METABOLIC EFFECTS ON ION FLUXES IN *NITELLA TRANSLUCENS*

I. ACTIVE INFLUXES

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Summary

The effects, on the light-dependent active ion fluxes in *Nitella translucens*, of interference with the normal patterns of photosynthetic electron flow and associated phosphorylation has been further studied. The results provide confirmatory evidence for the picture previously proposed.

In conditions in which only cyclic photophosphorylation is possible, in far-red light, in CO_2-free nitrogen, or in the presence of low concentrations of dichlorophenyldimethyl urea, there is inhibition of the active chloride influx without any effect on the active potassium influx. In conditions in which the electron flow is uncoupled from phosphorylation, in the presence of ammonium ions, imidazole, or carbonyl cyanide *m*-chlorophenyl hydrazone, there is inhibition of potassium influx, but no effect, or even a marked stimulation, of chloride influx. It is necessary to postulate that these effects are on the active component of potassium influx rather than on the passive component.

These results support the hypothesis that the potassium transport is dependent on ATP from photophosphorylation, but that the chloride transport is directly coupled to some electron transfer reaction, perhaps close to the second light reaction of photosynthesis, and is independent of ATP.

I. INTRODUCTION

It has been shown (MacRobbie 1962, 1964) that the ionic state of *Nitella translucens* is maintained by an active influx of potassium and efflux of sodium, and by an active influx of chloride; all these seem to act between the cytoplasm and the outside solution, presumably at the plasmalemma, although the membranes of the cellular organelles such as chloroplasts may also have powers of ionic regulation. Both the potassium influx and the chloride influx are strongly light-dependent. The chloride flux is reduced by a factor of 17 in the dark, but even so this dark influx must be almost entirely an active flux supported by respiration. The potassium influx is reduced by a factor of 8–10 in the dark, but it is not clear how much of the dark influx is active and how much is passive. Since chloride ions are so far from electrochemical equilibrium all the chloride influx must be active.

Thus ion transport in plant cells, in general, and in the same plant cell, in particular, may be driven either by respiration or by photosynthesis. Two possible links between ion transport and the driving process have been argued for many years—a direct coupling with electron-transfer reactions, or an indirect effect through the use of ATP produced in respiration by oxidative phosphorylation in the mitochondria, and in photosynthesis by photophosphorylation in the chloroplasts.

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Lundegårdh (for example, 1960) has argued in favour of the direct link between salt accumulation and electron transfer in a cytochrome system. Robertson (1960) has also developed the discussion of ion transport as a consequence of a primary separation of positive and negative charge in the process of respiratory electron transfer, and has suggested that a similar separation of charge in the early stages of photosynthesis may lead to a transfer of Cl^- across the cytoplasm and into the vacuole. Others have taken the view that the close linkage between salt accumulation and respiration is more likely to reflect the consumption, in the process of ion transport, of ATP produced in oxidative phosphorylation, with a consequent stimulation of the respiratory rate (Laties 1957; Sutcliffe 1959).

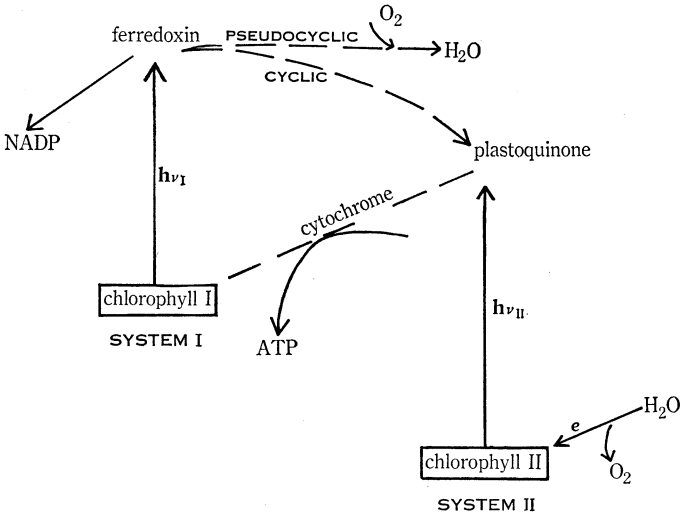

Fig. 1.—Postulated scheme for the electron-transfer reactions of photosynthesis, showing two light reactions driving electrons against the thermochemical gradient, and the subsequent downhill electron transfers driven by these light reactions, with the probable site of the associated phosphorylation. The postulated cyclic, pseudocyclic, and non-cyclic paths are shown.

A green tissue in which ion transport is linked to photosynthesis rather than to respiration offers a possibility of distinguishing between the two hypotheses, and experiments bearing on this question have already been published (MacRobbie 1965). Interpretation of specific light effects on ion fluxes in terms of the current scheme of photosynthetic electron transfer (shown in Fig. 1), suggested that chloride uptake was directly coupled to electron flow and did not depend on the associated phosphorylations. The present paper discusses the effects on the processes of ion transport of further interference with the normal patterns of electron flow and phosphorylation. As these are closely linked with the previous work, the earlier results will be summarized here. Normal photosynthetic electron flow involves two light reactions ($\mathbf{h}\nu_{\mathrm{I}}$ and $\mathbf{h}\nu_{\mathrm{II}}$) in series with one another, linked by a series of dark, electron-transfer reactions, down the thermochemical gradient, to which phosphorylation is coupled.

Light of wavelength greater than 705 mμ is incapable of exciting system II, whereas system I is functional up to 730 mμ; hence in far-red light only cyclic photophosphorylation is possible. By means of filters it was possible to separate the two light reactions, and the effect of the transition from filter I (approximately equal numbers of quanta below 705 mμ and between 705–730 mμ) to filter II (very few quanta <705 mμ) is seen in Table 1. It appears that under conditions when only cyclic photophosphorylation is possible, chloride uptake is inhibited but potassium uptake is unaffected. Thus it was argued that potassium uptake simply needs ATP,

TABLE 1

INFLUXES OF POTASSIUM, CHLORIDE, AND PHOSPHATE AND RATE OF CO_2 FIXATION FOR DIFFERENT LIGHT CONDITIONS

Filter*	Influxes (pmoles cm^{-2} sec^{-1})			Rate of CO_2 Fixation‡ (pmoles cm^{-2} sec^{-1})
	Potassium†	Chloride†	Phosphate‡	
FI	1·0 ±0·1	2·1 ±0·1	0·9	5·0
FI/4	1·05±0·12	1·9 ±0·2	—	—
FII	1·10±0·13	0·58±0·11	0·9	1·2
FIII	0·28±0·03	0·29±0·07	0·6	0·7
Dark	0·12±0·02	0·18±0·04	0·6	0·6

* The relative numbers of quanta below 705 mμ ($\mathbf{h}\nu_{II}+\mathbf{h}\nu_{I}$) and between 705 and 730 mμ ($\mathbf{h}\nu_{I}$ only) under filters FI, FII, and FIII are as follows:

	$\lambda<730$ mμ	$\lambda<705$ mμ	λ 705–730 mμ
FI	100	46	54
FI/4	25	11·5	13·5
FII	40	37	3·0
FIII	12·4	12·1	0·3

† See MacRobbie (1965).

‡ See Smith (1965).

irrespective of its source, whereas chloride uptake is somehow more directly linked to electron flow and is independent of ATP (MacRobbie 1965). Smith (1965) has done comparable experiments on the uptake of phosphate and on the fixation of carbon dioxide in *Nitella*, and these results are also shown in Table 1. These effects lend weight to the postulated biochemical states; in far-red light when non-cyclic photophosphorylation is prevented then CO_2 fixation is inhibited, but phosphate uptake remains unaffected. The effects of low concentrations of dichlorophenyldimethylurea (DCMU) were in all cases similar to those of far red light.

A further study on the effects of inhibitors on potassium and chloride influxes is now reported, with results which confirm the suggestion that chloride uptake does not depend on ATP, but is more directly linked with photosynthetic electron transfer. On this basis it should be possible to observe a stimulation of active chloride uptake in the presence of an uncoupling agent, in conditions in which there is stimulation of electron flow but a suppression of phosphorylation. This has now been observed.

II. Methods

Methods were similar to those described previously (MacRobbie 1964, 1965). Experiments were done on single internodal cells of *Nitella translucens*, which were 3–8 cm long and 600–1100 μ in diameter. Cells remained healthy in the laboratory in artificial pond water for some months, and only turgid cells showing rapid protoplasmic streaming were used.

The basic artificial pond water contained 1·0 mM NaCl, 0·1 mM KCl, and 0·1 mM $CaCl_2$. For uptake experiments this solution was labelled with the isotopes ^{42}K, ^{22}Na, or ^{36}Cl. Isotopes were assayed by Geiger counting. ^{36}Cl was dried down (in alkaline solution) on planchets and counted on an automatic, anti-coincidence, low background set-up (Isotope Developments Ltd.). ^{42}K was assayed either by liquid Geiger counting or in the automatic arrangement. In experiments in which both ^{42}K and ^{36}Cl were used, ^{36}Cl was counted after the decay of the short-lived ^{42}K, and ^{42}K was determined by liquid counting; a correction to the activity in the liquid tube for the ^{36}Cl activity was made, but the low specific activity of the chloride and the low efficiency of liquid counting for this isotope ensured that the correction was only 0·1–1·0% of the total.

Cells were soaked in the radioactive solution in a tank at 20±0·5°C, illuminated by two 40-W "warm-white" fluorescent tubes, giving a total incident light intensity of 13,400 erg cm^{-2} sec^{-1}.

Table 2

Effect of CCCP* on potassium and chloride influxes

CCCP Concn. (M)	Chloride Influx (pmoles cm^{-2} sec^{-1})		Potassium Influx (pmoles cm^{-2} sec^{-1})
	Light	Dark	
0	1·0 ±0·1	0·33±0·08	0·44±0·09
5×10^{-6}	0·77±0·09	0·52±0·07	0·14±0·01

* Carbonyl cyanide *m*-chlorophenyl hydrazone.

III. Results

(*a*) *Effects of CCCP*

Carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) has been shown to act as an uncoupler of both oxidative phosphorylation and photosynthetic phosphorylation (Heytler 1963). Its effects on the influxes of potassium and chloride to the cell are shown in Table 2; in the light there was very little effect on chloride influx but a marked inhibition of potassium influx, and there was a clear stimulation of the dark chloride influx in the presence of 5×10^{-6}M CCCP.

Spanswick (personal communication) has found that the potential between vacuole and medium becomes more negative for an hour or more after the addition of CCCP to the bathing solution, and that during this time there is also a decrease in electrical resistance; the decrease in potassium influx is observed during this initial

period and hence cannot be due to a change in passive influx caused by a potential change. It is necessary to suppose an effect on the active influx of potassium.

(*b*) *Effects of Imidazole and Ammonium Ion*

Imidazole and ammonium ion have been shown to uncouple photosynthetic phosphorylation in isolated chloroplasts (Good 1960; Hind and Whittingham 1963).

It was shown that imidazole inhibited potassium influx in *Nitella* without having any effect on chloride influx, measured on the same cells in an experiment

TABLE 3

EFFECT OF IMIDAZOLE ON POTASSIUM AND CHLORIDE INFLUXES

Imidazole Concn. (M)	Chloride Influx (pmoles cm^{-2} sec^{-1})			Potassium Influx (pmoles cm^{-2} sec^{-1})
	Expt. 1	Expt. 2	Expt. 3	
0	2·50±0·13	2·0 ±0·16	2·30±0·14	1·01±0·15
10^{-4}	3·63±0·30	3·44±0·23	2·16±0·18	0·20±0·05
10^{-3}	—	—	2·28±0·06	0·14±0·02

using ^{42}K and ^{36}Cl together (MacRobbie 1965). In further experiments a marked stimulation of chloride influx has now been found in the presence of imidazole. These results are shown in Table 3; the imidazole was used at 10^{-3}M or 10^{-4}M as imidazole-H_2SO_4 buffer at pH 7·2.

TABLE 4

POTASSIUM AND CHLORIDE INFLUXES IN AIR AND CO_2-FREE NITROGEN

Conditions of Experiment	Chloride Influx (pmoles cm^{-2} sec^{-1})	Potassium Influx (pmoles cm^{-2} sec^{-1})
Air	1·5 ±0·1	1·06±0·08
CO_2-free nitrogen	0·69±0·06	0·86±0·13
CO_2-free nitrogen	0·52±0·06*	—

* Result for experiment with different bubbling rate, to which a low rate of pseudocyclic flow would be particularly sensitive.

The substitution of 0·4 mM ammonium sulphate for an equivalent amount of sodium sulphate had comparable effects; the potassium influx in light was reduced (to 12% of the control value), while the chloride influx was stimulated (to 140% of the control value). With ammonium ion, a part of the effect on potassium could be the result of competition between NH_4^+ and K^+ rather than uncoupling, but no such explanation is tenable for the effect on chloride influx. The results are consistent with

the view that both imidazole and ammonium ion are acting as uncoupling agents in photosynthetic phosphorylation.

(c) *Effects of CO_2-free Nitrogen*

In CO_2-free nitrogen, in the absence of a terminal acceptor for a non-cyclic path of electron flow, only cyclic photophosphorylation should be possible. The effects on the ion fluxes of bubbling the solution with CO_2-free nitrogen are shown in Table 4. There is a marked inhibition of chloride influx although not to the dark level. It is likely, however, that the difference reflects the impossibility of maintaining a green cell completely anaerobic in light, with moderately fast but not excessive rates of bubbling; a certain amount of oxygen may well be recycled, maintaining a low rate of a pseudocyclic flow. There is no significant effect of nitrogen bubbling on potassium influx.

IV. Discussion

The results reported here are consistent with the picture previously proposed. In conditions in which only cyclic photophosphorylation is possible, in far-red light, in CO_2-free nitrogen, or in the presence of low concentrations of DCMU, there is inhibition of the active chloride influx without any effect on potassium influx. Thus it appears that the Cl^- uptake requires the participation of the second light reaction, and does not simply need ATP.

Under conditions in which the electron flow is uncoupled from phosphorylation, in the presence of ammonium ions, imidazole, or CCCP, there is inhibition of potassium influx but no effect, or even a marked stimulation, of chloride influx. This stimulation lends strong support to the hypothesis that chloride uptake is directly coupled to some electron transfer reaction, and is independent of the associated phosphorylation.

It was argued that the effect of CCCP on potassium influx, taken with Spanswick's electrical measurements on cells treated with CCCP, can only be interpreted as an effect of the uncoupler on the active component of potassium influx. This is consistent with the partition of potassium influx in *Nitella translucens* into active and passive components suggested earlier (MacRobbie 1962).

In the light of the CCCP results one must suppose that a major part of the normal potassium influx is by an active mechanism—with some 70–80% of the flux active, and only 20–30% by a passive mechanism. This is in contrast to the state in *Chara australis* where probably most of the potassium influx is passive. But in *N. translucens* only a limited decrease in influx could be achieved by cutting down the passive component of influx without any effect on the active flux, the major influx component. Hence the effects of dark and DCMU seem also to be on the active component of influx. This interpretation is lent credence by the observation that in *C. australis* there is no significant difference between the potassium influxes in light and dark (Hope 1963). It is also consistent with the observations of Hope (1965) in *Chara* and Spanswick (personal communication) in *Nitella*, that, in normal pond water, there is no effect on the resistance of the plasmalemma on going from light to dark.

The results of Smith (1965) on phosphate uptake are also consistent with this interpretation. Thus Smith found inhibition of phosphate uptake by CCCP, and by

imidazole and ammonium ion, but not by CO_2-free nitrogen or by low concentrations of DCMU; as the dark influx of phosphate is some 65–70% of the light influx it is interesting that the photosynthetic uncouplers, imidazole and ammonium ion, reduced the phosphate influx in light to the normal dark level, whereas the general uncoupler CCCP reduced it considerably further, to about 6% of the light level.

This association of chloride uptake with the second light reaction is interesting in view of the fact that it is this particular part of photosynthesis for which Cl^- (or Br^-) is an essential cofactor (Bové *et al.* 1963). This suggests some capacity for at least temporary chemical association of chloride with some component of the photosynthetic chain, and this may have relevance for the active uptake process. It is, however, possible that it is some reduced compound produced in a non-cyclic photosynthetic path which is responsible for chloride uptake, and the locus is not precisely defined by the work to date.

Another point is the distinction between chloride and phosphate in respect of both light sensitivity, and dependence on ATP. Chloride is strongly light-dependent and independent of ATP; phosphate is stimulated in light but the dark influx is still 65–70% of the light value, and the transport seems to depend on phosphorylation (Smith 1965). This suggests a mechanism for phosphate uptake independent of that for chloride or bromide (which is also strongly light-dependent and stimulated by uncoupling). It would be interesting to look at other anion fluxes, such as nitrate or sulphate, to see to what extent a general "anion pump" may be invoked.

The question of a direct link between the initial anion uptake to the cell and electron transfer reactions in other plant cells was discussed previously (MacRobbie 1965); it was argued that a similar picture to that proposed here was tenable in higher plant tissues, and that the available evidence could be read in support of a two-stage salt accumulation—an initial anion uptake into the cytoplasm, directly linked with electron transfer reactions, and a metabolic transfer of salts from cytoplasm to vacuole which may depend on ATP.

The nature of the link between chloride uptake to the cell and specific electron transfer reactions remains obscure. The Robertson hypothesis that such a direct link in mitochondria leads to chloride accumulation within the mitochondria, followed by discharge of ions to the vacuole as the mitochondria come temporarily in contact with the tonoplast, is difficult to transfer to *Nitella*. The light dependence seems to require a direct link with electron shifts in components bound to the internal membranes of the chloroplasts, but the chloroplasts are stationary and remote from the tonoplast, and a direct discharge to the central vacuole seems to be impossible. Thus considerable modification of the hypothesis is necessary, but it is not yet possible to construct a concrete model on the available experimental evidence. It is hoped that further work might provide a firmer base for speculation.

V. Acknowledgments

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