Light Stimulation of Active Transport in *Hydrodictyon africanum*

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ABSTRACT The mechanism of light stimulation of active K and Cl influx and active Na efflux, in *Hydrodictyon africanum* has been investigated using different wavelengths of red light and different gas mixtures, and the inhibitors DCMU and CCCP. The active Cl influx requires photosystem 2, since its relative quantal efficiency falls with increasing wavelength of red light, and it is as sensitive to the inhibitor DCMU as is photosynthesis; it is relatively insensitive to the uncoupler CCCP. The active K influx and active Na efflux are inhibited by CCCP, but the relative quantal efficiency of these processes increases with increasing wavelength of red light, and they are relatively insensitive to DCMU. These cation fluxes can be supported by cyclic photophosphorylation, whereas Cl influx needs photosystem 2 but probably not ATP.

INTRODUCTION

Light-stimulated ion transport in plants is mediated by light absorbed through the chlorophyll system (van Lookeren Campagne, 1957; Tazawa, 1961; Simonis and Mechler, 1963). The mechanism of this stimulation does not seem to be via net photosynthesis, since light-dependent ion transport is not inhibited in CO_2-free air (van Lookeren Campagne, 1957; Marrê, Forti, Bianchetti, and Parisi, 1963). Hence the light stimulation must involve some partial reaction(s) of photosynthesis.

It is generally held that two distinct light reactions are involved in photosynthesis in plants which contain chlorophyll *a*. This has suggested both the possibility that light-dependent processes in green plants may be powered by one or another of these light reactions independent of the other, and the means of experimentally testing this possibility. The recent reviews of Witt et al. (1965) and of Duysens (1964) interpret the recent results in terms of the popular "series" formulation. The results presented in this paper are interpreted in terms of this series scheme, but are also consistent with the various other schemes discussed by Clayton (1965).

The two light reaction systems may be separated in green plants by means of their different relative absorptions in the 680–730 mμ region of the spec-

trum. The proportion of the total absorbed light which sensitizes photosystem 2 (concerned with the oxidizing end of photosynthetic electron transport) decreases beyond 700 mμ; the proportion absorbed by photosystem 1 increases. Photosystem 1 is concerned with the reducing end of photosynthetic electron transport, and powers cyclic photophosphorylation. This effect is seen as a drop in the quantum efficiency of a process which needs photosystem 2, with or without photosystem 1, in the range 690–730 mμ, and an increase in the quantum efficiency of a process which needs only system 1 (Hoch and Martin, 1963; Sauer and Biggins, 1965; Sauer and Park, 1965). Thus the wavelength-dependence of the process in the red-far-red region can give information as to whether or not photosystem 2 is involved.

The photosynthetic inhibitor DCMU (3′-(3,4-dichlorophenyl), 1′,1′ dimethylurea; see Bishop, 1958) is believed to inhibit photosystem 2 (Gingras, Lemasson, and Fork, 1963; Gingras and Lemasson, 1965; Gould and Bassham, 1965). This inhibitor provides an additional method of finding out whether photosystem 2 is required in a given process. These methods have been applied to active ion transport processes in *Nitella translucens* by MacRobbie (1965, 1966) and Smith (1966). They concluded that Cl influx, like complete photosynthesis, required photosystem 2, while K influx and H_2PO_4 influx could occur under conditions where only photosystem 1 was operative. The results of Simonis (1964) indicate that H_2PO_4 influx in *Ankistrodesmus braunii*, presumably an active process, can be supported by photosystem 1 alone. The processes which can be supported by photosystem 1 alone (K, H_2PO_4 influx) were much less sensitive to the removal of $CO_2 + O_2$ from the external solution than was the Cl influx which requires system 2. Presumably Cl influx requires a non- or pseudocyclic electron flow with CO_2 or O_2 as terminal electron acceptor, while the processes which can be supported by cyclic electron transport alone do not.

Simonis (1964) has presented evidence, based on experiments with DCMU, and with various light wavelengths and gas mixtures, that the light-enhanced incorporation of ^{32}P into organic combination in intact *Ankistrodesmus* can proceed by a cyclic process as well as a non- or pseudocyclic one. The results of Forti and Parisi (1963), based on measurement of ATP content, also indicate the occurrence of cyclic photophosphorylation in vivo. The results of MacRobbie (1966) and of Smith (1966) with the uncoupling agent CCCP (carbonyl cyanide *m*-chlorophenyl hydrazone; see Heytler and Pritchard, 1962; Gould and Bassham, 1965) indicate that the ion transport processes which can be driven by system 1 alone are more sensitive to this uncoupler than is Cl influx, which requires system 2. These results show that Cl influx in *Nitella* occurs by a process which is apparently ATP-independent but which needs system 2, while K and H_2PO_4 influx need ATP but not system 2.

Previously Raven (1967) showed that light can stimulate both influx and

efflux of K, Na, and Cl in *Hydrodictyon africanum*. The greatest light stimulation is found in the case of the active fluxes (K and Cl influx and Na efflux). The way in which light energy powers these active fluxes is discussed in this paper. Experiments were carried out in which the effects on the active fluxes of light wavelength, metabolic inhibitors, and different gas mixtures were compared with their effects on photosynthesis.

MATERIALS AND METHODS

H. africanum was cultured as described previously (Raven, 1967). Methods of measuring the tracer influx of K and Cl, the tracer efflux of Na, and the photosynthetic fixation of $^{14}CO_2$ were as described in that paper.

The light filters used to separate the two photosystems were of the type used by Mapson (1964), MacRobbie (1965), and Smith (1966). They were of the "cut off" type, which transmits above but not below a given wavelength. Filters 1, 2, and 3 transmit 20% of the incident light at 670, 710, and 725 mμ respectively. The percentage transmission of the filters as a function of wavelength is set out in Table I.

The light source used with the filters was a 500 w tungsten filament lamp. Total energy flux incident on the filters at the standard distance of 50 cm was 3.10^5 erg. cm^{-2} sec^{-1}, measured with a solarimeter of the type described by Monteith (1959; MacRobbie, 1965). The total energy flux incident on the filters was varied by altering the distance between the filter arrangement and the lamp, and by the use of perforated zinc neutral filters.

Crude absorption spectra of the fragments obtained by grinding *H. africanum*, or of chloroplasts obtained by differential centrifugation of ground cenocytes, were measured with a Hilger spectrophotometer. The red absorption peak was at 675–685 mμ. Scattering made the rest of the red spectrum difficult to interpret, so the averaged absorption spectra of Emerson and Lewis (1943) and Brown and French (1959) for *Chlorella* were used; these spectra also peak at 675–685 mμ. From this absorption spectrum and the known transmission properties of the filters, the relative quantal absorption by the cells was calculated for different values of the light intensity incident on the filters (MacRobbie, 1965).

CO_2-free air was obtained by pumping air through two wash bottles of concentrated KOH solution, then $Ca(OH)_2$ solution to check that all the CO_2 had been removed, and finally through water at the temperature of the experiment before passing through the experimental solutions. O_2-free N_2 from a cylinder was passed through a soda-lime tower to remove traces of CO_2, then through water, and finally through the experimental solutions. Control experiments were also aerated (with normal air).

Cells were pretreated for 2 hr under the same conditions of light wavelength and intensity as were used in the measurements before the flux measurements were made. This pretreatment was sufficient to establish steady fluxes under all the illumination conditions.

Cells were pretreated for 2 hr in inactive DCMU solutions before the experiments in tracer solutions. DCMU at 50 μM is not toxic to the cenocytes over a period of 2–3 days, and does not affect the membrane PD over the longest experimental period

of 8 hr. CCCP causes an initial slight hyperpolarization at the concentrations used; a pronounced depolarization occurs after 3 hr. CCCP experiments were therefore limited to 2½ hr, including pretreatment. All experiments were carried out at 14°C.

RESULTS

The results shown in Table II indicate that when aeration by CO_2-free air replaced normal air, none of the light-stimulated fluxes investigated (K and Cl influx, Na efflux) was significantly affected. Thus these ion transport processes are not dependent on net photosynthesis. (All results are quoted as the mean ± the standard error of the mean.)

TABLE I

Composition	Filter 1 Ilford 205 Wratten 34	Filter 2 Ilford 206 Ilford 806	Filter 3 Ilford 206 Wratten 45
Relative quantal intensity transmitted			
Below 730 mμ	100	40	12.6
Below 705 mμ	46	3.1	0.3
705–730 mμ	54	37.0	12.3

TABLE II

ION FLUXES IN CO_2-FREE AIR IN LIGHT

Flux	Air	CO_2-free air
pmoles cm⁻² sec⁻¹		
Cl influx	2.04±0.34	2.09±0.33
K influx	1.24±0.21	1.04±0.19
Na efflux	0.37±0.05	0.36±0.04

Fig. 1 illustrates the "red drop" phenomenon in photosynthetic $^{14}CO_2$ fixation by *H. africanum*. For equal quantal absorption of light-limiting incident intensities, the photosynthetic rate decreases with increasing wavelength in the range 680–730 mμ. This is attributable to increasing rate limitation by photosystem 2, the relative absorption of which decreases in this range of wavelengths. Fig. 2 shows that a similar red drop occurs for light-stimulated Cl influx, thus implicating photosystem 2 in this process. The relative difference between filter 1 and filter 2 appears to be lower for Cl influx than for photosynthesis, probably because in the case of Cl influx the lowest light intensity used under filter 1 was still almost sufficient to saturate the process. Fig. 1 shows that this intensity of filter 1 light was well below that needed to saturate photosynthesis.

Figs. 3 and 4 show that, for K influx and Na efflux, the situation seen for photosynthesis and Cl influx is reversed. For equal quantal absorption at the

lowest intensities, the light transmitted by the long wavelength filter 3 is more efficient than that transmitted by the short wavelength filter 1; filter 2 is intermediate. In no experiment were these cation fluxes less efficiently supported, per absorbed quantum, under filters 2 and 3 than under filter 1. These results are consistent with an ability of system 1 to support these cation fluxes. The relative light absorption by system 1 increases over the range of wavelengths (680–730 mμ) covered by the three filters.

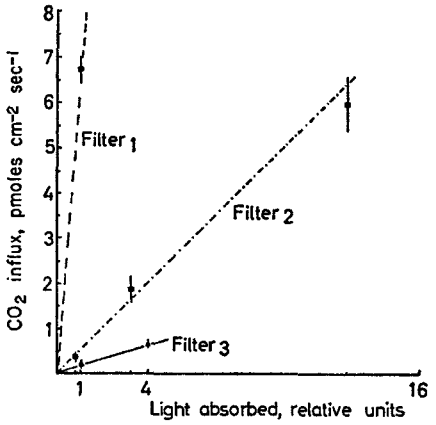

FIGURE 1. $^{14}CO_2$ fixation from 1 mM total CO_2, pH 7.4. Effect of light transmitted by the three light filters. $^{14}CO_2$ fixation saturated under filter 1 at 30 pmoles cm^{-2} sec^{-1}, with 25 units of absorbed light. $^{14}CO_2$ fixation in the dark in this experiment was less than 0.01 pmoles cm^{-2} sec^{-1}.

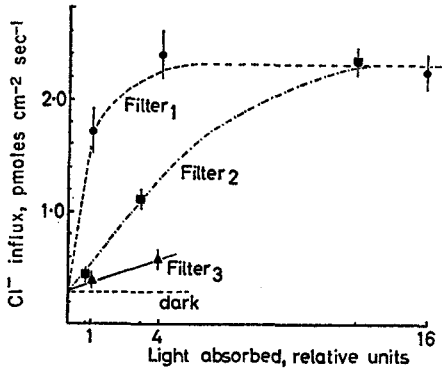

FIGURE 2. ^{36}Cl influx. Effect of light transmitted by the three light filters, and of darkness.

The finding of a clear differential effect of the three light filters on CO_2 fixation and Cl influx on the one hand, and on K influx and Na efflux on the other, indicates that photosystem 2 is needed for Cl uptake as it is for photosynthesis, but not for the active cation fluxes. This conclusion is independent of any uncertainties as to the absorption spectrum of *H. africanum*, or the extent to which light of various wavelengths suppresses the dark component of the uptake.

Figs. 1–4 also show that the light-saturated fluxes were the same under all the wavelengths which could be tested with the light intensity available. These

light-saturated values were the same as those under fluorescent illumination (compare the results of van Lookeren Campagne, 1957, on Cl influx in *Vallisneria*, and of Pickett and Myers, 1966, on *Chlorella* photosynthesis).

Fig. 5 illustrates the effect of various concentrations of the inhibitor DCMU on $^{14}CO_2$ fixation, K and Cl influx, and Na efflux. It will be seen that those processes for which the experiments with filters suggested a system 2 requirement are inhibited almost to the dark rate by 10^{-7} M DCMU, while K influx

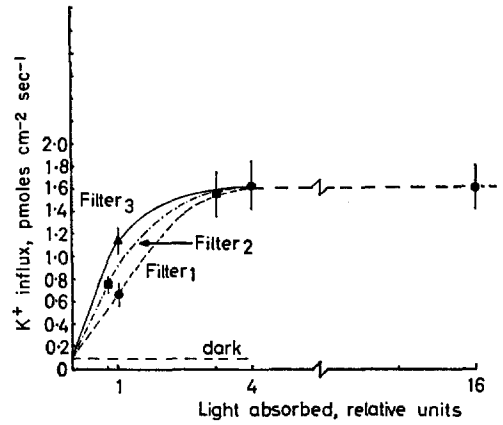

FIGURE 3. ^{42}K influx. Effect of light transmitted by the three light filters, and of darkness.

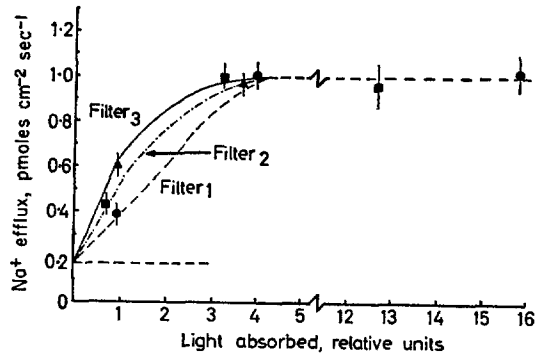

FIGURE 4. ^{22}Na efflux. Effect of light transmitted by the three light filters, and of darkness.

and Na efflux are not significantly inhibited at this concentration. In some experiments K influx is slightly inhibited by 10^{-7} M DCMU, with the main inhibition not occurring until 5.10^{-7} M, as shown in Fig. 5. Thus it is possible that a component of the K influx has an energy source similar to that powering the Cl influx. However, the main component of 80% or more of the K influx is much less sensitive to DCMU than is the Cl influx.

Table III compares the effect of CO_2- and O_2-free nitrogen gas with that of air on K and Cl influx and Na efflux. The removal of both potential exogenous terminal electron acceptors (CO_2 and O_2) has a greater inhibitory

effect on Cl influx, which cannot be supported by photosystem 1 (i.e. cyclic electron flow) than on K influx or Na efflux, which can be so supported.

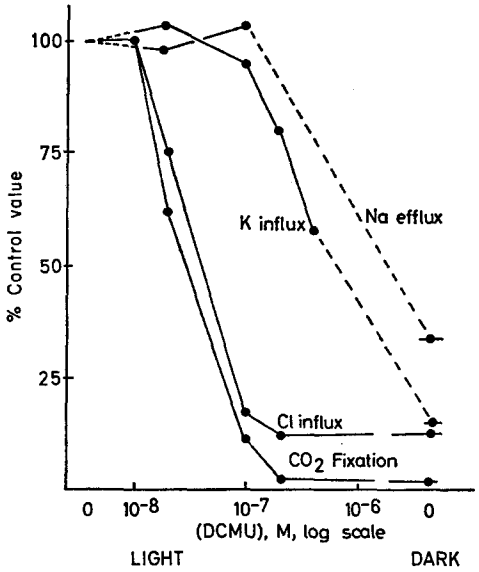

FIGURE 5. Ion fluxes and $^{14}CO_2$ fixation. Effect of various concentrations of DCMU in the light, compared with effects in light and darkness in the absence of the inhibitor. For each ion flux, and for $^{14}CO_2$ fixation, control values (=100%) are, in pmoles cm^{-2} sec^{-1}

$^{14}CO_2$ fixation: 18.1 ± 2.3 (1 mM total CO_2, pH 6.0)
^{36}Cl influx: 2.42 ± 0.38
^{42}K influx: 1.25 ± 0.18
^{22}Na efflux 0.60 ± 0.09

Per cent standard error of the mean for the various treatments is not greater than that for the respective light controls.

TABLE III
ION FLUXES IN N_2 IN LIGHT

Flux	Air	CO_2- and O_2-free N_2
pmoles cm^{-2} sec^{-1}		
Cl influx	2.04±0.34	1.03±0.17
K influx	1.18±0.19	0.85±0.08
Na efflux	0.37±0.05	0.33±0.09

Fig. 6 shows the effect of various concentrations of the uncoupler CCCP on CO_2 fixation, K and Cl influx, and Na efflux. CO_2 fixation in the light is almost completely inhibited at 5.10^{-6} M CCCP; this is consistent with an uncoupling role for CCCP in *H. africanum*. K influx and Na efflux are almost as sensitive, the maximal effect of CCCP being reached at 5.10^{-6}–10^{-5} M. In

contrast, Cl influx is much less sensitive to this inhibitor; when the control influx is over 2.0 pmoles cm^{-2} sec^{-1}, 10^{-5} M CCCP can cause an inhibition of the influx of up to 35%. It would thus appear that the cation fluxes are, like photosynthesis, much more sensitive to a treatment which lowers the rate of ATP synthesis than is Cl influx.

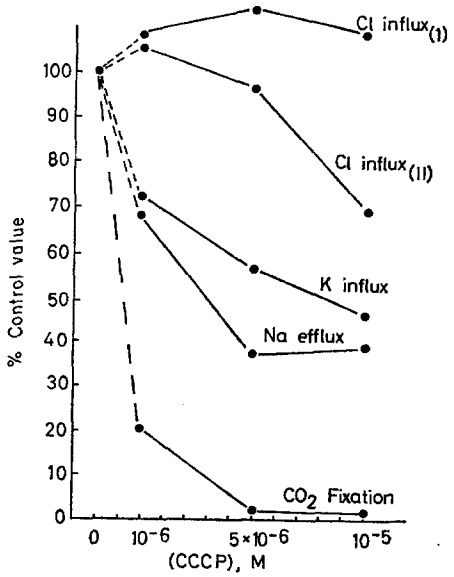

FIGURE 6. Ion fluxes and $^{14}CO_2$ fixation. Effect of various concentrations of CCCP in the light. For each ion flux, and for $^{14}CO_2$ fixation, control values (=100%) are, in pmoles cm^{-2} sec^{-1}

$^{14}CO_2$ fixation: 25.6 ± 2.8 (1 mM total CO_2, pH 6.0)
^{36}Cl influx: 1.11 ± 0.11 (I); 2.12 ± 0.23 (II)
^{42}K influx: 1.23 ± 0.18
^{22}Na efflux: 0.32 ± 0.03

Per cent standard error of the mean for the various treatments is not greater than that for the respective light controls.

The effect of CCCP on K influx and Na efflux in the light appears to be an effect on the metabolic component of these fluxes. In an experiment at 4°C in the light when the control K influx was 0.31 ± 0.04 pmole cm^{-2} sec^{-1}, the corresponding flux in the presence of 5.10^{-6} M CCCP was 0.36 ± 0.07 pmole cm^{-2} sec^{-1}. In the light at 3°C, when the control Na efflux was 0.075 ± 0.014 pmole cm^{-2} sec^{-1}, the flux in the presence of 5.10^{-6} M CCCP was 0.082 ± 0.009 pmole cm^{-2} sec^{-1}. It has been found that the level to which CCCP inhibits the K influx and Na efflux is the same as the ouabain-insensitive flux. In experiments on cells from the same net as were used in the CCCP experiments shown in Fig. 6, 0.5 mM ouabain inhibited the K influx in the light to 55% of

the control value; the Na efflux in the light was inhibited to 40% of the control flux. These two inhibitors added together had no greater effect than either added alone.

DISCUSSION

The red drop in quantum efficiency of Cl influx in *H. africanum* reported in this paper is similar to the phenomenon noted by Bishop and Gaffron (1963) for H_2 photoevolution in H_2-adapted *Scenedesmus*, and for acetate photometabolism in green algae other than *Chlamydobotrys* (Weissner, 1964). Other processes in vivo have been shown to require photosystem 2 on the grounds of their action spectra are Cl influx in *Nitella translucens* (MacRobbie, 1965), long term glucose uptake in *N. translucens* (Smith, 1967), nitrite photoreduction in *Anaebena* (Fujita and Hattori, 1963), and photophobotaxis in *Phormidium* (Nultsch, 1962 *b*). It would be expected that the processes (H_2 photoevolution and nitrite photoreduction) which require transfer of reducing equivalents from water to an electron acceptor require system 2. The requirement for photosystem 2 in acetate and glucose photometabolism, and in Cl influx, is less easily explained, since it might be expected that the ATP or redox energy required could be produced by photosystem 1 alone.

An increased quantum efficiency at far-red wavelengths, such as is seen for K influx and Na efflux in *H. africanum*, has been shown for CO_2 fixation in H_2-adapted *Scenedesmus* (Bishop and Gaffron, 1962), acetate photometabolism in *Chlamydobotrys* (Weissner, 1965), and anaerobic glucose photometabolism in *Chlorella* (Weissner, 1966). Other in vivo processes believed, from their action spectra, to be supportable by photosystem 1 alone include nitrite photoreduction in H_2-adapted *Anaebena* (Fujita and Hattori, 1963), photokinesis in *Phormidium* (Nultsch, 1962 *a*), K and H_2PO_4 uptake in *Nitella translucens* (MacRobbie, 1965; Smith, 1966), H_2PO_4 uptake in *Ankistrodesmus braunii* (Simonis, 1964), and anaerobic glucose conversion to polysaccharide and anaerobic synthesis of isocitrate lyase in *Chlorella* (Syrett, 1966). All these processes, except possibly nitrite photoreduction, seem to require ATP, and the function of photosystem 1 is probably to produce ATP by cyclic photophosphorylation. Nitrite photoreduction, and possibly CO_2 fixation, in H_2-adapted algae, may need noncyclic electron flow in photosystem 1.

Thus the light-stimulated active ion fluxes in *H. africanum* contain representatives of the possible classes of photosynthetic partial processes, those which require photosystem 2 and those which do not. The conclusions drawn from the filter experiments are supported by the effects of DCMU, and the influence of N_2. The slight inhibition by N_2 of the processes (K influx, Na efflux) which can be supported by cyclic electron flow indicates that under the conditions of the experiment with N_2 the fluxes were partly supported by pseudocyclic electron flow and the accompanying phosphorylation. An alter-

native explanation is that cyclic electron flow in *H. africanum* needs O_2 for redox poising of intermediate electron carriers (Whatley, 1963; Zweig and Avron, 1965). The O_2 requirement for Cl influx is consistent with O_2 acting as terminal electron acceptor in pseudocyclic electron flow. An isotopic O_2 exchange, dependent on photosystem 2, and consistent with the in vivo occurrence of pseudocyclic electron flow, is known in green algae (Brown and Weis, 1959; Hoch, Owens, and Kok, 1963; Govindjee, Owens, and Hoch, 1963). The finding that solute transport processes which appear from experiments with different light wavelengths to require photosystem 2 are more sensitive to lack of O_2, and to DCMU, than those which require only photosystem 1, is in agreement with the findings of MacRobbie (1965, 1966) and of Smith (1966, 1967). Other reports of an O_2 requirement for light-stimulated Cl influx are those of van Lookeren Campagne (1957), working with *Vallisneria*, and of Neilson (1964), who used *Chlorella*.

The inhibition of K influx by CCCP is consistent with a requirement for ATP, or some high energy precursor, in these active transport processes. Such a requirement for ATP, together with the linkage found between active K influx and active Na efflux, and the sensitivity of these two fluxes to ouabain (Raven, 1967), suggests the operation of a ouabain-sensitive ATPase system similar to that postulated in animal cells (see review by Skou, 1964).

Since most of the light-stimulated portion of the K influx is ouabain-sensitive, the effects of light wavelength, DCMU, and CCCP on light-stimulated K influx will predominantly be effects on the ouabain-sensitive portion of this flux. The effects of these treatments on the ouabain-insensitive portion of the K influx in the light are difficult to determine in experiments in which ouabain is not used. In several such experiments a slight (10–20%) inhibition of K influx was found at a lower concentration of DCMU than was needed to produce the major part of the inhibition (MacRobbie, 1965). Other experiments (Raven, data to be published), in which ouabain was used in conjunction with other inhibitors and with different light wavelengths, indicate that this effect of DCMU was an effect on a component of the K influx which was stimulated by light but which was insensitive to ouabain.

The insensitivity of Cl influx to CCCP, when the effect of this uncoupler on Cl influx is compared with its effect on active cation fluxes, suggests some degree of independence of Cl influx and ATP formation. Cl influx is not significantly inhibited at a concentration of CCCP which completely inhibits light-dependent $^{14}CO_2$ fixation. If the Cl influx does require ATP, then it is difficult to bring the observation that the Cl influx is not supported by cyclic photophosphorylation into line with the finding that the Cl influx is much less sensitive to CCCP than are the active cation fluxes. It would be necessary to assume that ATP produced by cyclic photophosphorylation is in some way unavailable to the pump, while ATP produced by non- or pseudocyclic photophosphorylation is more readily available to Cl influx than to the cation

pump or to CO_2 fixation under ATP-limiting conditions. This would involve a physical separation of the ATP produced in cyclic and noncyclic photophosphorylation, with only the latter available to the Cl pump, and also an affinity of the Cl pump for ATP higher than that of the cation pump or of the Calvin cycle enzymes which use ATP. Alternatively, further compartmentalization must be invoked, with ATP produced in noncyclic photophosphorylation being specifically available to Cl influx rather than to other processes if ATP production is limited by CCCP. It seems perhaps more reasonable to take the results with CCCP at their face value, and postulate an ATP-independent Cl pump, as MacRobbie (1965, 1966) has done for *Nitella* on the basis of insensitivity of the Cl influx to presumed uncouplers, and a requirement for photosystem 2.

Hope, Simpson, and Walker (1966) have shown that the light inhibition of Cl efflux in *Nitella translucens* has responses to metabolic inhibitors similar to those of the light-stimulated Cl influx. They suggest that the large efflux of Cl in the dark occurs via the Cl carrier when its energy supply is removed. *H. africanum* has a Cl pump similar to that in *Nitella* (MacRobbie, 1965, 1966), but does not have a light-inhibited Cl efflux (Raven, 1967). Hence the mechanism for the light-inhibited Cl efflux suggested by Hope, Simpson, and Walker (1966) cannot be an essential part of the Cl influx mechanism.

MacRobbie (1965) has suggested that the requirement for photosystem 2 for Cl influx in *Nitella* may be associated with the action of Cl as a cofactor in photosystem 2 in isolated chloroplasts (Bové, Bové, Whatley, and Arnon, 1963). Such an explanation would presumably restrict the site of the Cl pump to a chloroplast membrane. However, the site of the Cl pump is not known with certainty (Raven, 1967). The stoichiometry of ^{36}Cl influx and $^{14}CO_2$ fixation under light-limiting conditions is about 1 to 1; this is less than would be expected for a 1 to 1 stoichiometry of a univalent anion flux with electron flow (Robertson, 1960; Lundegårdh, 1961). This could be due to the mechanism operating at a reduced efficiency, but it may be significant that the observed stoichiometry of Cl influx and CO_2 fixation is similar to the observed turnover of the DCMU-sensitive sites of Izawah and Good (1965), and the Mn-dependent sites of Eyster (1964), relative to the rate of O_2 evolution. Both these sites, like Cl influx, are linked to photosystem 2.

Cl influx in *Chlorella* (Neilson, 1964) is light-stimulated. A portion of the influx is relatively sensitive to DCMU, and the total influx is rather less sensitive to CCCP than is photosynthesis. This suggests that a part of the Cl influx in *Chlorella* is similar to that in *H. africanum*, but that the other part has greater similarities (with respect to energy supply) to the cation-regulating system in *H. africanum*.

Thus it may be concluded that cation regulation (K influx, Na efflux) in *Hydrodictyon africanum* in the light is ATP-powered, and that this ATP can be produced by cyclic photophosphorylation. Active Cl influx in the light is

powered by a different mechanism, which requires photosystem 2 rather than ATP.

I am very grateful to Dr. E. A. C. MacRobbie for her considerable advice and encouragement. This work was carried out during the tenure of a Science Research Council Studentship, and a Title A Fellowship from St. John's College, Cambridge.

Received for publication 14 November 1966.

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