

THE EFFECT OF DCMU ON ION UPTAKE AND PHOTOSYNTHESIS IN LEAVES OF *VALLISNERIA SPIRALIS* L.

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SUMMARY

The influence of DCMU on the photosynthesis and the uptake of chloride and rubidium ions by *Vallisneria* leaves was studied.

The photosynthesis was more reduced by DCMU than was the ion uptake. The rubidium uptake is somewhat more sensitive to DCMU than the chloride uptake.

The provisional conclusion is that both the rubidium and chloride uptake can be supported by cyclic photophosphorylation.

1. INTRODUCTION

The ion uptake by leaves of *Vallisneria spiralis* L. is stimulated by light in the presence or absence of carbon dioxide. This phenomenon, studied by Arisz and co-workers since 1943, has been called photoaccumulation. This effect of light has now been found in algae, mosses, and higher plants.

Light has generally been assumed to act as an energy source for the active uptake (ARISZ 1952; VAN LOOKEREN CAMPAGNE 1957; SOL 1958; MACROBBIE 1962; SMITH 1966; RAVEN 1967). But an influence of light on the permeability of the cell membranes (RAVEN 1968b; SMITH 1967b, 1968b) and on the membrane potential (HOPE 1965; NAGAI & TAZAWA 1962), causing an increase in passive penetration, has been considered as well.

In leaves of *Vallisneria* both the chloride and rubidium uptake are stimulated. As the presence of carbon dioxide is not a requisite (ARISZ 1947; VAN LOOKEREN CAMPAGNE 1955, 1957), it is the primary photosynthetic reactions, and not the formation of carbohydrates, which are responsible for the photoaccumulation.

The scheme for the photosynthetic reactions as has been recently described by AVRON & NEUMANN (1968) and HIND & OLSON (1968) will be taken for granted here and will serve as a starting point for further discussion.

As to the coupling between photosynthesis and ion uptake in *Nitella translucens*, MACROBBIE (1962, 1964, 1965, 1966; SMITH 1967b, RAVEN *et al.* 1969) came to the following concept: the active chloride influx which is linked to a monovalent cation influx is supported by photosystem 2 (PS-2), whereas the coupled active potassium influx and sodium efflux require ATP and can be supported by cyclic photophosphorylation.

The above concept was based on experimental results on the effect of DCMU, CCCP, imidazol, phlorizine and Dio-9¹ and light of different wavelengths on

the ion fluxes. Reducing the photosynthetic reactions in PS-2 by illuminating the cells with far red only or by applying the inhibitor DCMU resulted in a reduced chloride influx. The sodium and potassium fluxes were less reduced. On the other hand, uncouplers of the photophosphorylation, such as imidazole and CCCP, gave an inhibition of the potassium and sodium fluxes.

Essentially the same results were obtained with *Hydrodictyon* (RAVEN 1967a, b, 1968a, b, c, 1969; RAVEN *et al.* 1969) and with *Tolypella* (SMITH 1968b, RAVEN *et al.* 1969).

In the present article experiments on the influence of different concentrations of DCMU on both the uptake of chloride and rubidium ions and the photosynthesis in leaves of *Vallisneria* will be discussed.

2. METHODS AND MATERIAL

Plants of *Vallisneria spiralis* were cultivated as described by VAN LOOKEREN CAMPAGNE (1957) and WINTER (1961). The leaves were cut into 4×25 mm strips. The leaf strips were arranged into sets of eight and inserted into perspex frames as described by WINTER (1961). The sets were then kept for some 16 hours in the light in water containing calcium sulphate (0.5 mM.). This pretreatment in the light enhances the uptake in the absorption period that follows (ARISZ 1947; ARISZ & SOL 1956, SOL 1958).

After the pretreatment the sets were placed in perspex vessels in a solution containing a mixture of RbCl 1.0 mM., Rb HCO₃ 0.4 mM., Rb H₂PO₄ 0.6 mM. and CaSO₄ 0.5 mM. The rubidium and chloride ions were labelled with Rb-86 and Cl-36. The pH of this solution was 6.5.

The sets were allowed to absorb at a temperature of 24°C for three hours. The solution was stirred and aerated with normal air.

At the end of this absorption period the sets were transferred into an unlabelled solution of RbCl 2.0 mM. and CaSO₄ 0.5 mM. and kept in the dark for two hours in order to remove the labelled rubidium and chloride from the free space (WINTER 1961). Then the leaf strips were digested in 2 ml. concentrated HNO₃, containing AgNO₃ 0.05 N, at 95°C. Rb-86 activity of the supernatant was measured in a γ -spectrometer for liquid samples, whereas the Cl-36 activity of the AgCl precipitate was measured with a thin-window Geiger-Müller tube or a liquid scintillation counter. This latter technique was used exclusively except when in some experiments the chloride uptake was not determined by the isotope technique but by titrating the chloride of the surrounding solution electrometrically with AgNO₃.

The oxygen production was measured in separate experiments by means of a Clark type oxygen electrode in a closed perspex vessel. The solution used had the same composition as in the uptake experiments. Temperature, pH, and conductivity were also measured in the majority of these experiments. Only small changes in the pH were observed.

¹ DCMU = 3-(3,4 dichlorophenyl)-1,1 dimethyl urea; CCCP = carbonyl cyanide-m-chlorophenyl hydrasone.

The oxygen content of the solution was recorded continuously. In these experiments small amounts of the inhibitor were added at various time intervals with alternating light and dark periods. In this way the effect of increasing concentrations on the photosynthesis and respiration could be studied with the same set of plant material. The duration of a single experiment was about 8 hours.

Experiments were carried out at a somewhat reduced oxygen concentration (16–20%) as compared to the air saturated concentration (21%). Light was supplied by two 20 Watt fluorescent tubes, placed at a distance of about 60 cm from the perspex vessel. The light intensity used in both the uptake and photosynthesis experiments was 1000 Lux. At this intensity light is the limiting factor for both processes (unpublished results).

3. RESULTS

DCMU is an electron-transfer inhibitor of PS-2 (GINGRAS *et al.* 1963, 1965; AVRON & NEUMANN 1968, HIND & OLSON 1968). In the presence of DCMU, ATP formation occurs in cyclic photophosphorylation only (FORTI & PARISI 1963; URBACH & SIMONIS 1967).

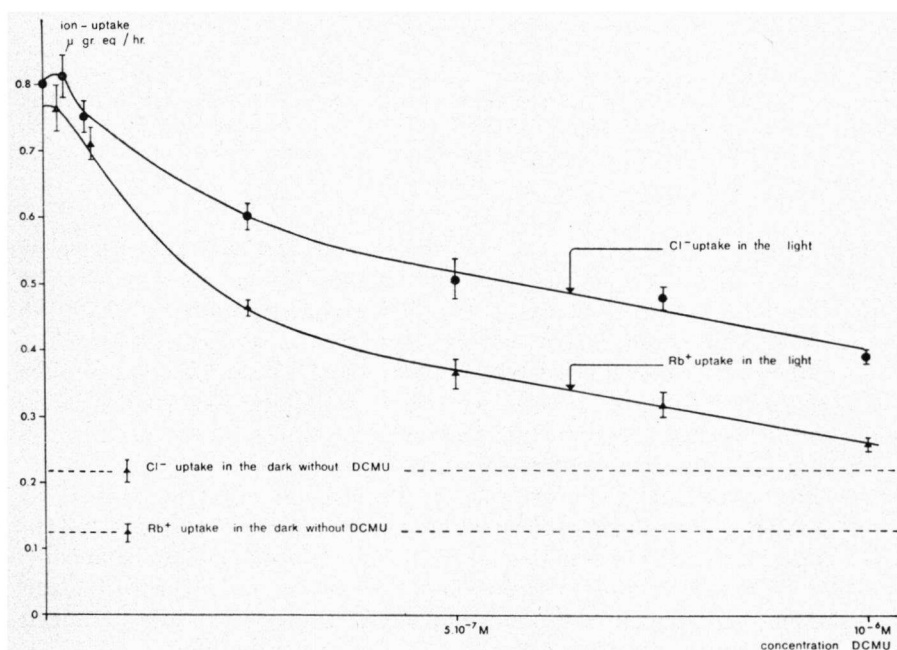


Fig. 1a: The effect of various concentrations of DCMU on the uptake of rubidium and chloride by *Vallisneria* leaves.

Absorption was from a solution containing a.o. 2 mM. rubidium and 1 mM. chloride labelled with Rb-86 and Cl-36.

Mean values of six experiments are given together with their standard error.

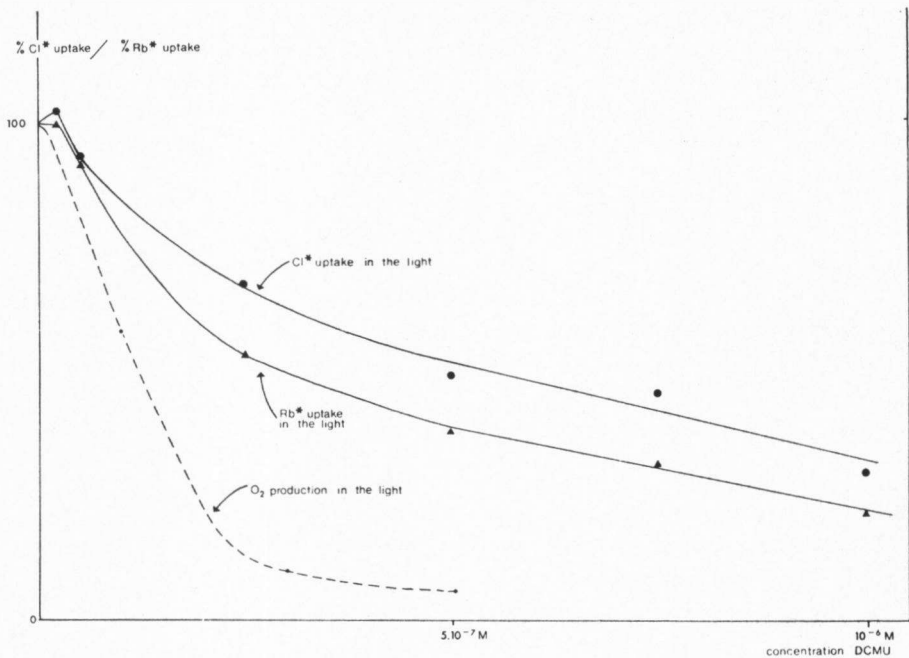


Fig. 1b: The effect of various concentrations of DCMU on photosynthesis and ion uptake. The results obtained in the experiments of *fig. 1a* were corrected for the uptake in the dark. The uptake in the light without DCMU was then taken as a reference. The oxygen production values were corrected for the respiration values in the dark.

The uptake of rubidium and chloride ions in the dark was not affected by DCMU 10^{-6} M. In the light, however, the uptake of both ions is reduced by DCMU (*fig. 1a*). A further increase of the DCMU concentration up to $2 \cdot 10^{-6}$ M. had no significant effect (data from other experiments, not included here).

As to the extent of the inhibition by DCMU, there is little difference between the chloride and rubidium uptake, whereas this inhibitor has a much larger influence on the oxygen production (*fig. 1b*). The values for the oxygen production given in *fig. 1b* are corrected for dark respiration.

The effect of DCMU on the chloride uptake increases with time, as is shown in *fig. 2*. After 4 hours there is still an increase of the inhibitory effect of DCMU. In this figure it can also be seen that, at a low concentration, DCMU stimulated the chloride uptake in short experiments (1.5 hours). After longer periods this stimulation disappears and the chloride uptake is reduced. In these experiments chloride was absorbed from a 1.0 mM. potassium chloride solution.

The effect of DCMU is only partially reversible (*table 1*). After a period of 16 hours in the dark in a DCMU free solution there is still a marked inhibition of the chloride uptake caused by the DCMU treatment in the previous period, as becomes apparent in the following experiment.

EFFECT OF DCMU ON ION UPTAKE AND PHOTOSYNTHESIS

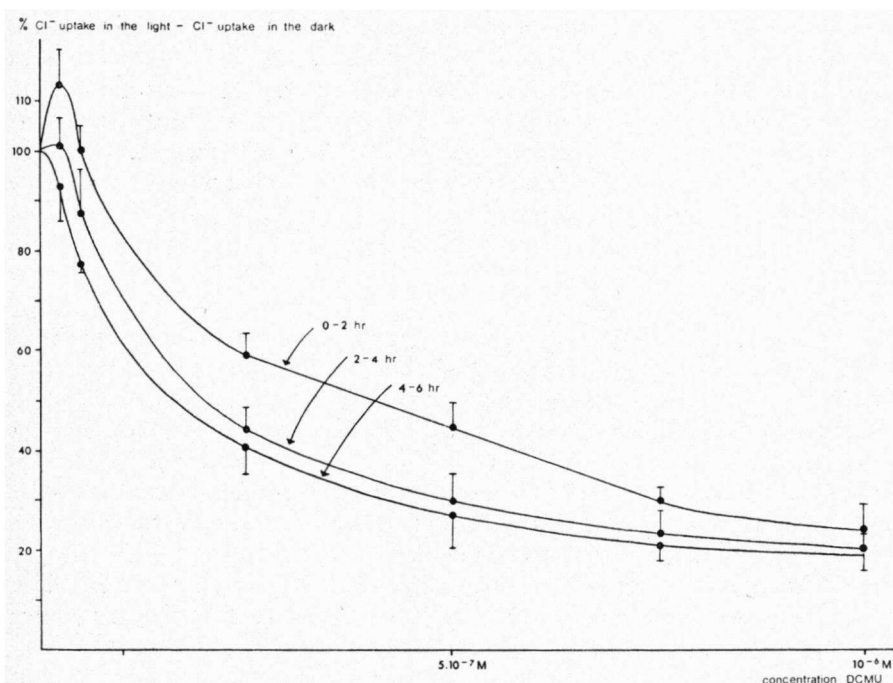


Fig. 2. The increase with time of the inhibitory effect of DCMU.

The chloride uptake in the light with different concentrations of DCMU was measured over three successive 2-hour periods. Mean values of four experiments are given with their standard error (given in only one direction for the sake of clarity). The uptake figures were corrected for the uptake in the dark. Absorption was from a 1 mM. potassium chloride solution and was determined indirectly by titrating the external solution.

Table 1. The reversibility of the inhibitory effect of DCMU on the chloride absorption by *Vallisneria* leaves.

In period A both sets were placed on the light on a 1 mM. KCl solution for 1.5 hours. Then the sets were placed in the dark on a 0.5 mM. calcium sulphate solution for 16 hours (period B).

During period C the sets were again allowed to absorb from a chloride solution for 1.5 hours.

DCMU was added to a concentration of 10⁻⁶ M. during the first period only to the second set, as indicated.

	Set 1	Set 2
Period A	100%	13% (+ DCMU)
Period B	no absorption	no absorption
Period C	94%	47%

After the usual pretreatment in a calcium sulphate solution sets were allowed to absorb in the light from a potassium chloride solution (1.0 mM.) with or without DCMU for 1.5 hours (period A). The sets were then transferred to a calcium sulphate solution (0.5 mM.) for 16 hours in the dark in order to remove the DCMU from the plant material (period B). They were then transferred to a DCMU free potassium chloride solution (1.0 mM) and allowed to absorb for another 1.5 hours with light (period C).

4. DISCUSSION

For determining the chloride uptake two techniques were employed. With the first technique the chloride content of the surrounding solution was measured by titrating with silver nitrate. In this way the net uptake of the chloride is determined. MacRobbie described this as the flux from the outside into the cytoplasm (M_{oc}) minus the flux from the cytoplasm to the outside (M_{co}).

With the isotope technique the influx from the outside into the cytoplasm (M_{oc}) is measured, provided that the specific activity of the ions in the cytoplasm remains relatively low during the experiment.

According to WINTER (1961) and ARISZ (1963a, b) the influx of chloride is not accompanied by an efflux of chloride. This could be confirmed by the present author in experiments not described here. If this holds in the presence of DCMU, the implication is that with both techniques essentially the same phenomenon was studied (*fig. 1b* and *fig. 2*). It can therefore be concluded that DCMU mainly reduces the influx, although it remains possible that DCMU also causes a slight efflux.

In *Nitella* and *Hydrodictyon* a coupling is assumed between PS-2 and the active chloride influx on the one hand, and a coupling between the photo-phosphorylation and the active potassium influx on the other hand.

Comparing our results with those obtained in *Nitella*, *Hydrodictyon*, and *Tolypella*, we must bear in mind that there are some fundamental differences between the uptake conditions in the Characeae and those in *Vallisneria*. In our experiments, there is a net uptake of rubidium and chloride, as the influx of rubidium and chloride is not accompanied by an efflux. So the rubidium is not taken up by an exchange mechanism, such as the potassium-sodium pump in the Characeae.

Further the action of PS-2 seems not to be essential for the chloride uptake in *Vallisneria*. Even if the oxygen production is almost completely inhibited by DCMU, there remains a light dependent chloride uptake of 30–40% of the control (light without DCMU = 100%). The conclusion is that the chloride uptake, as well as the rubidium uptake, can be supported by the cyclic electron flow.

It remains possible that under normal conditions one part of the uptake is linked to PS-2 and the other one to phosphorylation. At any rate, it seems reasonable to assume that both chloride and rubidium uptake require ATP, which is produced, at least in part, by means of cyclic electron flow.

No significant difference between the chloride and rubidium uptake with respect to the sensitivity for DCMU was found. The rubidium uptake tends to be slightly more sensitive. A difference between the potassium influx and the chloride influx as found in *Nitella* in the present experiments with *Vallisneria* was not found for rubidium and chloride.

BARBER (1968 a-e) assumed that the potassium and chloride uptake in *Chlorella pyrenoidosa* depends on photophosphorylation. With respect to the sensitivity of photosynthesis and ion uptake for DCMU our results are very similar to those of BARBER (1968b), but in *Chlorella*, as in the Characeae, the influx is accompanied by an efflux.

JESCHKE (1966, 1967) and JESCHKE & SIMONIS (1967) assumed also that the chloride uptake in *Elodea* depends on photophosphorylation.

Our provisional conclusion is that the absorption of both chloride and rubidium ions by *Vallisneria* leaves is stimulated by light as a result of ATP formation by cyclic and non-cyclic photophosphorylation. To what extent light can stimulate the absorption-process in another way, e.g. by coupling to the electron transfer mechanism of PS-2, remains to be studied.

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