

## ION ABSORPTION IN *ATRIPLEX* LEAF TISSUE

### III.\* SITE OF METABOLIC CONTROL OF LIGHT-DEPENDENT CHLORIDE SECRETION TO EPIDERMAL BLADDERS

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#### Summary

The nature and location of the light-stimulated active transport of chloride to the epidermal bladders of *A. spongiosa* leaves was examined. Chloride transport to the bladder vacuole was found to have properties similar to those for light-dependent uptake to the mesophyll cell vacuole. It was almost totally inhibited by  $10^{-6}M$  3-(3,4-dichlorophenyl)-1,1-dimethylurea but was unaltered or stimulated in the presence of *m*-chloro- or *p*-trifluoromethoxy derivatives of carbonyl cyanide phenylhydrazone at concentrations which totally inhibited chloride uptake in the dark. Apparently active chloride uptake in the light is dependent on non-cyclic photosynthetic electron transport.

Evidence presented here and elsewhere indicates that chloroplasts of the stalk cell and bladder cytoplasm are not very active. Light-dependent active transport of chloride to the bladder vacuole is probably driven by mesophyll chloroplast photosynthesis. The role of the symplasm and the gland-like structure of the bladder complex is discussed.

#### I. INTRODUCTION

Large epidermal bladders are a feature of *Atriplex* and *Chenopodium* leaves. They are attached to the epidermis by gland-like stalk cells and ions are secreted from the lamina to the bladder via these cells. The anatomical and physiological characteristics of this ion transport have been described (Osmond *et al.* 1969) and in particular it was shown that chloride transport to the bladder was strongly stimulated by light. Chloride transport in the light was against an electrochemical potential gradient and it was concluded that an active pumping mechanism was involved within the multicellular system. However, we were unable to specify the location of the chloride pump within a particular cell or particular membrane system.

Other aspects of ion transport in the mesophyll cells of *Atriplex* leaves have been described (Osmond 1968; Lüttge, Pallaghy, and Osmond 1969). This paper relates metabolic aspects of ion uptake in mesophyll cells and chloride transport to the bladder, and provides evidence that light-stimulated chloride uptake to the bladder is dependent on photosynthetic electron transport in the chloroplasts of mesophyll cells.

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## II. MATERIALS AND METHODS

*Atriplex spongiosa* seedlings were grown in water culture as described earlier (Osmond *et al.* 1969). Leaf strips 3 by 12 mm were cut and floated on experimental solutions. Ion transport into the bladders of the lower epidermis was measured after uptake by brushing the bladders from the surface of frozen leaf slices as described earlier. Bladders and lamina were fixed to aluminium planchets with propanol-0.1% gelatin and after drying at 70°C, were counted in a Nuclear Chicago gas flow counter with a Micro-mil end window.

All experimental solutions contained 0.5 mM CaSO<sub>4</sub>. Uptake solutions containing KCl were labelled with <sup>36</sup>Cl. The free space was exchanged for two periods of 15 min in ice-cold unlabelled solution. <sup>14</sup>CO<sub>2</sub> fixation in lamina and bladders was followed during 15 and 30 min of exposure to 0.23 mM KH<sup>14</sup>CO<sub>3</sub> (containing approx. 1 μCi <sup>14</sup>C) in the presence of salt solutions. Bladders and lamina were separated as described above and <sup>14</sup>C not fixed by the tissue was released by the addition of 10N formic acid during drying. Uptake rates quoted were measured from the slope of the linear portions of uptake curves in time course experiments. Triplicate samples were used for each interval. Rates are expressed as μmoles per hour per gram fresh weight of tissue on the basis of external specific activity.

*Light-dependent chloride uptake and transport* is defined as uptake or transport in the light minus uptake or transport in the dark. Subtraction is permissible only if it can be shown that dark-dependent processes continue to operate in the light. This has been demonstrated in experiments with 0.5-mm leaf slices in which  $2 \times 10^{-6}$ M DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] inhibits chloride uptake in the light to the level of uptake in the dark. With this concentration of DCMU present, chloride uptake in the light further responds to uncouplers in a manner identical to chloride uptake in the dark (Lüttge, Pallaghy, and Osmond 1969). Similarly *light-dependent CO<sub>2</sub> fixation* is defined as fixation in the light minus fixation in the dark.

The DCMU was provided by Dr. N. K. Boardman. The uncouplers used, namely the *m*-chloro- and *p*-trifluoromethoxy derivatives of carbonyl cyanide phenylhydrazone, were supplied by Dr. P. Heytler.

## III. RESULTS

(a) *Diffusion in Leaf Strips of Various Widths*

Earlier experiments established that the optimum width of leaf strips for ion-uptake experiments was about 0.5 mm (Smith and Epstein 1964; Osmond 1968). However, the preservation of intact bladders on leaf strips demanded the use of wider sections which introduced diffusion problems in uptake. The diffusion limitation on ion uptake in slices 3 mm wide was shown by comparison with uptake in slices 0.5 mm in width. The ratio of chloride uptake rates in mesophyll cells in the light was approximately 10 for the narrower strips compared with the wider strips, showing that a large percentage of cells in the strips 3 mm wide were not in contact with the external solution (5 mM KCl).

(b) *Time Course of Chloride Uptake into Bladders and Lamina*

The time course of chloride uptake in the light and in the dark into the lamina and transport to the bladders of *Atriplex* leaves has been described earlier (Osmond *et al.* 1969). In the light, three phases may be distinguished:

Phase I : comparatively low rates of transport from 0 to  $\simeq$  16 hr after transfer of the 3 mm wide leaf strips to the labelled uptake solution;

Phase II : more rapid rates between  $\simeq$  16 and  $\simeq$  40 hr;

Phase III: a levelling off after  $\simeq$  40 hr.

In the experiment shown in Figure 1 uptake did not level off, even after 60 hr. This experiment indicates, however, that the slow rates in phase I were not due to the diffusion problem mentioned above. When the 3 mm wide leaf strips which had been in 5 mM KCl for 17 hr in the dark were transferred to the light (arrows in Fig. 1) there was still a lag phase of at least 7 hr before light stimulation of chloride uptake and transport became evident. Experiments with 0.5 mm wide strips have confirmed the finding that treatment of the tissue with light tends to lead to increased rates of chloride uptake. The mechanism of this adaptation to light is not known.

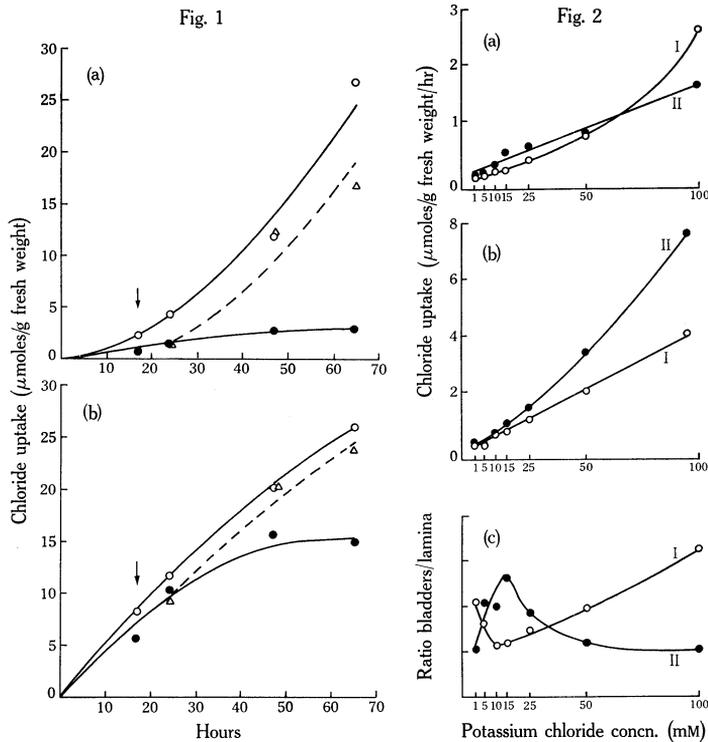


Fig. 1.—Time course of chloride transport to the bladders (a) and uptake by the lamina (b) in the light (O) and in the dark (●). After 17 hr in the dark (arrows) some tissue was transferred to the light ( $\Delta$ ). External concentration 5 mM KCl.

Fig. 2.—(a) and (b) Relation between rates of chloride uptake and transport in the light by bladders (a) and lamina (b) and external concentration during initial (phase I) and later (phase II) uptake (for explanation of phases see text). (c) Efficiency of transport to the bladders (i.e. ratio of bladders to lamina) in relation to external concentration during phases I and II of uptake.

(c) *Chloride Uptake into Bladders and Lamina in Relation to External Potassium Chloride Concentration*

The relation between rates of ion uptake by plant tissues and the external salt concentration is described by the well-known double hyperbolic isotherm (Epstein 1966). This relationship has been confirmed in thin slices of corn leaf tissue (Rains and Epstein 1967; Rains 1968).

Figure 2 shows that with 3 mm wide strips of *A. spongiosa* leaves isotherms in the high concentration range (1–100 mM KCl) were not hyperbolic. The rates of uptake given in Figures 2(a) and 2(b) were obtained from the slope of time curves, and isotherms are plotted from phase I and phase II rates (see Fig. 1). Interestingly, the efficiency of chloride accumulation into the bladders, as represented by the ratio of bladders to lamina and calculated from the data of Figures 2(a) and 2(b), changed with concentration and time [Fig. 2(c)]. In phase II of uptake the highest efficiency was found at about 15 mM KCl. In phase I efficiency increased with concentration up to 100 mM KCl.

(d) *Effect of Uncouplers on Chloride Uptake*

Table 1 shows the effect of both uncouplers used on chloride uptake by 3 mm leaf strips in experiments where bladders and lamina were not separated. The rates of uptake reported were calculated from the slopes of uptake versus time curves which were linear between 5 and 15 hr after transferring the leaf strips to the labelled solution. Both uncouplers had no effect on light-dependent chloride uptake and may have enhanced it at moderate concentrations. However, at concentrations of  $2 \times 10^{-6}$  M, the *m*-chloro- derivative inhibited light-dependent  $^{14}\text{CO}_2$  fixation to 13.5% of control in 0.5 mm wide strips, suggesting that it was an effective uncoupler of photophosphorylation. By contrast, chloride uptake in the dark was highly sensitive to both uncouplers. The specific effects of the uncouplers on chloride uptake in the dark and light rule out the possibility that they were destructive in these lengthy experiments.

TABLE 1  
CHLORIDE UPTAKE INTO LAMINA PLUS BLADDERS IN THE PRESENCE OF UNCOUPLERS  
External concentration 5 mM KCl

Uncoupler	Uncoupler Concn. (M)	Chloride Uptake in the Light ( $\mu\text{mole/hr/g}$ )	Chloride Uptake in the Dark ( $\mu\text{mole/hr/g}$ )	Light- dependent Chloride Uptake
Control	—	0.17	0.06	0.11
<i>m</i> -Chloro derivative	$1 \times 10^{-6}$	0.14	0.00	0.14
	$2 \times 10^{-6}$	0.12	0.01	0.11
	$3 \times 10^{-6}$	0.12	0.00	0.12
<i>p</i> -Trifluoromethoxy derivative	$5 \times 10^{-7}$	0.15	0.01	0.14
	$1 \times 10^{-6}$	0.09	0.002	0.09
	$2 \times 10^{-6}$	0.10	0.003	0.10

(e) *Effect of DCMU on Chloride Uptake and Translocation*

DCMU, an inhibitor of electron transport near the photosynthetic system II (Vernon and Avron 1965), had a marked effect on light-dependent chloride uptake by the lamina and on light-dependent chloride translocation to the bladders (Table 2).

Under certain conditions ( $1 \times 10^{-6}$ – $2 \times 10^{-6}$ M DCMU, 100 mM KCl) light-dependent chloride transport was entirely inhibited, i.e. chloride uptake and translocation in the light are reduced to dark rates. DCMU was not effective in the dark.

TABLE 2  
LIGHT-DEPENDENT CHLORIDE UPTAKE INTO THE LAMINA AND TRANSPORT TO  
THE BLADDERS IN THE PRESENCE OF DCMU  
Values are percentages of the controls

DCMU Concn. (M)	Bladders		Lamina	
	5 mM KCl	100 mM KCl	5 mM KCl	100 mM KCl
$5 \times 10^{-7}$	29	25	57	19
$1 \times 10^{-6}$	14	0	26	6
$2 \times 10^{-6}$	14	0	0	0

(f) *Effect of DCMU on CO<sub>2</sub> Fixation*

Figure 3 shows that light-dependent CO<sub>2</sub> fixation was affected by DCMU in the same way in the bladders and lamina. DCMU at a concentration of  $2 \times 10^{-6}$ M reduced CO<sub>2</sub> fixation in the light to the level of dark fixation in mesophyll and bladder cells.

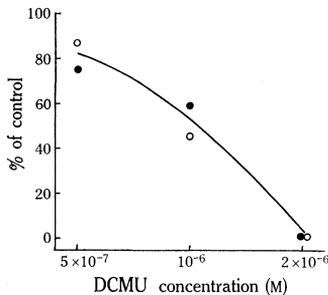


Fig. 3.—Effect of DCMU on the amount of <sup>14</sup>C found in bladders (●) and lamina (○) due to light-dependent <sup>14</sup>CO<sub>2</sub> fixation by the leaf strips.

The ratio, light-dependent CO<sub>2</sub> fixation to dark fixation, was very similar in sections of lamina 0.5 and 3 mm wide, but this ratio was considerably smaller for the bladders (Table 3). Leaf strips 0.5 mm wide fixed less CO<sub>2</sub> per unit fresh weight than leaf strips 3 mm wide. This indicates that minimizing the diffusion path for ion uptake does not facilitate CO<sub>2</sub> fixation by the lamina cells. The apparent lower rates of CO<sub>2</sub> fixation in strips 0.5 mm wide may have been due to a higher percentage of damaged cells.

#### IV. DISCUSSION

Uptake of ions into the mesophyll cells of *Atriplex* leaves and transport to the bladder vacuole is analogous to uptake by root cortical cells and subsequent ion transport to the xylem (Osmond *et al.* 1969). Double isotherm kinetics have been used to investigate the linkage between uptake from the solution and transport to the

xylem in root tissues (Lüttge and Laties 1966, 1967*a*, 1967*b*). This approach was based on the hypothesis that the double isotherm represents the control of ion transport at the plasma membrane and tonoplast of vacuolated cells (Torii and Laties 1966; Osmond and Laties 1968). We have been unable to use this approach to determine whether ions moving to the bladder vacuole of *Atriplex* leaves move through the mesophyll symplasm as shown in the root system. In fact, using the 3 mm wide strips necessary to preserve bladder structure, the typical double isotherm for ion uptake into mesophyll cells found in other leaf tissues (Rains and Epstein 1967; Rains 1968) could not be demonstrated. The diffusion of ions in thick leaf slices evidently complicates the kinetic relationship and makes this approach untenable.

TABLE 3  
CO<sub>2</sub> FIXATION BY BLADDERS AND LAMINA IN THE LIGHT AND DARK

Tissue	Width of Tissue Slices (mm)	CO <sub>2</sub> Fixation (μmoles/g/hr)		Light-dependent CO <sub>2</sub> Fixation (μmoles/g/hr)	Ratio Light/Dark
		Light	Dark		
Lamina	0.5	4.01	0.24	3.77	16
Lamina	3	11.25	0.62	10.63	17
Bladders	3	0.183	0.017	0.166	10

Consequently we have endeavoured to identify the site of the active chloride pump, demonstrated earlier (Osmond *et al.* 1969), using other techniques. Metabolic inhibitors have been used to analyse the light-dependent ion absorption processes in 0.5 mm slices of *Atriplex* mesophyll cells (Lüttge, Pallaghy, and Osmond 1969). These compounds have qualitatively similar effects in 3 mm leaf slices and justify similar conclusions about the energetic coupling of ion transport in the light.

The effect of DCMU on light-dependent chloride uptake and transport to the bladders shows that both processes are closely linked to photosynthesis. It is generally accepted that at the concentrations used DCMU inhibits electron transport near the photosynthetic system II (MacRobbie 1965; Vernon and Avron 1965; Jeschke and Simonis 1967). However, as has been pointed out by Jeschke and Simonis (1967), inhibition of transport by DCMU is not a sufficient argument for an ion pump coupled to photosystem II. Similarly, response of chloride uptake to wavelengths in the far red end of the spectrum, which suggests a participation of photosystem II in light-dependent ion uptake by 0.5 mm wide *A. spongiosa* leaf strips (Lüttge, Pallaghy and Osmond 1969), does not allow unambiguous distinction between energy provided by electron transport and phosphorylation (Jeschke 1967; cf. Lüttge 1969). In intact cells of higher plants and in the presence of CO<sub>2</sub>, cyclic electron flow and phosphorylation seem to be virtually inoperative when photosystem II is inhibited by DCMU. A chloride pump, dependent on energy derived from cyclic photophosphorylation, would also be inhibited by DCMU. However, as pointed out in discussion of light-dependent ion uptake by 0.5 mm wide *A. spongiosa* leaf strips (Lüttge, Pallaghy, and Osmond 1969) and also emphasized by others (Smith and West 1969), the effects of carbonyl cyanide *m*-chlorophenylhydrazone provide evidence

against an ATP-driven mechanism of light-dependent ion transport. Light-dependent chloride uptake and transport to the bladders is unaffected or even enhanced by concentrations of the uncouplers which almost entirely inhibit chloride transport in the dark (Table 1) and light-dependent CO<sub>2</sub> fixation in 0.5 mm strips. Therefore, light-dependent chloride uptake into the lamina and light-dependent chloride transport to the bladders is powered by a pump which is dependent on photosystem II electron transport. The data do not allow distinction between dependence on electron flow *per se* or the production of reducing potential (NADPH<sup>+</sup>). Similar chloride pumps have been shown to operate in algae (MacRobbie 1965, 1966; Raven 1967, 1968, 1969) but have not been detected in angiosperm aquatic plants (Jeschke 1967; Weigl 1967).

Although stalk cells and the bladder cytoplasm contain chloroplasts (Osmond *et al.* 1969), several observations indicate that these may not be very active photosynthetically:

- (1) The light-triggered, transient changes of membrane electrical potential between bladder cell vacuole and external solution (Osmond *et al.* 1969), shown to be dependent on photosystem II (Lüttge and Pallaghy 1969), are not observed in bladders attached to the isolated epidermis of *Chenopodium album* leaves. This observation suggests that the transients are due to events in mesophyll cells and not to light responses in bladder or stalk cell plastids.
- (2) Light-dependent CO<sub>2</sub> fixation, relative to dark fixation, is low in the bladders compared with the lamina (Table 3).
- (3) The value of 0.015 for the ratio of bladders to lamina of light-dependent CO<sub>2</sub> fixation is very small compared with the value of 0.2–1.0 for the ratio of bladders to lamina for light-dependent chloride uptake—see also Table 3 and Figures 1 and 2. This comparison indicates that photosynthetic reactions in the stalk cell and bladder cytoplasm may not be sufficiently active to support the chloride transport observed.

For these reasons it is very unlikely that the driving force for the light-dependent active transport of chloride to the bladder vacuole is located in the stalk cell or bladder cell cytoplasm. Rather, the active mechanism for the transport of chloride to the bladder seems to be located in the leaf mesophyll cells, and is linked to non-cyclic electron transport in the chloroplasts of these cells.

This conclusion focuses attention on an important unsolved problem. It is extremely difficult to explain how electron transport and charge separation on mitochondrial and chloroplast membranes may directly drive ion uptake (without involvement of ATP) at the plasmalemma and tonoplast of the same cell. Robertson (1968, pp. 72–4) speculates on the role of small vesicles moving in the cytoplasm. It is even more difficult to explain the linkage of light-dependent chloride transport in one cell to chloroplast electron transport in another. And yet, the results presented indicate that such a phenomenon exists.

Downhill symplasmatic transport, facilitated far beyond the rates of normal diffusion by an unknown mechanism, which is not directly linked with metabolic energy yet depends on maintenance of cytoplasmic structures by the cell metabolism,

occurs in many unspecialized tissues (Arisz and Wiersema 1966; see also reviews by Helder 1967 and Lüttge 1969). We have pointed out earlier that the gland-like ultrastructure of the *Atriplex* bladder system, in particular the numerous vesicles in the stalk and bladder cytoplasm, may have an important role in ion transport to the bladder vacuoles (Osmond *et al.* 1969). Structural elements and organelles of gland cells are not unique to these cells. However, glands appear to be specialized in terms of the amounts and arrangement of cytoplasmic organelles. It may be assumed that the specialized cytoplasm of gland cells, at a critical locality in the multicellular system, has a specific role in processes which lead to secretion (Lüttge and Krapf 1969).

The probable function of symplasmatic transport in connecting bladders and mesophyll in the *Atriplex* leaf system was discussed earlier (Lüttge and Pallaghy 1969; Osmond *et al.* 1969). The connection between chloroplast and symplasm is obscure but one is reminded of the dynamic relationships between chloroplasts and cytoplasmic elements shown by Hongladarom, Honda, and Wildman (1965). So far, symplasmatic transport is the only mechanism which can be envisaged to link an active transport process to an event of translocation or secretion powered by it, but separated far from it in a complex multicellular system.

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