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EVIDENCE FOR AN ELECTROGENIC ION PUMP IN *NITELLA TRANSLUCENS*

II. CONTROL OF THE LIGHT-STIMULATED COMPONENT OF THE MEMBRANE POTENTIAL

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SUMMARY

1. It was established previously that there is a light-stimulated electrogenic ion pump in *Nitella translucens* (Spanswick R. M. (1972) Biochim. Biophys. Acta 288, 73). The effects of CO_2 and inhibitors have been used to study the relationship of the pump to photosynthesis under conditions (pH 6.0, 0.5 mM external K^+) where it produces a hyperpolarization beyond the limit for a diffusion potential.

2. Under CO_2-free conditions the light-stimulated hyperpolarization is maximal and constant. Addition of 1 mM ($CO_2 + HCO_3^-$) to the external solution produces a slow reversion of the membrane potential and resistance to the dark levels. If 1 mM ($CO_2 + HCO_3^-$) is present throughout, light has only a small effect. It was concluded that the products of CO_2 fixation are not involved in supplying energy to the pump.

3. Further experiments with inhibitors were conducted under CO_2-free conditions. 3,3-Dichlorophenyl-*N*,*N*-dimethylurea (DCMU) at concentrations of 0.5 and 1.0 μM had no significant effect on the membrane potential in the light but the higher concentration increased the membrane resistance. These experiments suggested that the pump was not directly linked to non-cyclic electron flow.

4. The remaining possibility was that the pump is driven by ATP produced by cyclic photophosphorylation. This was consistent with the effect of the uncoupler carbonylcyanide *m*-chlorophenylhydrazone (CCCP) which, at a concentration of 1.0 μM, depolarized the cell to the dark level and increased the resistance. 1 mM azide also reversibly depolarized the cell but its site of action is not known.

5. Dicyclohexylcarbodiimide (DCCD) produced a permanent depolarization to the dark level and reduced the subsequent response of the potential to external pH changes. It is suggested that it may affect the ATPase in the cell membrane.

6. 710-nm light also produces a hyperpolarization, confirming the dependence on cyclic photophosphorylation.

Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; DCMU, 3,3-dichlorophenyl-*N*,*N*-dimethylurea; DCCD, dicyclohexylcarbodiimide; MES, 2-morpholinoethane sulfonic acid.

7. CO_2 depolarizes the cell in the light even under conditions where CO_2 fixation is minimal (DCMU or 710-nm light). It is suggested that this is due to over-oxidation of a redox component of photosystem one.

8. There is no effect of 1 mM ouabain and no immediate effect of Cl^--free solutions on the membrane potential. This confirms the previous hypothesis that the electrogenic pump does not involve one of the major ions but possibly H^+. The hypothesis was based largely on an estimate of the flux through the pump (at least 33 pmoles·cm^{-2}·s^{-1} under CO_2-free conditions) which is an order of magnitude too large to be due to a major ion.

INTRODUCTION

In the first paper in this series [1], the presence of an electrogenic ion pump in the plasmalemma of *Nitella translucens* was established by demonstrating that the membrane potential in the light could be more negative than the theoretical negative limit for a diffusion potential. The large effect of temperature on the membrane potential in the light was consistent with this hypothesis. An estimate of the flux through the pump was made by measuring the current required to depolarize the membrane to the negative limit of the diffusion potential set by the K^+ equilibrium potential. The estimated flux was an order of magnitude too large to be due to one of the major ions. It was therefore suggested that the flux could be due to the H^+ pump postulated by Kitasato [2]. As an alternative to the large passive flux of H^+, postulated by Kitasato [2] to account for the effect of external pH on the membrane potential, a model for an electrogenic Na^+–K^+ pump [3] was modified to describe the H^+ pump. An expression for the EMF of the pump, E_p, was also obtained:

$$E_p = \frac{\Delta\bar{\mu}_p}{F\nu_H} - \frac{RT}{F} \ln \frac{H_i^+}{H_o^+} \tag{1}$$

where $\Delta\bar{\mu}_p$ is the free-energy change for the non-transported components of the reaction, ν_H is a stoichiometric coefficient and H_i^+ and H_o^+ are the internal and external H^+ concentrations, respectively. If the pump is in parallel with the passive diffusion pathways (Fig. 11 in ref. 1) and if the conductance of the pump is much greater than the passive conductance, the membrane potential would be approximately equal to E_p. This would provide an alternative explanation for the dependence of the potential on H_o^+. It also suggests that the membrane potential would be dependent on the cytoplasmic pH and the energy supply for the pump.

The main results reported here are the effects of CO_2 and inhibitors on the light-stimulated component of the membrane potential. Further confirmation of the presence of an electrogenic pump is provided and the source of its energy is deduced.

METHODS

The membrane potential and resistance were measured using the methods described previously [1]. The changes in membrane potential used to calculated the membrane resistance were below the threshold for the hyperpolarizing response.

The value of the potassium equilibrium potential, E_K, for individual cells was calculated by subtracting 75 mV from the membrane potential in artificial pond water (pH 6) plus 10 mM KCl. At this concentration the cell membrane appears to behave as a potassium electrode [1]. The experiments were performed in a room maintained at 20 °C. The light intensity was 1.0 $mW \cdot cm^{-2}$.

Except where indicated otherwise, the solution used in these experiments was artificial pond water (pH 6) plus 0.4 mM KCl. The ionic concentrations in artificial pond water (pH 6) were [1]: 0.1 mM K^+, 0.1 mM Ca^{2+}, 0.1 mM Mg^{2+}, 1.0 mM Cl^-. NaOH was added to adjust the pH, the final Na^+ concentration being approximately 1.0 mM. The solution was buffered with 1.0 mM 2-morpholinoethane sulfonic acid (MES). Some inhibitors were dissolved in 1 ml of ethanol before addition to 1 l of solution. Control experiments revealed no effect of 0.1% ethanol on the membrane potential.

CO_2-free air was obtained by bubbling air through two flasks of 1.0 M KOH and one of water. CO_2-free solutions were obtained by passing CO_2-free air through them for at least 30 min.

710-nm light was obtained using an apparatus similar to that described by Robinson [4]. The interference filter was obtained from Baird-Atomic Inc., Bedford, Mass.

RESULTS

The effect of CO_2 on the membrane potential and resistance

Considerable variation in the light-stimulated component of the membrane potential has been observed in the period following the initial hyperpolarization. During this period there is often some depolarization and, in extreme cases, the potential may revert to the dark level (Fig. 1). Investigation on the factors involved in this phenomenon led to the conclusion that it was independent of the buffer strength of the external solution at buffer concentrations of 1 mM and above and was, therefore, not due to changes in the pH at the membrane surface. However,

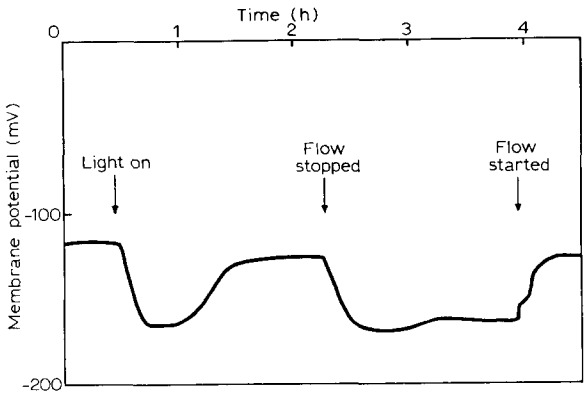

Fig. 1. The effects of flow rate on the membrane potential of a cell in a solution containing a small amount of CO_2. The hyperpolarization in the light is transient but the cell repolarizes when the flow is stopped. It depolarizes when the flow is started again.

it was observed to depend on the rate of flow of the external solution; stationary solutions usually produced a repolarization (Fig. 1). Two possibilities were considered: (a) that the flowing solution led to a net loss of H^+ from the cells and an increase in cytoplasmic pH or (b) that the flowing solution provided a continuous supply of dissolved CO_2. In case (a) the increase in cytoplasmic pH would affect the EMF of the electrogenic pump (Eqn 1) and in case (b) the CO_2 might compete with the electrogenic pump for the same energy source. In stationary solutions the cell would fix most of the CO_2 in its immediate vicinity and the amount available by diffusion from the surface and from respiration could be low enough for CO_2 to be rate-limiting for photosynthesis.

The possibility of an effect on cytoplasmic pH has not been investigated since it was found that solutions bubbled with CO_2-free air or N_2 produced an effect similar to stationary solutions, the potential returning to a value close to the maximum initial hyperpolarization (Fig. 2). Conversely, cells initially in CO_2-free solutions could be depolarized by addition of 1 mM ($CO_2 + HCO_3^-$) to the solution (Fig. 3).

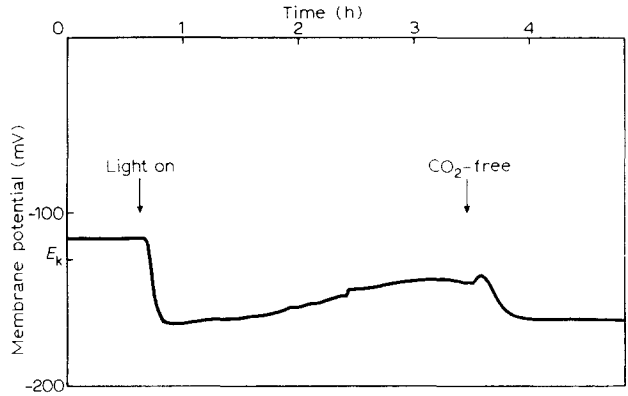

Fig. 2. The effect of CO_2-free solutions on the membrane potential in the light. In a solution containing a small amount of CO_2 the light-stimulated hyperpolarization is partly transient. In CO_2-free solution the potential returns to a value close to the maximum initial hyperpolarization and remains constant.

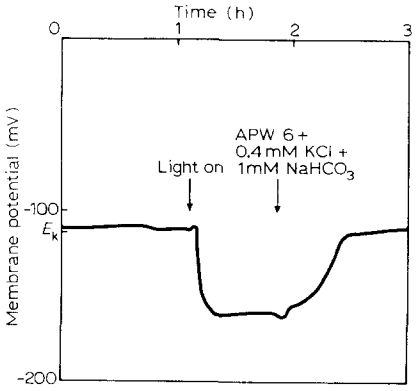

Fig. 3. The effect of 1 mM (CO_2 + HCO_3^-) on the membrane potential of a cell initially in CO_2-free artificial pond water (pH 6) plus 0.4 mM KCl.

Comparison of the effects of darkness and light plus CO_2 on the membrane potential and resistance suggest that the two conditions are very similar (Table I). The similarity extends to the existence of the hyperpolarizing response which at high values of the membrane resistance is normally observed only in the dark [1]. As might be expected, the presence of CO_2 during the dark period eliminates the hyperpolarization when the light is turned on and the resistance remains high (Table II).

TABLE I

EFFECT OF LIGHT AND CO_2 ON THE MEMBRANE POTENTIAL AND RESISTANCE

Mean values for 7 cells ± S.E. E_K for this group of cells was 123 ± 3 mV.

Conditions	Membrane potential (mV)	Membrane resistance ($k\Omega \cdot cm^{-2}$)
Dark, CO_2-free	−116 ± 4	72 ± 26
Light, CO_2-free	−161 ± 4	12 ± 2
Light, 1 mM ($CO_2 + HCO_3^-$)	−117 ± 4	58 ± 15

TABLE II

EFFECT OF LIGHT ON THE MEMBRANE POTENTIAL AND RESISTANCE IN ARTIFICIAL POND WATER (pH 6) PLUS 0.4 mM KCl plus 1 mM $NaHCO_3$

E_K = 123 ± 4 mV.

Conditions	Membrane potential (mV)	Membrane resistance ($k\Omega \cdot cm^{-2}$)
Dark	−113 ± 5	146 ± 52
Light	−116 ± 5	113 ± 49

At pH 5, addition of CO_2 in the light had no effect on the membrane potential but increased the membrane resistance by about 60%. At pH 7 the effects were similar to those at pH 6 but smaller and more variable. Further reduction in the effect was observed at pH 8 and the effects at pH 9 were not significant. Thus the reduced effect above pH 6 appears to parallel the reduction in the rate of CO_2 fixation observed by Smith [5, 6].

These experiments suggest that the products of CO_2 fixation are not involved in supplying energy to the pump. Further experiments were therefore designed to determine whether ATP or reducing power is the energy source.

The effect of inhibitors on the membrane potential and resistance

1. *DCMU.* MacRobbie [7] showed that DCMU at a concentration of 0.6 μM inhibited the Cl^- influx in *N. translucens* to 36% of the control value but only inhibited the K^+ influx to 75% of the control. The effects of DCMU on the membrane potential in the light given in a preliminary report [8] were variable, probably due to the presence of CO_2. However, in the absence of CO_2, there is no effect on the

potential at concentrations of 0.5 or 1.0 μM over periods of at least 1 h (Table III). There is, however, an increase in the membrane resistance at the higher concentration. Addition of CO_2 in the presence of the inhibitor produces a depolarization and an increase in resistance similar to that observed in its absence (Tables I and III). Smith [5] has shown that DCMU at these concentrations reduces CO_2 fixation in *N. translucens* to the dark level. Since DCMU blocks electron flow from System 2 to System 1 in photosynthesis and therefore prevents reduction of NADP, these experiments suggest that the electrogenic pump is driven by ATP produced by cyclic photophosphorylation. The action of CO_2 under these conditions will be considered in the discussion.

TABLE III

EFFECT OF DCMU ON THE MEMBRANE POTENTIAL AND RESISTANCE OF CELLS INITIALLY IN CO_2-FREE ARTIFICIAL POND WATER (pH 6) PLUS 0.4 mM KCl IN THE LIGHT

For this group of 7 cells, $E_K = -119 \pm 2$ mV.

Condition	Membrane potential (mV)	Membrane resistance (k$\Omega \cdot$cm^2)
CO_2-free	-156 ± 3	16 ± 2
CO_2-free, 0.5 μM DCMU	-158 ± 3	17 ± 3
CO_2-free, 1.0 μM DCMU	-154 ± 3	45 ± 10
1 mM (CO_2 + HCO_3^-), 1.0 μM DCMU	-117 ± 2	105 ± 32
CO_2-free, recovery	-161 ± 4	16 ± 2

2. *CCCP.* CCCP is generally recognized as an effective inhibitor of cyclic photophosphorylation [9]. MacRobbie [10] showed that a concentration of 5 μM inhibited the influx of K^+ much more severely than the influx of Cl^-. In the present work it was found that exposure of cells to 5 μM CCCP for long periods produced irreversible damage. However, 1.0 μM CCCP was effective without killing the cells. At this concentration, Smith [5] showed that there was only a small inhibition of CO_2 fixation.

The effect of CCCP is shown in Table IV. The membrane potential is reduced to the dark level and the membrane resistance is increased. Recovery on removal of CCCP is slow and variable. These results are consistent with a dependence on cyclic photophosphorylation as suggested above. A similar effect, under somewhat different conditions, has been reported by Vredenberg and Tonk [11].

3. *DCCD.* DCCD is an energy-transfer inhibitor that inhibits H^+ extrusion in *Streptococcus faecalis* [12]. In *Hydrodictyon africanum* it inhibits respiration and the light-stimulated K^+ influx [13]. It appears to bind covalently to the membrane-bound ATPase system from *Streptococcus* [14] and inhibits the plasma membrane-bound ATPase from oat roots [15]. However, it will bind to any membrane-bound ATPase so a positive effect does not define the site of action.

The time taken to depolarize the membrane potential in *Nitella* was variable. This may reflect both the difficulty in dissolving the inhibitor and the slow rate of binding that is observed in isolated systems [14]. However, once the cell had depolarized

TABLE IV

EFFECT OF INHIBITORS ON THE MEMBRANE POTENTIAL AND RESISTANCE IN CO_2-FREE ARTIFICIAL POND WATER (pH 6) PLUS 0.4 mM KCl IN THE LIGHT

Results expressed as the mean ± S.E. (number of cells).

Inhibitor	Membrane potential (mV)			Membrane resistance (kΩ·cm^2)			E_K (mV)
	Initial	−inhibitor	Recovery	Initial	−inhibitor	Recovery	
1.0 μM CCCP	−156 ± 4 (9)	−120 ± 3 (9)	−133 ± 5 (9)	14 ± 2 (8)	68 ± 13 (8)	21 ± 4 (8)	−123 ± 3 (8)
50 μM DCCD	−154 ± 4 (8)	−112 ± 3 (8)	−109 ± 5 (8)	22 ± 3 (8)	48 ± 14 (8)	65 ± 13 (8)	−120 ± 2 (8)
1.0 mM azide	−153 ± 6 (7)	− 89 ± 5(7)	−150 ± 6 (7)	16 ± 2 (8)	17 ± 2 (7)	46 ± 8 (7)	−122 ± 5 (7)
1.0 mM ouabain	−152 ± 3 (7)	−151 ± 3 (7)	−150 ± 4 (7)	14 ± 2 (7)	15 ± 2 (7)	18 ± 2 (7)	−117 ± 1(7)

the effect was usually irreversible (Table IV). Even many hours after the removal of the inhibitor from the external solution the membrane potential was insensitive to light. Concomitant with this was a marked decrease in the response to a change in external pH from 5 to 7. Before treatment, the mean change in potential for seven cells was 75 ± 3 mV. After removal of the inhibitor the change in potential was only 17 ± 3 mV.

DCCD also increased the membrane resistance (Table IV) and the hyperpolarizing response can be observed with applied currents producing hyperpolarizations greater than about 15 mV. The resistance measurements given in Table IV were made below the threshold of the hyperpolarizing response.

4. *Azide*. There is a large and rapid effect of azide on the membrane potential of *Neurospora crassa* [16]. Coster and Hope [17] found that azide inhibited the Cl^- influx in *Chara corallina* by about 60% and van Lookeren Campagne [18] showed that it inhibited Cl^- uptake in *Vallisneria spiralis*. Apart from this, little use seems to have been made of this inhibitor in photosynthetic systems except for occasional reports of its effect on the Hill reaction [19].

In Table IV it can be seen that 1 mM azide has a large and readily reversible effect on the membrane potential, depolarizing the cell to a potential more than 30 mV more positive than the E_K. There appears to be little effect of the inhibitor on the membrane resistance. However, the membrane potential is reduced to a value where there is often a decrease in the resistance and this may have obscured any increase due to the inhibitor.

The effect of 710-nm light on the membrane potential and resistance

MacRobbie [7] found that illumination of *N. translucens* with light predominantly in the waveband 705–730 nm resulted in an inhibition of the Cl^- influx but no reduction in the K^+ influx in comparison to the usual values in the light. Smith [20] has shown that CO_2 fixation is severely inhibited by the same light source. It therefore appears that the K^+ influx can be supported by ATP from cyclic photophosphorylation in far red light.

The results of the inhibitor experiments described above are consistent with energy from cyclic photophosphorylation being used to drive the pump. To add further confirmation to the inhibitor experiments, the effect of 710-nm light on the membrane potential was investigated. The results in Table V show that light of this wavelength can provide energy to power the electrogenic pump, thus providing

TABLE V

EFFECT OF 710-nm LIGHT ON THE MEMBRANE POTENTIAL AND RESISTANCE

The light intensity was 0.55 mW·cm^{-2}. For this group of 7 cells E_K was 121 ± 3 mV.

Condition	Membrane potential (mV)	Membrane resistance (kΩ·cm^2)
Dark, CO_2-free	-117 ± 2	124 ± 27
710-nm light, CO_2-free	-165 ± 4	23 ± 4
710-nm light, 1 mM (CO_2 + HCO_3^-)	-115 ± 2	51 ± 5

further evidence that ATP is involved. CO_2 also depolarized the cell under these conditions.

The effect on the resistance was similar to that of white light.

The effect of inhibiting the Na^+–K^+ pump and the Cl^- pump

Although the previous estimate of about 20 pmoles·cm^{-2}·s^{-1} for the flux through the electrogenic pump appeared to rule out a pump for one of the major ions [1], the possibility of a contribution to the potential from either of these pumps was investigated.

1. *The Na^+-K^+ pump.* MacRobbie [21] showed that ouabain inhibited the K^+ influx in *N. translucens* and has also shown that it inhibits the Na^+ efflux [22]. It was observed previously that there was no effect of 0.05 mM ouabain on the membrane potential in artificial pond water [23]. A similar result was reported by Stolarek [24].

Addition of 1 mM ouabain to CO_2-free artificial pond water (pH 6) plus 0.4 mM KCl also had no significant effect on the membrane potential or resistance in the light (Table IV). Thus the Na^+–K^+ pump does not appear to be electrogenic.

2. *The Cl^- pump.* It seems unlikely that the Cl^- influx is the electrogenic process in view of the fact that conditions which inhibit the Cl^- pump (far-red light, DCMU) permit the electrogenic pump to function and conditions which inhibit the electrogenic pump (CCCP) do not inhibit the Cl^- influx [10]. This was confirmed by using a solution in which SO_4^{2-} was substituted for Cl^-. After 20 min in Cl^--free solution the average membrane potential for 10 cells was -167 ± 4 mV compared to -164 ± 4 mV for the same group of cells in CO_2-free artificial pond water (pH 6) plus 0.4 mM KCl. Thus there was no immediate effect on the potential as would be expected if the Cl^- pump were electrogenic. However, there was often a slow depolarization over a longer time period (Fig. 4).

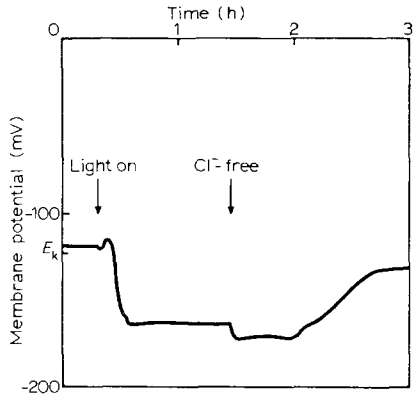

Fig. 4. The long-term effect of Cl^--free, CO_2-free artifical pond water (pH 6) plus 0.2 mM K_2SO_4 on the membrane potential.

DISCUSSION

The effect of CO_2 on the membrane potential is important for two reasons. Firstly, it accounts for a source of variability in measurements of the membrane

potential in green tissues. Secondly, it indicates that there may be competition between CO_2 fixation and ion transport for photosynthetic energy in this system. An alternative possibility is that there is an electrogenic bicarbonate pump as suggested by Hope [25]. However, such a pump would produce a hyperpolarization rather than a depolarization and there was no immediate effect on the membrane potential upon addition of bicarbonate at constant pH [26] (Fig. 3). The decreasing effect of 1 mM ($CO_2 + HCO_3^-$) as the pH is raised above 6 suggests that it is not the HCO_3^- that is important but CO_2 fixation.

Using CO_2-free solutions it was then possible to study the effects of inhibitors on the membrane potential in the light without the uncertainty introduced by the drift in potential that occurs in the presence of small and variable amounts of CO_2 (Figs 1 and 2). The negligible effect of DCMU on the membrane potential, at concentrations that inhibit CO_2 fixation [20], suggests that electron flow, and hence NADPH or an equivalent reductant, is not involved in driving the electrogenic pump. Since cyclic photophosphorylation may occur in the presence of DCMU, it is reasonable to postulate that ATP provides the energy for the pump. The apparent inhibition of the pump by CCCP is consistent with this hypothesis. So is the effect of DCCD, whether it inhibits photophosphorylation or an ATPase in the membrane, or both. The basis for the effect of azide is not clear though the results suggest that it too may affect photophosphorylation.

Some caution should be expressed concerning adoption of this interpretation of these results at face value. While the inhibitor effects now appear to be consistent, it has yet to be shown that they have their postulated effects, or lack of effect, on this system. However, it seems reasonable to postulate that ATP is the energy source for the electrogenic pump and this work has defined a set of conditions under which it will be possible to test this hypothesis by measuring ATP levels.

Further evidence for the involvement of ATP is provided by the ability of 710-nm light to support the electrogenic pump. Light of this wavelength gives low rates of CO_2 fixation in this species [20], but promotes glucose and phosphate uptake [20, 27] both of which appear to be ATP-dependent. Again, this postulated dependence on ATP should be tested directly.

As anticipated, there was no effect of ouabain on the membrane potential and no immediate effect of removing Cl^- from the external solution. This is not surprising since the fluxes through the Na^+–K^+ and Cl^- pumps are in the range 1–2 pmoles$\cdot$cm$^{-2}\cdot$s^{-1} whereas the pump can produce a current equivalent to a flux of about 20 pmoles$\cdot$cm$^{-2}\cdot$s^{-1}, at least when the membrane potential is reduced to E_K [1]. This calculation may be checked for CO_2-free solutions using the data given in Table I. If current is applied to reduce the membrane potential to E_K the passive flux should be near zero and the applied current will provide a minimum estimate of the flux through the pump [1]. Since the current–voltage relationship in the light is linear [1], the current through the pump may be calculated by dividing the difference between the resting potential and E_K (38 mV) by the membrane resistance (12 k$\Omega\cdot$cm^2). The calculated current is 3.2 μA$\cdot$cm^{-2} which is equivalent to a flux of about 33 pmoles$\cdot$cm$^{-2}\cdot$s^{-1}. This is, in fact, an underestimate because the resistance measurement includes the tonoplast resistance.

The previous assertion [1] that the electrogenic pump does not transport a major ion is supported by these results. Identification of the ion as H^+ is still

by a process of elimination, though the work of Lucas and Smith [28], which demonstrates a light-stimulated acidification of the external medium, does suggest that there is in fact an H^+ efflux.

The depolarization produced by long exposure to Cl^--free solutions requires some explanation. If Smith's hypothesis [29] of a Cl^-–OH^- exchange mechanism for *Chara* also applies to *Nitella*, the removal of external Cl^- might block the OH^- efflux and this would lead to an increase in cytoplasmic pH. According to Eqn 1 this would make E_p more positive. Verification of this hypothesis calls for a separate investigation of cytoplasmic pH.

If DCCD affects the pump directly, its effect on the subsequent response to external pH changes is relevant to the applicability of Eqn 1. A direct effect on the pump would decrease g_p as observed (Table IV) and this would lead to a reduction in the contribution of E_p to the membrane potential. Thus, this observation would be consistent with an effect of pH on the pump EMF rather than on a diffusion potential associated with a large passive flux of H^+ as suggested by Kitasato [2].

Vredenberg and Tonk [11] have suggested that in their work g_p may be less than g_m. This may be true since they used conditions that were quite different than those used here, including a higher pH (6.9), the presence of 0.1 mM $KHCO_3$ and short periods of illumination with the resistance measurements made in succeeding dark periods. Their values for the membrane resistance are in the range observed here for darkness or light plus CO_2 and their calculated pump currents are only one tenth of that calculated here for CO_2-free artificial pond water (pH 6) plus 0.4 mM KCl. Their results are, therefore, not inconsistent with the hypothesis that $g_p > g_m$ under conditions of prolonged illumination. It should be noted that, under the present conditions, inhibition of the pump by CCCP does produce an increase in membrane resistance (Table IV) whereas under Vredenberg's conditions there was little effect [30].

It should also be pointed out that Vredenberg and Tonk's treatment [11] of the equivalent circuit in Fig. 1 of ref. 1 is not straightforward in that the current i_p, defined by them as $E_p/(R_m + R_p)$ where $R_p = 1/g_p$ and $R_m = 1/g_m$, is not in general the current through the pump, i.e. R_p. In fact, i_p will only represent the actual current through the pump when $E_m = 0$, a most unlikely situation. The current through either arm of the circuit, one arm of which represents the pump, in the absence of net current flow is $(E_p - E_m)/(R_m + R_p)$. This points out the essential feature of this circuit which is that, in the absence of an applied current, the current through the pump is a function of E_m and will only be zero when $E_m = E_p$. According to Vredenberg and Tonk [11], i_p would be zero when $E_p = 0$. This is particularly confusing since they refer to i_p as the 'electrogenic' or 'active' membrane current.

The depolarization produced by CO_2 in the presence of DCMU or 710-nm light also deserves comment. Since CO_2 fixation is minimal under these conditions, it might be expected that CO_2 would have little effect on the membrane potential. However, parallel observations have been made by Jeschke and Simonis [31] with regard to the effect of CO_2 on Cl^- uptake by *Elodea densa*. They suggest that the presence of CO_2 leads to a change in the redox potential of the electron acceptor or an associated component of photosystem one. Qualitatively this can be looked on as CO_2 fixation draining off electrons from photosystem one. In the presence of DCMU or far-red light the electrons could not be replaced from photosystem

two at an appreciable rate and this would reduce the rate of cyclic photophosphorylation, i.e. the appropriate redox component would become too oxidized ('over-oxidized') to feed electrons into the cyclic system. A similar regulatory system has been used by Raven [32] to explain the interaction between CO_2 and K^+ uptake in *Hydrodictyon africanum*.

In summary, the evidence presented above is consistent with the presence of an electrogenic H^+ pump driven by ATP hydrolysis. Apart from the experiments on ATP levels and cytoplasmic pH suggested above, it will be interesting to see whether Smith's hypothesis [29] of a Cl^- influx driven by a pH gradient set up by the H^+ pump, which now appears to be driven by ATP in *N. translucens*, can be used to explain the Cl^- influx in *N. translucens* which appears to be dependent on electron flow rather than photophosphorylation [7, 10].

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