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Inhibition of Electrogenic Transport Associated with the Action Potential in *Chara*

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Summary. The membrane conductance and potential difference (PD) of *Chara* were measured as a function of the time elapsed since initiation of an action potential. The usual large conductance increase associated with the action potential was observed. The conductance measured several seconds later, however, was found to be significantly decreased relative to its value prior to initiation of the action potential. This decrease in conductance was only temporary, and after several minutes the conductance eventually regained its original value. The magnitude of this conductance decrease was enhanced by increased hyperpolarization of the resting membrane PD prior to the action potential, and diminished by depolarization. This was checked by varying the external pH and illumination, and by the addition of sodium azide. A plausible explanation is that the decrease in conductance is a consequence of inhibition of the electrogenic proton pump. If this inhibition is complete, then the pump conductance for illuminated cells at pH 5.5 is 0.37 ± 0.04 S/m^2, or about 20% of the resting membrane conductance.

Key words action potential · *Chara* · conductance · electrogenic proton pump

Introduction

Under some conditions the membrane potential difference (PD) in charophyte cells can be considerably more hyperpolarized than is explicable by the known concentration gradients of any ionic species. This is usually referred to as the "hyperpolarized state." Kitasato (1968), followed by others (e.g., Spanswick, 1972, 1974*a*, *b*; Saito & Senda, 1973*a*, *b*, 1974; Richards & Hope, 1974) have shown that this hyperpolarized state appears to be a consequence of an electrogenic proton pump. These, and other studies (e.g., Pickard, 1973), generally agree that the hyperpolarized state is

(a) enhanced with increasing illumination

(b) usually not evident for external pH < 6 and is enhanced considerably at pH 7.5

c) removed by the presence of metabolic inhibitors.

The pump can, however, still be operating even when the hyperpolarized state is not evident.

Studies with perfused cells indicate that the presence of internal ATP is necessary for both the hyperpolarized state (Shimmen & Tazawa, 1977; Kawamura, Shimmen & Tazawa, 1980; Smith & Walker, 1981) and for proton extrusion (Shimmen & Tazawa, 1980). Thus the process involved in the production of the hyperpolarized state might be described as primary active transport, whereby ATP provides the energy for proton translocation against an electrochemical gradient.

There is, however, disagreement in the literature about the contribution that this electrogenic proton pump makes towards the total measured membrane conductance. Some experiments suggest that most of the measured conductance is that of the electrogenic proton pump (e.g., Keifer & Spanswick, 1978). Other experiments have suggested that the electrogenic proton pump makes only a small contribution (e.g., Richards & Hope, 1974).

Two main techniques have been used to estimate the pump conductance. In the first technique metabolic inhibitors are applied to the cell and the measured decrease in conductance is equated to the pump conductance. The second technique ascribes the pump conductance to the difference between the total measured electrical conductance and that interpreted from the measured fluxes of radioactive tracers (except hydrogen). Both techniques, however, ignore the presence of passive proton fluxes and the possibility of secondary active transport involving proton movement. In the steady state, inward proton fluxes must balance the efflux of protons produced by the pump. The neglect of such factors may be in part responsible

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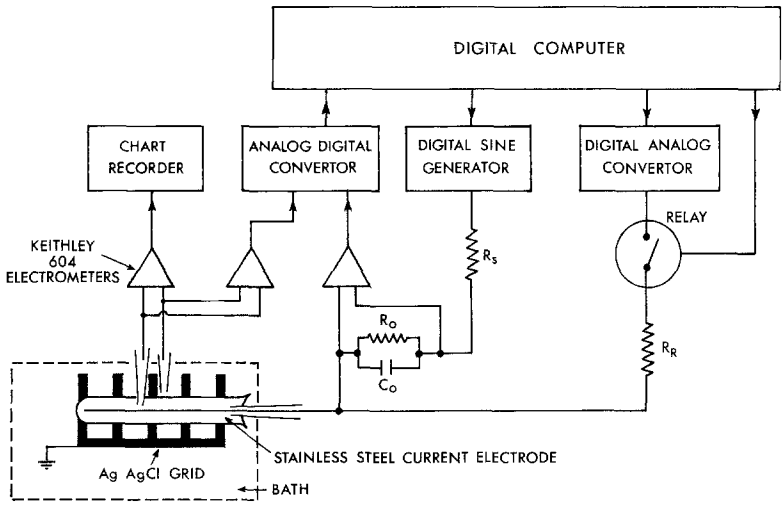

Fig. 1. Schematic diagram of the experimental set-up. A 5-Hz sinusoidal current was passed through the cell membrane via a large series resistor R_s (10 MΩ). Comparison of the PD developed across the membrane with that developed across a known impedance permit calculation of the membrane conductance. R_o and C_o in parallel constituted the known impedance (individual values measured on a Wayne-Kerr B224 bridge), and were chosen to match the equivalent parallel conductance and capacitance of the cell membrane under study. The depolarizing current pulse that initiated the action potential was applied via a relay and a series resistor R_R (1 MΩ)

for the widely varying estimates of the pump conductance in the literature.

Once the membrane PD is sufficiently depolarized, an action potential is triggered. This is an all-or-none excitatory response associated with a large transient increase in membrane permeability to chloride ions. Recent experiments (Beilby & Coster, 1979*a*) have revealed that in *Chara* an additional transient increase in plasmalemma permeability occurs to a different ionic species, possibly calcium. These transient permeability changes can manifest themselves as large increases in the membrane conductance (e.g., Cole & Curtis, 1938; Findlay & Hope, 1964; Findlay, 1970; Kishimoto, 1972).

It has been reported previously that production of the hyperpolarized state can be temporarily inhibited after an action potential (Hope, 1965; Spanswick, 1972; Bisson & Walker, 1981). This suggests that the conductance associated with the electrogenic proton pump might temporarily decrease after an action potential. If this indeed occurs, a comparison of the membrane conductance measured immediately prior to the initiation of an action potential with that measured several seconds later should reveal a decrease in conductance with magnitude corresponding to the degree of inhibition of the electrogenic proton pump. Such a technique would allow rapid precise measurements that could be repeated severally, and may possess additional advantages over techniques used previously.

We present here the results of experiments which investigate the behavior of the membrane conductance after an action potential and how it is affected by illumination, external pH and the presence of sodium azide. From these measurements we estimate the conductance attributable to the electrogenic proton pump.

Materials and Methods

Plants of *Chara australis* (R. Br.) were originally collected from the Nepean River (Camden-Sydney) and grown in the laboratory. Young whorl cells of length 2–6 mm and diameter 0.7–1.5 mm were used. Their surface area was estimated via a microscope and calibrated graticule (approximate error 10–20%). During experiments cells were bathed in a flowing solution of artificial pond water (APW – 0.1 mM KCl, 1 mM NaCl, 0.5 mM $CaCl_2$) at 20–22 °C. For most experiments this APW was unbuffered with a pH of 5.5. For experiments at pH 4.5 and 7.5 the APW was buffered with 5 mM MES[1] and HEPES, respectively. Bright illumination (approx. 50 $\mu Em^{-2} sec^{-1}$ was provided by a microscope light source. In dim illumination cells were exposed to approx. 0.5 $\mu Em^{-2} sec^{-1}$.

A schematic diagram of the electrical apparatus and electrode arrangement used is shown in Fig. 1. Cells were mechanically restrained by a grid of silver wire (coated with AgCl) which also served as the external current electrode. Current was passed through the cell membranes via a stainless steel wire (diameter = 12 μm) inserted longitudinally via a glass micropipette manipulated into the cell. This wire extended at least 90% of the length of the cell, thus ensuring space-clamping (a spatially uniform PD across the membranes) and obviating any need to correct for cable properties. The PD developed across the membrane (i.e., plasmalemma + tonoplast in series) was measured between two glass micropipettes filled with 2 M KCl. One micropipette was located immediately adjacent to the cell exterior, the other (tip diameter < 1 μm) was placed into the vacuole. A chart recorder was used to record the shape of each action potential and also monitor the resting PD.

The four-terminal digital impedance-measuring technique used has been described in detail previously (Bell, Coster & Smith, 1975; Coster & Smith, 1977). In this technique a computer generates a sinusoidally varying current which is passed through the cell membrane and a known impedance connected in series. Comparison of the PD measured across the known impedance with that developed across the membrane yields the equivalent parallel capacitance and conductance of the membrane. The precision of these conductance measurements at 5 Hz is better than 0.3% (*see* Ashcroft, Coster & Smith, 1981).

The conductance measurements reported here were all made with a 5-Hz sinusoidal current. This frequency was chosen

[1] *Abbreviations:* MES, 2(N-morpholino)ethanesulphonic acid; HEPES, N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid.

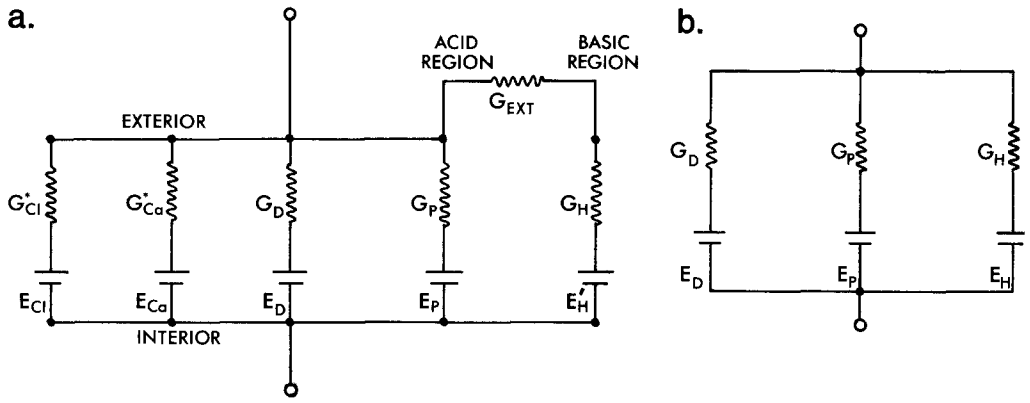

Fig. 2. Suitable equivalent circuits for the plasmalemma of *Chara* when both acid and basic regions are present. *a*) Suitable both during and after an action potential. *b*): A simplified version only suitable after an action potential

as a compromise between being sufficiently low for the measured AC conductance to accurately reflect that of the membrane at very low frequencies (e.g., the conductances at 5 and 0.05 Hz differ by only 5–10% – Smith & Coster, 1980), yet being sufficiently high to permit rapid measurement of the single cycles of voltage and current required to calculate the membrane conductance. At 5 Hz each conductance measurement took 200 msec. For high precision measurements it is essential to minimize the contribution of any voltage response additional to that caused by the injected 5-Hz sinusoidal current. For this reason measurements were only commenced approximately 2 sec after initiation of the action potential, the rate of change of the membrane potential then being sufficiently small to ensure high precision measurements. The amplitude of the sinusoidal oscillations in membrane potential was kept below 5 mV rms.

Measurements were commenced approximately 30 min after electrode insertion. For each set of measurements the following procedure was adopted. The computer first measured the membrane conductance. An action potential, which occurred simultaneously over the entire cell surface, was then initiated by application of a depolarizing current pulse of 100–200 msec duration. This pulse was derived from a digital-to-analogue converter which was temporarily connected to the internal current electrode via a magnetic reed relay. A single cycle of voltage and current was then sampled and stored at 15 equally spaced time intervals after initiation of the action potential. The conductance at these 15 equally spaced intervals was then calculated.

A Suitable Equivalent Circuit for the Plasmalemma

To provide a basis for the interpretation of the conductance measurements it is necessary to consider a suitable equivalent circuit for the plasmalemma. In this paper we will not explicitly consider the distinct electrical properties of the tonoplast, but will assume that the electrical properties measured via vacuolar electrodes reflect primarily those of the plasmalemma. Simultaneous measurements with cytoplasmic and vacuolar electrodes (e.g., Coster & Smith, 1977) indicate that this is a reasonable first approximation (*see also* Discussion). To construct a suitable equivalent circuit the major paths for current flow through the plasmalemma must be considered. When a particular current pathway is dominant the membrane can be considered to be in a distinct electrochemical "state". In characean cells the plasmalemma is capable of displaying four main types of distinct electrochemical behavior (e.g., Walker, 1980; Bisson & Walker, 1982). These can be summarized as follows:

Depolarized or D State. This appears to be the "default" state for the plasmalemma. Current flow through the plasmalemma is then dominated by passive potassium fluxes. The high membrane permeability to potassium is indicated by both the sensitivity of the resting PD to changes in the external potassium concentration (e.g., Hope & Walker, 1961) and by the measured radioactive tracer fluxes (e.g., Walker & Hope, 1969). The passive fluxes of sodium, chloride, and calcium ions for the unexcited plasmalemma are included in this element, as are any secondary active transport processes (e.g., the H^+, Cl^- symport – Beilby & Walker, 1981). Measurements in the dark with the pump inhibited indicate that $G_D = 0.2$–0.5 S/m^2 and that the equilibrium PD in this state, E_D, is approx -100 to -120 mV for the APW used in these experiments. This PD is depolarized from that which would be expected for potassium alone, presumably because of the other pathways involved.

Hyperpolarized or P state. As discussed in the introduction, the hyperpolarized state appears to be a consequence of the operation of an ATP-driven electrogenic proton pump. The equivalent conductance of the pump, G_p, is still controversial. The equilibrium PD, E_p, appears to be approx. -230 mV (e.g., Smith & Walker, 1981). This is approx. the most hyperpolarized membrane PD evident for the cells used in this laboratory.

Basic (Alkaline) or *H* State. When the external pH is raised above pH 9–10 (Bisson & Walker, 1980, 1981) the plasmalemma of *Chara* can become highly permeable to protons (or alternatively hydroxyl ions). The conductance, G_H, associated with this passive proton flux, can be as large as 5–10 S/m^2 at pH 11.

Excited State. During an action potential the plasmalemma becomes transiently (for a few seconds) highly permeable to both chloride ions ($G^*_{Cl} = 4$ S/m^2) and to another species that appears to be calcium ($G^*_{Ca} = 13$ S/m^2) (Beilby & Coster, 1979*a*, *b*).

An equivalent circuit for the plasmalemma of *Chara* incorporating the four possible states of electrochemical behavior, i.e., the four main equivalent circuit elements, and thus suitable both during and after the action potential, is shown in Fig. 2*a*. Illuminated characean cells are known to develop alternating acid and basic regions in the external medium adjacent to the cell surface (e.g., Spear, Barr & Barr, 1969; Lucas & Smith, 1973). The membrane PD of the basic regions can be depolarized by approx. 5–10 mV with respect to the acid regions, and consequently large electric currents can circulate between these

regions (Walker & Smith, 1977). For this reason an additional conductance element G_{EXT} is shown connecting the acid and basic regions in the external bathing solution. Additionally the membrane conductance of *Chara* is not uniform over the cell surface, the area-specific conductance of the basic regions being much greater than that of the acid regions (Chilcott, Coster, Ogata & Smith, 1983; Smith & Walker, 1983). The internal longitudinal electrode used for the measurements reported here ensures space-clamping, and thus the spatially-averaged properties of the whole cell are studied. The simplified circuit of Fig. 2*b* should then be an adequate approximation once the conductance increase associated with an action potential has decayed away. The total plasmalemma conductance, G_m, is then given by

$$G_m = G_p + G_H + G_D \quad (1)$$

The plasmalemma PD V_m without external current is

$$V_m = (G_D E_D + G_H E_H + G_p E_p)/G_m \quad (2)$$

where

$$E_H = E'_H + V_{AB} \quad (3)$$

and V_{AB} is the PD between the acid and basic regions in the external solution.

Because V_{AB} is typically less than 5 mV, $E_H \approx V_r$ where V_r is the resting membrane PD in the acid regions. Substituting back into Eq. (2) then gives

$$V_r = (G_P E_P + G_D E_D)/(G_P + G_D) \quad (4)$$

If a linear relationship holds between G_P and G_H, i.e., $G_H(V_m) = \gamma G_P(V_m)$, Eq. (1) can be rearranged into

$$G_P = (G_m - G_D)/(\gamma + 1) \quad (5)$$

where G_D has been assumed independent of V_m.

Results

The experiments revealed slow variations in the membrane conductance over a period of several hours. These appeared to be a consequence of variations in the fraction of the plasmalemma in the basic state. In order that such conductance variations might not obscure any small conductance differences under investigation, the data is often presented as the relative conductance, being normalized with respect to that measured immediately prior to the action potential.

Figure 3 shows representative results of the variation of the membrane PD and relative conductance during and immediately after an action potential. Figure 3(*a*) shows the response of the membrane PD to both a subthreshold pulse and to a pulse of amplitude just sufficient to generate an action potential. Figure 3(*b*) shows the relative membrane conductance measured simultaneously with the membrane PD. The decay of the the very large conductance increase associated with production of an action potential is still evident several seconds after its initiation. When this conductance increase has completely decayed away, however,

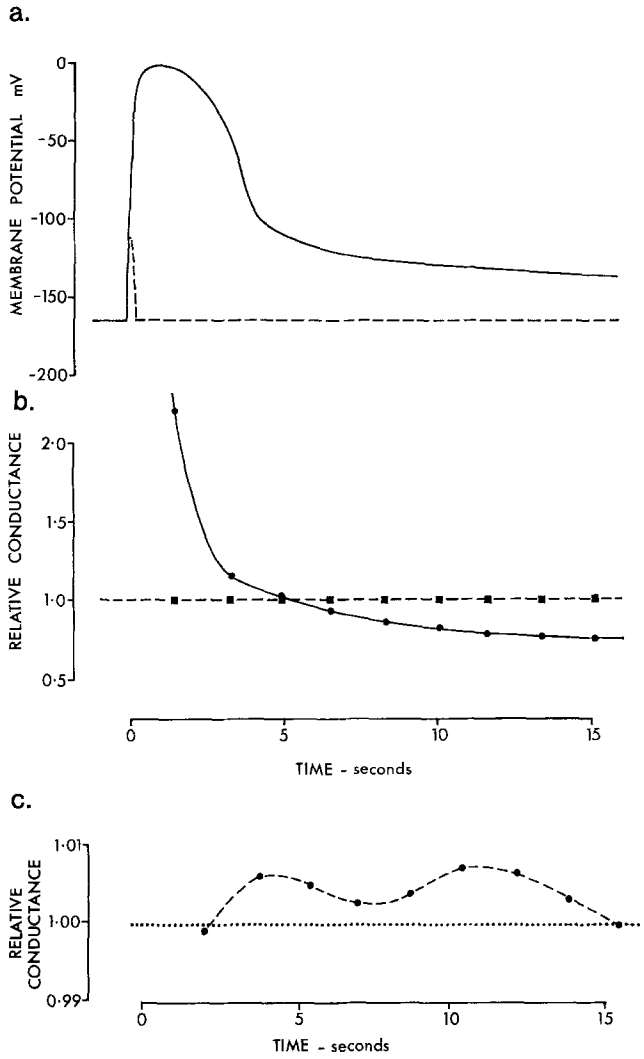

Fig. 3. The variation of membrane PD (*a*) and relative conductance *b*) during and immediately after an action potential (full curves). For comparison, the responses to a subthreshold (90% of threshold) depolarizing current pulse are also shown (dashed lines). (*c*): The same data for a subthreshold pulse as *b*, but replotted on a scale magnified 50×. The membrane PD responses are taken from the chart recorder. In this and subsequent plots of the action potential, the small 5-Hz sinusoidal oscillation has been removed for clarity. The conductance data have been normalized with respect to their values measured immediately prior to application of the depolarizing current pulse

the conductance has decreased from its value immediately prior to the action potential. Subthreshold pulses produced no variation in conductance visible on the scale of Fig. 3(*b*). Figure 3(*c*) shows the conductance of a subthreshold pulse on a scale enlarged 50×. Only a very slight (<1%) conductance change is apparent as a consequence of the subthreshold pulse. The decrease in conductance is thus apparently a consequence of the action potential.

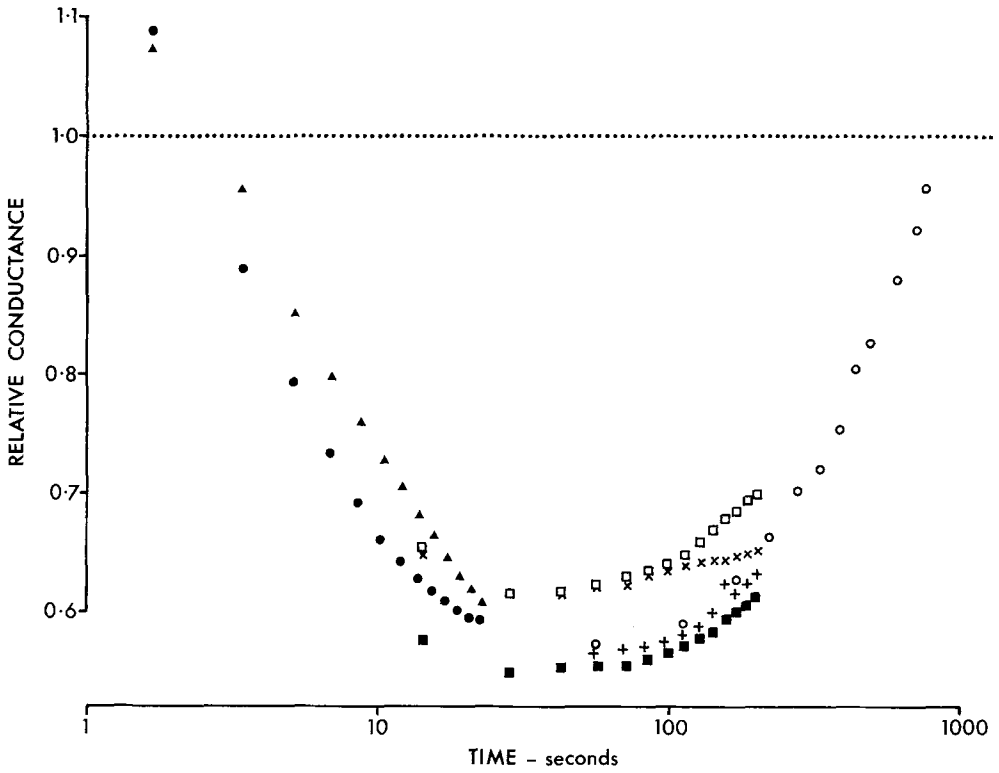

Fig. 4. The relative membrane conductance as a function of time elapsed since initiation of an action potential. The results shown are from seven consecutive action potentials on the same cell initiated at 15-min intervals over a period of approx. 100 min. Each different symbol refers to the 15 data points from a different action potential. Note that the time axis is logarithmic

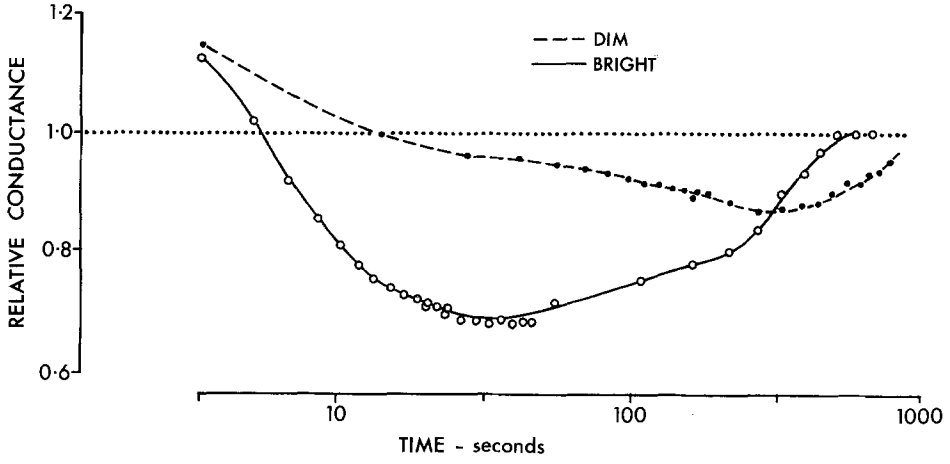

Fig. 5. The effect of varying illumination. The relative membrane conductance is shown as a function of time elapsed after initation of an action potential in bright illumination (continuous line) and dim illumination (dashed line) on the same cell. Note that the time axis is logarithmic

Figure 4 presents the results of conductance measurements continued for longer periods on several successive action potentials. They indicate that the decrease in conductance is only temporary and that the conductance eventually returns to its value prior to initiation of the action potential. This temporary decrease in conductance was apparent after every action potential initiated in cells bathed in normal APW at pH 5.5 and at room temperature – a total of 181 action potentials on 24 cells.

Effect of Varying Illumination

Figure 5 shows the relative conductance as a function of time after initiation of an action potential in bright and dim illumination. It is apparent that the temporary decrease in conductance is enhanced by increased illumination, and occurs more rapidly. A slight hyperpolarization (approx. 8 mV) was noticed when the illumination was increased from dim to bright. Table 1 summarizes the effects of

Table 1. The effect of varying illumination upon membrane parameters

Illumination ($\mu Em^{-2}\ sec^{-1}$)	G_{min}/G_r	T_{min} (sec)	T_x (sec)	T_r (sec)	G_r (S/m^2)	G_{DIFF} (S/m^2)	V_m (mV)
Bright (50)	0.80±0.02(39)	97±13(40)	12±2(24)	500±50(13)	1.60±0.08(133)	0.37±0.04(70)	−150±3(133)
Dim (0.5)	0.87±0.01(52)	150±10(54)	26±3(29)	520±50(17)	1.42±0.08(112)	0.23±0.02(51)	−142±3(112)

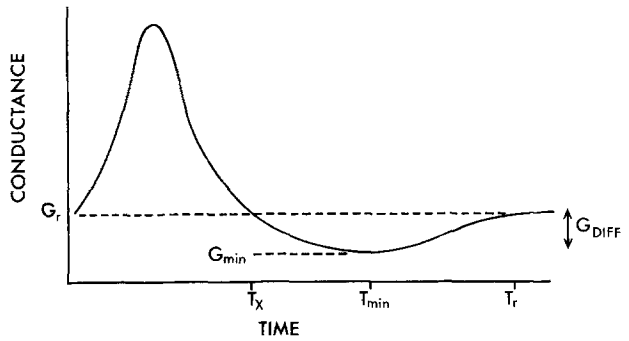

Fig. 6. An idealized plot of the time dependence of the conductance as a function of time indicating the following parameters used to summarize the experimental data. G_r = membrane conductance immediately prior to the initiation of an action potential; G_{min} = minimum value of the membrane conductance after an action potential; G_{DIFF} = difference between the conductance measured immediately before and after an action potential, i.e. $G_{DIFF} = G_r - G_{min}$; T_{min} = time taken for the conductance to reach its minimum value (i.e. G_{min}); T_x = time taken for the conductance increase associated with the action potential to decay to G_r; T_r = time taken for the conductance to regain the value G_r after undergoing the minimum in conductance

illumination in terms of the parameters defined in Fig. 6 and expressed as mean±standard error (number of samples).

Effect of Sodium Azide

Sodium azide at 0.5 mM concentration was added to the bathing APW (pH 5.5) of four cells. In each case the total membrane conductance was found to decrease, with a time constant of about 15 min, towards a final value that was 20–30% of its initial value. Concurrently the membrane potential was depolarized by 20–30 mV (sometimes after a brief transient hyperpolarization). The shape of the action potential remained substantially unaffected, however (*see* Fig. 7(*a*)). The addition of azide was found to essentially remove the temporary decrease in conductance after the action potential. The inhibition of this decrease appeared to be more rapid in bright illumination. Removal of the sodium azide from the bathing APW substantially reversed all of the above effects (for example, *see* Fig. 7(*b*)).

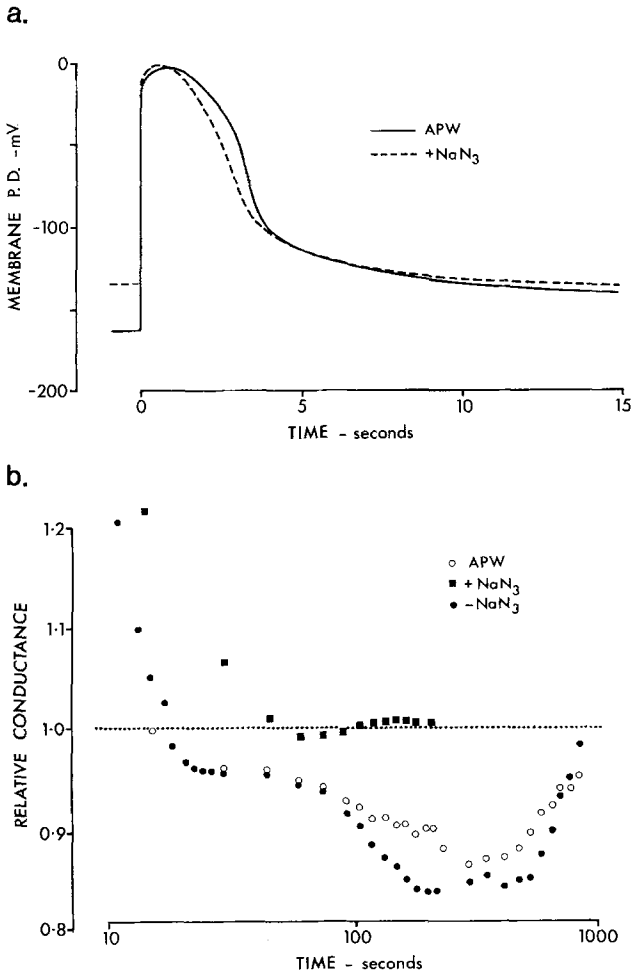

Fig. 7. The effect of the addition of 0.5 mM sodium azide. *a*: The action potential in APW (continous line) and another initiated 30 min after the addition of 0.5 mM sodium azide (dashed line) to the bathing solution are shown. *b*: The relative membrane conductance is shown as a function of the time elapsed since the initiation of the two action potentials shown in *a*. The conductance data for another action potential initiated after removal of the sodium azide from the bathing solution is shown in *b* to demonstrate that the effect of azide was reversible. Note that the time axis of *b* is logarithmic

Variation of External pH

On five cells the pH of the external bathing APW was increased from 5.5 to 7.5 (*see* Fig. 8). This produced a marked hyperpolarization of the mem-

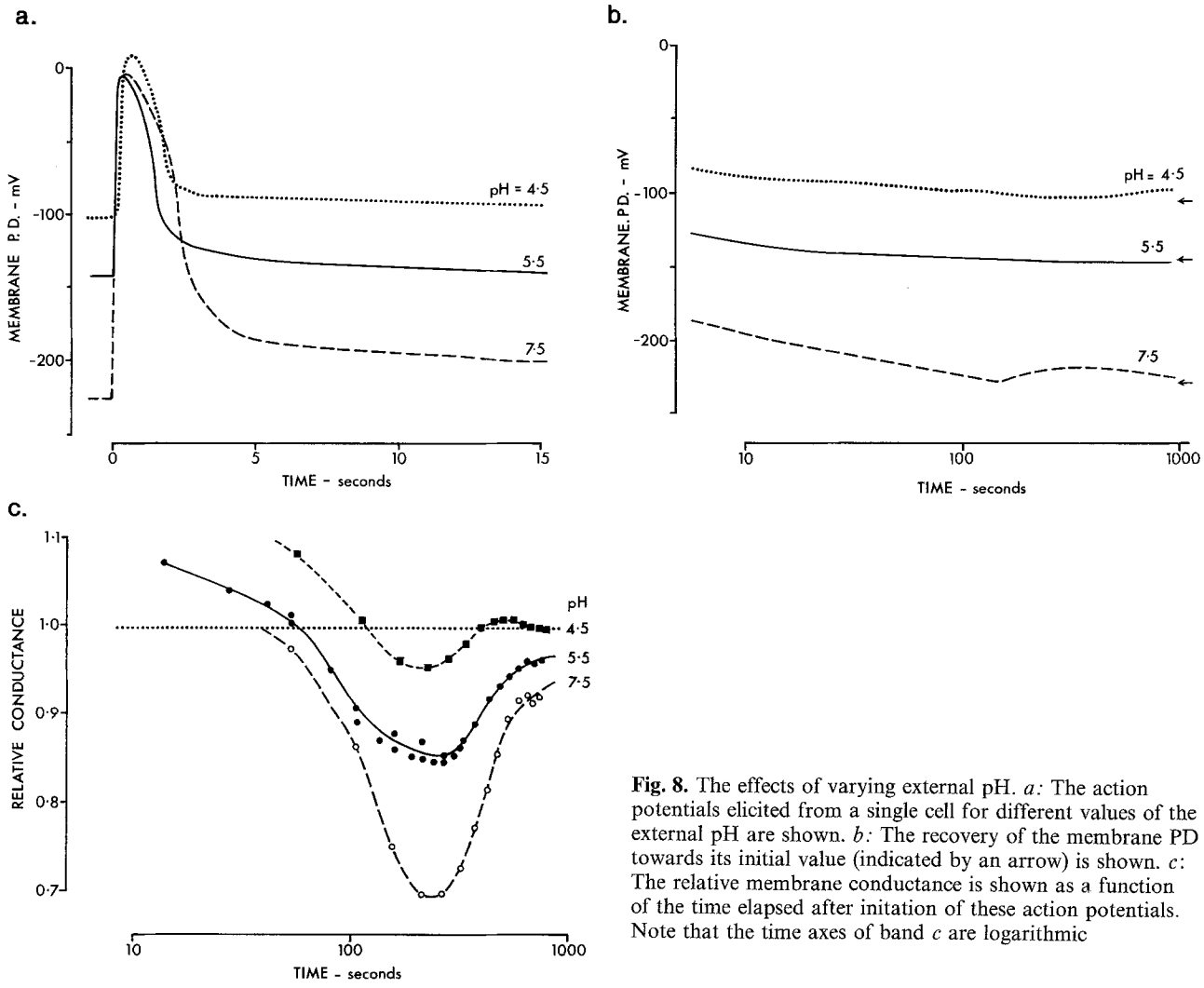

Fig. 8. The effects of varying external pH. *a:* The action potentials elicited from a single cell for different values of the external pH are shown. *b:* The recovery of the membrane PD towards its initial value (indicated by an arrow) is shown. *c:* The relative membrane conductance is shown as a function of the time elapsed after initation of these action potentials. Note that the time axes of band *c* are logarithmic

Table 2. The effect of varying external pH upon the membrane parameters of cells with dim illumination

pH	G_{min}/G_r	T_{min} (sec)	T_r (sec)	G_{DIFF} (S/m^2)	G_r (S/m^2)	V_m (mV)
4.5	0.98±0.01 (6)	–	–	0.03±0.02 (6)	1.03±0.05 (6)	−115±5 (6)
5.5	0.87±0.01(52)	150±10(54)	520±50(17)	0.23±0.02(51)	1.42±0.08(112)	−142±3(112)
7.5	0.69±0.03(15)	210±30(15)	385±40 (4)	0.32±0.03(15)	1.03±0.1 (15)	−220±6 (15)

brane potential to an average value of −220 mV. The magnitude of the temporary decrease in conductance after the action potential was also greatly increased (by up to 270%). When the pH of the bathing APW was reduced from 5.5 to 4.5 the membrane PD was depolarized by approx. 35 mV. The decrease in conductance after the action potential was also effectively removed at pH 4.5. The effects of varying the external pH were found to be reversible. The pH results are summarized in Table 2.

The Conductance at Different Resting PD

Figure 9 and 10, respectively, show how the measured values of G_{DIFF} and G_r varied when measured at different resting membrane PD for pH 4.5–5.5. The variations reflect those present both in individual cells and between different cells. The membrane PD was depolarized by either reducing the illumination, the addition of sodium azide, or by lowering the pH from 5.5 to 4.5. Figure 11 shows the relationship between G_{DIFF} and G_r.

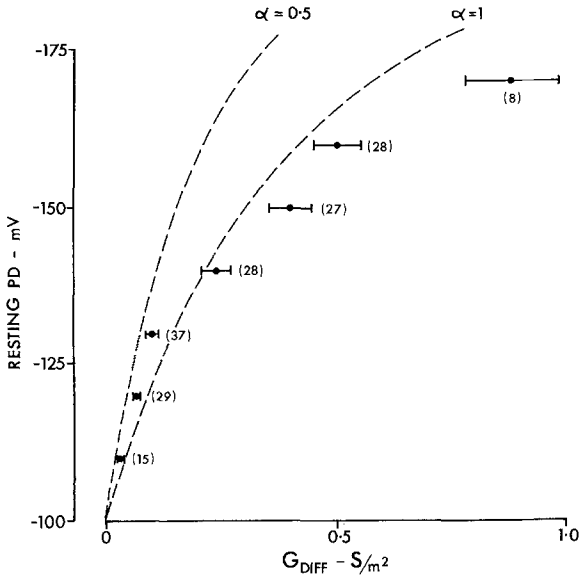

Fig. 9. The difference in conductance (G_{DIFF}) measured before and after an action potential is shown plotted against the resting membrane PD for pH 4.5–5.5. The data is from 24 cells and has been grouped into 10-mV subdivisions. The number next to each point refers to the number of samples. The error bars represent the standard errors. The dashed lines were calculated from Eq. (4). The parameters had the following values: $E_D = -100$ mV, $Ep = -230$ mV, $G_D = 0.5$ S/m^2, $G_{DIFF} = \alpha Gp$

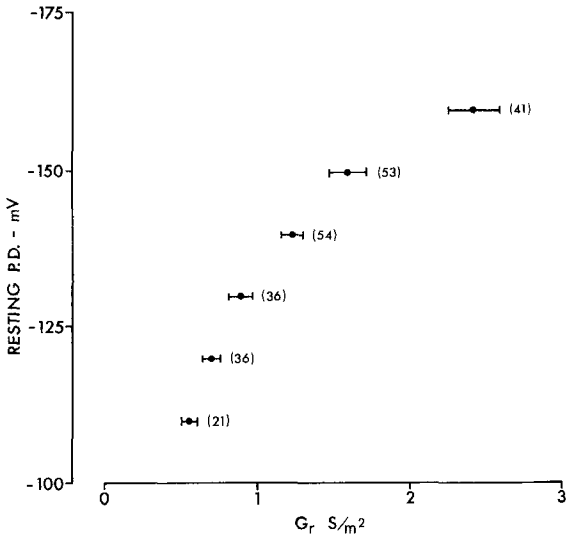

Fig. 10. The resting membrane PD plotted against the total measured membrane conductance (G_r) at that PD Otherwise the caption from Fig. 9 applies to this figure

Discussion

The results clearly show that a temporary decrease in the membrane conductance occurs as a consequence of the action potential. It now remains to discuss which conductance element in the equivalent circuit is temporarily inhibited.

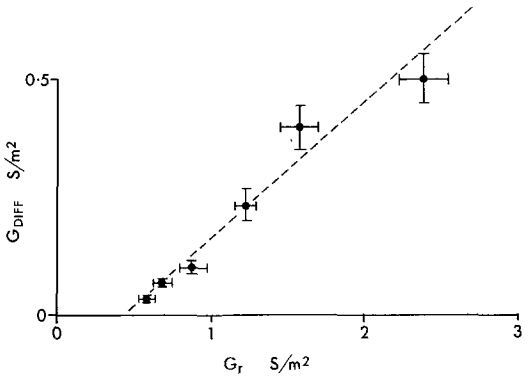

Fig. 11. The difference in conductance G_{DIFF} plotted against the total measured conductance G_r. The data is replotted from Figs. 9 and 10. The dotted line of best fit had a slope = 0.28

A striking feature was that the magnitude of the decrease in conductance (G_{DIFF}) was always well correlated with the degree of hyperpolarization of the resting plasmalemma PD prior to an action potential (*see* Fig. 9). Thus the effects of reduced illumination, decreased pH, and sodium azide which depolarized the plasmalemma PD decreased the magnitude of G_{DIFF}. Figure 9 shows the theoretical dependence of V_r upon G_{DIFF} calculated using Eq. (4) and assuming that $G_{DIFF} = \alpha Gp$. The agreement is best for $G_{DIFF} = Gp$, (i.e., $\alpha = 1$). Also $V_r \approx E_D$ when $G_{DIFF} = 0$. These two features strongly suggest that inhibition of the electrogenic proton pump is relatively complete and that G_{DIFF} approximates the total conductance of that pump.

Once an action potential occurs, the membrane PD V_m is perturbed from its resting value V_r, and should be described approximately by Eq. (2). The dependence of V_m upon Gp can then be significantly less than the dependence of V_r upon Gp. This is because the relatively large conductance element G_H becomes significant once V_m differs from E_H. Thus the value of V_m at T_{min} need not be particularly depolarized, even if G_P were totally inhibited. This is consistent with the experimental data (e.g., *see* Fig. 8*b*).

The membrane PD after the action potential was also found to be depolarized from the resting potential, usually to an extent that increased with increasing hyperpolarization of V_r (e.g., Figs. 7*a* and 8*a*). This depolarization was due primarily to the large conductances G^*_{Cl} and G^*_{Ca}, but the slower action potential across the tonoplast (e.g., Findlay & Hope, 1964) was also partially responsible.

Experiments with cytoplasmic electrodes (M.J. Beilby & J.R. Smith, *unpublished*) still revealed the conductance decrease, although it was faster and reduced somewhat in magnitude. Thus the conclu-

sions reached in this paper reflect predominantly on the plasmalemma.

The possibility that the reduction in magnitude of any other conductance elements is responsible for the results presented here is now considered. Inhibition of G_D would hyperpolarize the membrane PD, whereas inhibition of G_H should leave V_m relatively unaffected. The $V-I$ curves at short times of these two elements appear to be linear over the range of PD considered here (e.g., Bisson & Walker, 1980; Smith & Walker, 1981). However, the total membrane conductance has been usually found to increase slightly upon depolarization (e.g., Beilby & Coster, 1979*b*). Such an increase would produce a conductance *increase* after an action potential. In any case, the depolarization of V_m is slight at $T_{\min}$, and any voltage dependence would need to be very strong (and in an opposite direction to that normally reported) to significantly upset the conclusions reported herein.

Figure 10 demonstrates how the total measured conductance (G_r) was found to vary with the resting membrane PD. The dependence was found to be similar to that of G_{DIFF} (*see* Fig. 9). Figure 11 indicates that an approximately linear relationship existed between the potential dependence of G_{DIFF} and G_r. If as a first approximation G_D is assumed independent of the resting PD and the proton influx is entirely via passive uniport, then the intercept from Fig. 11 at $G_{\text{DIFF}}=(Gp)=0$ gives $G_D=G_r=0.45$ S/m^2. Using Eq. (5) the slope of the line of best fit in Fig. 11 suggests that $G_H(V_m)=2.5\,G_{\text{P}}(V_m)$ in a bathing solution of pH 4.5–5.5. That $G_H>G_{\text{P}}$ is not surprising because the proton flux back into the cytoplasm must, in the steady state, equal the efflux due to the proton pump. The basic zones, however, usually occupy only a small fraction of the membrane area, and their equilibrium PD for protons is close to the membrane PD (Bisson & Walker, 1980). Indeed, recent experiments (Smith & Walker, 1983; Chilcott et al., 1983) indicate that the area-specific membrane conductance of the basic zones can be 6–7 times greater than that of the acid zones.

The reason that this conductance decrease after an action potential has not been reported previously appears to be that previous measurements have been deficient in both their precision and duration after the action potential. However, the results of Findlay and Hope (1964) show such a conductance decrease for the tonoplast.

It is of interest to speculate on the mechanism responsible for the inhibition of the pump, particularly as the time course of pump inhibition is very similar to that of cytoplasmic streaming (e.g., Kishimoto & Akabori, 1959). A likely initiator appears to be the increase in cytoplasmic Ca concentration produced by the action potential. Although this increase is in itself both too small and too rapid (e.g., Williamson & Ashley, 1982) to directly inhibit the streaming and electrogenic transport, it can presumably act as a trigger for the inhibitory reaction which is responsible.

In conclusion our experimental data supports the concept that a large fraction of the conductance of the electrogenic proton pump is temporarily inhibited as a consequence of the generation of an action potential. This could prove to be a useful technique for investigating the electrical characteristics of this pump, as results are produced within a few minutes and a large number of sequential measurements are possible on the one cell. Conventional techniques involving various inhibitors can, however, take several hours. Incidently, these results also show that measurements taken within 10 min of an action potential need not necessarily reflect the steady-state membrane properties.

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References

Ashcroft, R.G., Coster, H.G.L., Smith, J.R. 1981. The molecular organisation of bimolecular lipid membranes. The dielectric structure of the hydrophilic/hydrophobic interface. *Biochim. Biophys. Acta* **643**:191–204

Beilby, M.J., Coster, H.G.L. 1979*a*. The action potential in *Chara corallina*. II. Two activation-inactivation transients in voltage clamping of the plasmalemma. *Aust. J. Plant. Physiol.* **6**:323–335

Beilby, M.J., Coster, H.G.L. 1979*b*. The action potential in *Chara corallina*. III. The Hodgkin-Huxley parameters for the plasmalemma. *Aust. J. Plant Physiol.* **6**:337–353

Beilby, M.J., Walker, N.A. 1981. Chloride Transport in *Chara*. 1. Kinetics and current-voltage curves for a probable proton symport. *J. Exp. Bot.* **32**:43–54

Bell, D.J., Coster, H.G.L., Smith, J.R. 1975. A computer based, four-terminal impedance measuring system for low frequencies. *J. Phys. E. Sci. Inst.* **8**:66–70

Bisson, M.A., Walker, N.A. 1980. The *Chara* plasmalemma at high pH. Electrical measurements show rapid specific passive uniport of H^+ or OH^-. *J. Membrane Biol.* **56**:1–7

Bisson, M.A., Walker, N.A. 1981. The hyperpolarization of the *Chara* membrane at high pH: Effects of external potassium, internal pH, and DCCD. *J. Exp. Bot.* **32**:951–971

Bisson, M.A., Walker, N.A. 1982. Transitions between modes of behaviour (states) of the charophyte plasmalemma. *In:* Plasmalemma and Tonoplast: Their Functions in the Plant Cell. D. Marme, E. Marre, and R. Hertel, editors. pp. 35–40. Elsevier Biomedical, Amsterdam

Chilcott, T.C., Coster, H.G.L., Ogata, K., Smith, J.R. 1983. Spatial variation in the electrical properties of *Chara:* II. Membrane capacitance and conductance as a function of frequency. *Aust. J. Plant Physiol. (submitted)*

Cole, K.S., Curtis, H.J. 1938. Electric impedance of *Nitella* during activity. *J. Gen. Physiol.* **22:**37–64

Coster, H.G.L., Smith, J.R. 1977. Low-frequency impedance of *Chara corallina:* Simultaneous measurements of the separate plasmalemma and tonoplast capacitance and conductance. *Aust. J. Plant Physiol.* **4:**667–674

Findlay, G.P. 1970. Membrane electrical behaviour in *Nitellopsis obtusa. Aust. J. Biol. Sci.* **23:**1033–1045

Findlay, G.P., Hope, A.B. 1964. Ionic relations of cells of *Chara australis.* VII. The separate electrical characteristics of the plasmalemma and tonoplast. *Aust. J. Biol. Sci.* **17:**62–77

Hope, A.B. 1965. Ionic relations of cells of *Chara australis.* X. Effects of bicarbonate ions on electrical properties. *Aust. J. Biol. Sci.* **18:**789–801

Hope, A.B., Walker, N.A. 1961. Ionic relations of cells of *Chara australis.* IV. Membrane potential differences and resistances. *Aust. J. Biol. Sci.* **14:**26–44

Kawamura, G., Shimmen, T., Tazawa, M. 1980. Dependence of the membrane potential of *Chara* cells on external pH in the presence or absence of internal adenosinetriphosphate. *Planta* **149:**213–218

Keifer, D.W., Spanswick, R.M. 1978. Activity of the electrogenic pump in *Chara corallina* as inferred from measurements of the membrane potential, conductance and potassium permeability. *Plant Physiol.* **62:**653–661

Kishimoto, U. 1972. Characteristics of the excitable *Chara* membrane. *Adv. Biophys.* **3:**199–226

Kishimoto, U., Akabori, H. 1959. Protoplasmic streaming of an internodal cell of *Nitella flexilis. J. Gen. Physiol.* **42:**1167–1187

Kitasato, H. 1968. The influence of H^+ on the membrane potential and ion fluxes of *Nitella. J. Gen. Physiol.* **52:**60–87

Lucas, W.J., Smith, F.A. 1973. The formation of acid and alkaline regions at the surface of *Chara corallina* cells. *J. Exp. Bot.* **24:**1–14

Pickard, W.F. 1973. Does the resting potential of *Chara brauni* have an electrogenic component? *Can. J. Bot.* **51:**715–724

Richards, J.L., Hope, A.B. 1974. The role of protons in determining membrane electrical characteristics in *Chara corallina. J. Membrane Biol.* **16:**121–144

Saito, K., Senda, M. 1973*a*. The light dependent effect of external pH on the membrane potential of *Nitella. Plant Cell Physiol.* **14:**146–156

Saito, K., Senda, M. 1973*b*. The effect of external pH on the membrane potential of *Nitella* and its linkage to metabolism. *Plant Cell Physiol.* **14:**1045–1052

Saito, K., Senda, M. 1974. The electrogenic ion pump revealed by the external pH effect on the membrane potential of *Nitella.* Influences of external ions and electric current on the pH effect. *Plant Cell Physiol.* **15:**1007–1016

Shimmen, T., Tazawa, M. 1977. Control of membrane potential and excitability of *Chara* cells with ATP and Mg^{2+}. *J. Membrane Biol.* **37:**167–186

Shimmen, T., Tazawa, M. 1980. Dependence of H^+ efflux on ATP in cells of *Chara australis. Plant Cell Physiol.* **21:**1007–1013

Smith, J.R., Coster, H.G.L. 1980. Frequency dependence of the AC membrane impedance of *Chara*: The effect of temperature. *In*: Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas, and J. Dainty, editors. pp 609–610. Elsevier/North Holland, Amsterdam

Smith, J.R., Walker, N.A. 1983. The membrane conductance of *Chara* measured in the acid and basic zones. *J. Membrane Biol. (in press)*

Smith, P.T., Walker, N.A. 1981. Studies on the perfused plasmalemma of *Chara corallina:* I. Current-voltage curves, ATP and potassium dependence. *J. Membrane Biol.* **60:**223–236

Spanswick, R.M. 1972. Evidence for an electrogenic ion pump in *Nitella translucens.* I. The effects of pH, K^+, Na^+, light and temperature on the membrane potential and resistance. *Biochim. Biophys. Acta* **288:**73–89

Spanswick, R.M. 1974*a*. Evidence for an electrogenic ion pump in *Nitella translucens.* II. Control of the light-stimulated component of the membrane potential. *Biochim. Biophys. Acta* **332:**387–398

Spanswick, R.M. 1974*b*. Hydrogen ion transport in giant algal cells. *Can. J. Bot.* **52:**1029–1034

Spear, D.J., Barr, J.K., Barr, C.E. 1969. Localisation of hydrogen ion and chloride ion fluxes in *Nitella. J. Gen. Physiol.* **54:**397–414

Walker, N.A. 1980. The transport systems of charophyte and chlorophyte giant algae and their integration into modes of behaviour. *In.* Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas, and J. Dainty, editors. pp. 287–304. Elsevier/North Holland, Amsterdam

Walker, N.A., Hope, A.B. 1969. Membrane fluxes and electrical conductance in Characean cells. *Aust. J. Biol. Sci.* **22:**1179–1195

Walker, N.A., Smith, F.A. 1977. Circulating electric currents between acid and alkaline zones associated with HCO_3^--assimilation in *Chara. J. Exp. Bot.* **28:**1190–1206

Williamson, R.E., Ashley, C.C. 1982. Free Ca^{2+} and cytoplasmic streaming in the alga *Chara. Nature (London)* **296:**647–650

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