West, 1969; Raven, 1968, 1969). It is difficult to explain the enhancement of light-dependent K^+ and Cl^- uptake by FCCP. The uncoupler may enhance cyclic electron flow (Teicher-Zallen & Hoch, 1967), which, like noncyclic electron flow, may drive ion uptake without the involvement of ATP. Since we have not determined rates of electron flow under our conditions, these discussions are only speculative.

Although phosphorylation is unlikely to be the energy source for light-dependent ion uptake into cells of *A. spongiosa* leaves, our data do not allow distinction between a direct coupling to electron transport and a dependence on NADPH. Similarly, experiments showing different rates of light-dependent ion uptake in the presence and absence of CO_2 (Hope & Lilley, *personal communication*) do not permit unequivocal association with NADPH, because of the arguments of Jeschke and Simonis (1967) discussed above. Further, it is probable that photosynthetically produced NADPH remains in the chloroplast and may not be involved in transport events at other membranes (Heber & Santarius, 1965). The mechanism could only be resolved if the effect of FCCP on electron flow was investigated by direct observation in the tissue. Our results tentatively suggest a direct linkage between ion transport and electron flow in *Atriplex* leaf cells in the light.

Summary of Ion Transport Processes in Mesophyll Cells

Earlier experiments established the active nature of anion uptake to the vacuole of *A. spongiosa* mesophyll cells in the light and probably also in the dark (Osmond *et al.*, 1969). The present experiments suggest two separate mechanisms for providing the energy required to drive these active absorption processes.

(1) A light-dependent Cl^- pump which is linked to light-dependent K^+ uptake. This process is driven by photosynthetic electron transport or by NADPH.

The poor stoichiometry between net Cl^- uptake and K^+ uptake in the light may be accounted for by H^+ efflux or HCO_3^- uptake. It was not accompanied by Na^+ loss. Ouabain (10^{-3} M) had no effect on any of the net fluxes, and there is at present no evidence to suggest a coupled K – Na pump of the type invoked by MacRobbie (1962) and by Raven (1968, 1969) in algal cells.

(2) A KCl-transporting system dependent on energy sources in the dark. Absorption of KCl by this process is accompanied by a loss of Na^+ and by H^+ uptake. It is evidently similar to processes described earlier in the same tissue but under different conditions (Osmond, 1968). The absorption of KCl by this process is very sensitive to FCCP, but we are unable to specify further the coupling with respiratory ATP formation (Budd & Laties, 1964; Weigl, 1963, 1964*a*) or with electron flow (Robertson, 1968; Atkinson & Polya, 1967, 1968; Polya & Atkinson, 1969; Cram, 1969).

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