J. Membrane Biol 68, 179–189 (1982)

The Journal of
Membrane Biology

# Electrogenic and Diffusive Components of the Membrane of *Hydrodictyon africanum*

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**Summary.** In cells of the freshwater alga *Hydrodictyon africanum*, in solutions where $[K^+]_o = 0.1$ mM and $pH_o > 7.0$, the membrane in the light is hyperpolarized. The membrane potential difference $\psi_m$ has values from $-180$ to $-275$ mV, more negative than any ion diffusion potential difference, and is predominantly a function of $pH_o$, and independent of $[K^+]_o$. The hyperpolarization of the membrane appears to arise from an electrogenic efflux of $H^+$, estimated from voltage-clamp data to be about 8 nmol $m^{-2}$ $sec^{-1}$ when $pH_o = 8.5$. In the light the membrane conductance $g_m$ is about 0.084 S $m^{-2}$. At light-off, $\psi_m$ becomes less negative, with a half-time for change of 15 to 30 sec and $g_m$ decreases by about 0.052 S $m^{-2}$. After dark periods of up to 300 sec, $\psi_m$ is largely independent of $pH_o$ for values greater than 6.0 and usually behaves as a combined $K^+$ and $Na^+$ diffusion potential with permeability ratio $P_{Na}/P_K = 0.05$ to 0.2. The membrane potassium conductance $g_K$ has either a low value of $2\text{–}6 \times 10^{-2}$ S $m^{-2}$, or a high value of up to $18 \times 10^{-2}$ S $m^{-2}$ depending on $[K^+]_o$, the transition from low to high values occurring when $\psi_m$ moves over a threshold value that is more negative than $\psi_K$, the electrochemical equilibrium potential for $K^+$. The time for half-change of the transition is about 30 sec. The results are consistent with a model of the membrane in which the pump electromotive force and conductance are in parallel with diffusive electromotive forces and conductances. When the pump is operating its properties determine membrane properties, and when it is inoperative, or running at a diminished rate, the membrane properties are determined more by the diffusive pathways. Changes in both pump rate and $g_K$ can account for a variety of characteristic changes in membrane PD and conductance occurring in response to light-dark changes, changes in light intensity, passage of externally applied electric current across the membrane and changes in ionic constituents of the external medium.

**Key words:** electrogenic transport · diffusive components · *Hydrodictyon* · membrane

## Introduction

Raven (1967) has shown that in the cells of the freshwater alga *Hydrodictyon africanum* the electric potential difference across the plasmalemma may arise from the diffusion mainly of $K^+$, but in a subsequent paper (Raven, 1968) suggested that under certain conditions a component of the potential difference might arise from an electrogenic influx of $HCO_3^-$. Hope (1965) had found previously that in *Chara corallina*, the addition of $HCO_3^-$ to the external medium, in the light, hyperpolarized the membrane, and concluded that there was an electrogenic $HCO_3^-$ influx. However, Spanswick (1970) showed that a similar hyperpolarization in *Nitella* can be produced by changes in external pH, with no $HCO_3^-$ present, and concluded that the hyperpolarization was an effect of changes of external pH. Spanswick (1973) further proposed that the hyperpolarization was caused by an electrogenic $H^+$ efflux pump. Indeed, it is becoming increasingly evident that the energy-requiring $H^+$ efflux pump plays a major role in ion and solute transport across plant cell membranes (Spanswick, 1981), and in the regulation of interacellular pH (Smith & Raven, 1979). In the light of accumulating evidence on the widespread existence of the $H^+$ efflux pump, it seems worthwhile to examine, in general, electrogenic transport in *Hydrodictyon africanum*, and in particular Raven's (1968) proposal for an electrogenic $HCO_3^-$ influx.

This paper describes experiments designed to investigate the electrical properties of the *Hydrodictyon* membrane under conditions where either electrogenic transport occurs and the electrical properties of the pump may determine, to a large extent, the electrical properties of the membrane, or where the transport is not operating or is operating at a reduced rate and the electrical behavior of the membrane is determined more by its passive permeability or diffusive properties.

## Materials and Methods

### *Material*

Coenocytes of *Hydrodictyon africanum* were grown either in the manner described by Raven (1967), in constant light and at 15 °C, or in sterile culture in 1-litre Ehrlenmeyer flasks again in constant

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0022-2631/82/0068-0179 $02.20

**Table 1.**

| Solution description | $[K^+ + Na^+]$ | Ionic concentrations (mM) | | | |
|---|---|---|---|---|---|
| | | $Cl^-$ | $Ca^{2+}$ | $HCO_3^-$ | Buffer |
| HPW 1 | 1.1 | 1.3 | 0.1 | 0.0 | 0.0 |
| HPW 2 | 1.5 | 1.3 | 0.1 | 0.4 | 0.0 |
| HPW 3 | 3.5, 10.5, 30.5 | 1.3 | 0.1 | 0.4 | 0.0 |
| HPW 4 | 10.5 | 10.3 | 0.1 | 0.4 | 0.0 |
| HPW 5 | 11.8 | 10.3 | 0.1 | 0.4 | 4.0 |

Particular solutions are specified with the $K^+$ concentration, e.g., HPW 1, K 0.1. In HPW 3, $[Na^+]$ was constant at 0.5 mM, and $[K^+]$ was either 3.0, 10.0 or 30.0 mM. Variants of these solutions are specified in detail in the text.

light and at 15 °C. These latter culture vessels were prepared by autoclaving 100 g of an acid loamy soil in 500 or 1,000 ml of a solution consisting of HPW 4 (as described later) together with trace elements, and were left to stand for several days to allow fine suspended material to settle. The starting materials for the culture were either zygotes or small nets from the Cambridge Algal Collection, or groups of small cells subcultured from previous cultures. Cells from nine separate cultures were used in the experiments described in this paper.

Samples of vacuolar sap for analysis of ionic content were obtained by first soaking the cells in a solution of 5 mM $CaCl_2$ for 1 min to remove extracellular $Na^+$ and $K^+$, followed by rinsing in distilled water for 1 min. The cells were then blotted dry, and cut open with a sharp razor blade on a greased surface. A 5–10 µl sample of vacuolar sap was then collected and added to 5 ml of distilled water for determination of $K^+$ and $Na^+$ concentrations by flame photometry.

*External Solutions.* Five basic artificial pond waters were used. The ionic constituents were $K^+$, $Na^+$, $Ca^{2+}$, $Cl^-$ and $HCO_3^-$. Where required, solutions were buffered with appropriate zwitterionic buffers, and the pH adjusted usually with either NaOH or HCl. In most of the experiments where the effects of different $[K^+]_o$ were examined, $[K^+ + Na^+]_o$ was kept constant, to eliminate changes in PD due to any Donnan system within the cell wall (Hope & Walker, 1961). The various solutions are listed in Table 1.

### *Electrical Measurements*

For most experiments, cells of diameter 3 mm or greater from cultures up to 9 months old were used. These cells had usually separated from one another, or could be separated by gentle pressure. The cells were transferred from the culture medium to an unbuffered artificial pond water consisting, basically, of NaCl 1.0 mM, KCl 0.1 mM and $CaCl_2$ 0.1 mM at a temperature of about 25 °C, usually $^1/_2$ to 1 hr before the insertion of microelectrodes.

The cells were illuminated by a tungsten light source, through the condenser system of the compound microscope used to view the cell.

Membrane potential differences were measured with 3 M KCl-filled glass microelectrodes (with extra internal capillary) of tip diameter 1–2 µm, inserted into the cell. With these microelectrodes successful insertions, where there was no visible loss of cell contents into the microelectrode, and where the cell retained its turgor pressure, were possible on a fairly routine basis. Under these conditions, the tip of the microelectrode most probably only penetrated into the cytoplasm, and thus the measured membrane PD should be the PD across the plasmalemma.

Membrane resistance was determined from the change in membrane PD resulting from the passage of a rectangular pulse of current from a separately inserted microelectrode, to the outside. The magnitude of the current pulse was adjusted so that the change in PD was usually less than 10 mV. The PD and current were recorded on a potentiometric pen-recorder. Voltage-clamp apparatus, used to maintain the PD constant, was similar to that described by Findlay and Hope (1976) with the current injected through a 3 M KCl-filled glass microelectrode.

## Results

### *(a) The Membrane Potential Difference ($\psi_m$) and Conductance ($g_m$) in the Light*

In seven separate batches of cells, totalling 66 cells in all, the steady value of $\psi_m$ for pH 5.0–6.0 and $[K^+]_o = 0.1$ mM was $-143 \pm 9$ mV. In three of these batches of cells the vacuolar concentrations of $K^+$ were 52.1, 53.3 and 15.5 mM, of $Na^+$ 12.8, 8.7 and 6.6 mM. The corresponding Nernst PD's were $\psi_K = -158$, $-158$ and $-127$ mV, and $\psi_{Na} = -64$, $-55$ and $-48$ mV for $[K^+]_o = 0.1$ mM and $[Na^+]_o = 1.0$ mM. In four batches of cells, totalling 16 cells, for pH 8.0–8.5 and $[K^+]_o = 0.1$ mM the steady value of $\psi_m$ was $-182 \pm 14$ mV. In three of these batches of cells $[K^+]_{vac} = 15.5$, 5.5 and 12.1 mM, $[Na^+]_{vac} = 6.6$, 1.5 and 2.4 mM, with $\psi_K = -101$, $-121$ and $-128$, and $\psi_{Na} = -10$, $-22$ and $-41$ mV.

Figure 1 shows $g_m$ as a function of the steady value of $\psi_m$ for cells in the light with $[K^+]_o = 0.1$ mM. Data from cells in a number of external media have been combined. It can be seen that $g_m$ decreases as $\psi_m$ becomes more negative.

### *(b) Responses of $\psi_m$ and $g_m$ to Light-off and Light-on*

In hyperpolarized cells (defined as those in which $\psi_m$ is more negative than any ionic diffusion potential) with $[K^+]_o = 0.1$ mM, $\psi_m$ at light-off changed to a less negative value after a latent period of $< 1.0$ sec, reaching either a peak or a plateau within 1.5 to 4 min. In some cells a small hyperpolarization lasting about 3 sec followed the latent period but preceded the main change. The mean half-time of rise of $\psi_m$ between the steady light level, and the peak dark level in five batches of cells totalling 22 cells in all was $24.4 \pm 4.3$ sec. After the initial depolarization of the membrane in the dark, there was some variation in the time course of $\psi_m$. In most cells, $\psi_m$ remained at the peak level, or slowly became more negative over periods of time of up to 4 min before moving more rapidly towards a level often intermediate between the peak dark level and the steady light level. In a few cells $\psi_m$ remained at the peak dark level for up to 10 min. A representative set of results is shown in Fig. 2, for periods of darkness from 2 to 17 min.

The light-off response was produced largely by the removal of red light ($\lambda > 690$ nm) from the source of illumination, while the removal, with filters, of light of wavelength < 540 nm had no effect on $\psi_m$.

The response of $\psi_m$ to light-on was more complex than the light-off response. Within 1–2 sec of the light being switched on, the membrane showed either a rapid depolarization or hyperpolarization preceding the major hyperpolarization which eventually returned $\psi_m$ to the light level.

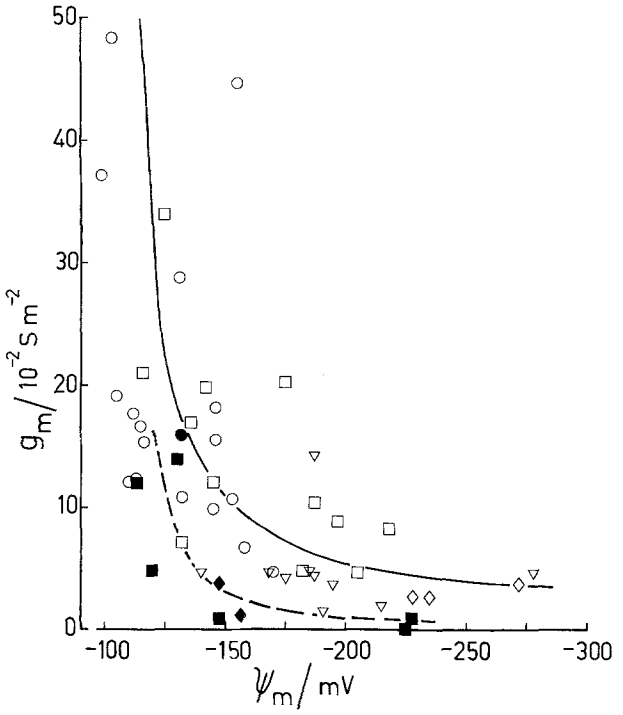


**Fig. 1.** Relationship between $\psi_m$ and $g_m$ in the light (open symbols) and in the dark (closed symbols) with $[K^+]_o = 0.1$ mM. The results are from 45 cells in the light, each point being a measurement from one cell made when $\psi_m$ had reached a steady value, and from nine of these cells after 2 min of darkness. (○, ●): cells in HPW 1 K 0.1; (□, ■): cells in HPW 2 K 0.1; (◇, ◆): cells in HPW 5 K 0.1. (○): cells in KCl 0.1 $NaHCO_3$ 0.4 $CaCl_2$ 0.1. The continuous and dashed lines have been fitted by eye to the data for light and dark conditions, respectively

At light-off and light-on $\psi_m$ and $g_m$ changed in the manner shown in the first pair of curves in Fig. 4. The usual result when the light was turned off was a decrease in $g_m$ to a quasi-steady dark level. In one batch of six cells, in the light, $g_m$ was $0.084 \pm 0.002$ (6) S m$^{-2}$ and $\psi_m$ $-213 \pm 15$(6) mV, while the dark levels were $0.032 \pm 0.006$(6) S m$^{-2}$ and $-102 \pm 20$(6) mV, respectively. The time for half change of $g_m$ was $21.5 \pm 2.1$(6) sec; when the light was turned on after short periods of darkness (up to 4–5 min) $g_m$ almost invariably increased to a value greater than the original steady light value, before decreasing to near its value prior to the dark period. The peak value of $g_m$ occurred about 100 sec after the light was turned on.

### *(c) $\psi_m$ as a Function of $[HCO_3^-]_o$ and $pH_o$*

Hyperpolarized cells were held in buffered solution at pH = 8.0 and $[HCO_3^-]_o$ was varied from 0 to 3 mM with each level being held for 15–20 min. Such changes had little effect on $\psi_m$ or on the light-off and light-on responses of $\psi_m$. On the other hand, $\psi_m$ varied with $pH_o$. Figure 3 shows $\psi_m$ as a function of $pH_o$ in the light and in the dark.

### *(d) Responses of $\psi_m$ and $g_m$ to Light-off and Light-on as a Function of $[K^+]_o$*

*(i) $\psi_m$ and $g_m$ as a Function of Time.* Figure 4 shows $\psi_m$ and $g_m$ in one cell as functions of time during light-off and light-on for a range of values of $[K^+]_o$ from 0.1 to 10 mM. The external medium was buffered at pH 8.5, and the membrane in the light was hyperpolarized for all values of $[K^+]_o$. In this particular cell, $\psi_m$, at light-off, became less negative, and $g_m$ decreased for $[K^+]_o$ up to 1.0 mM. For $[K^+]_o = 3.0$, 10 mM, $g_m$ subsequently rose again, while the curve

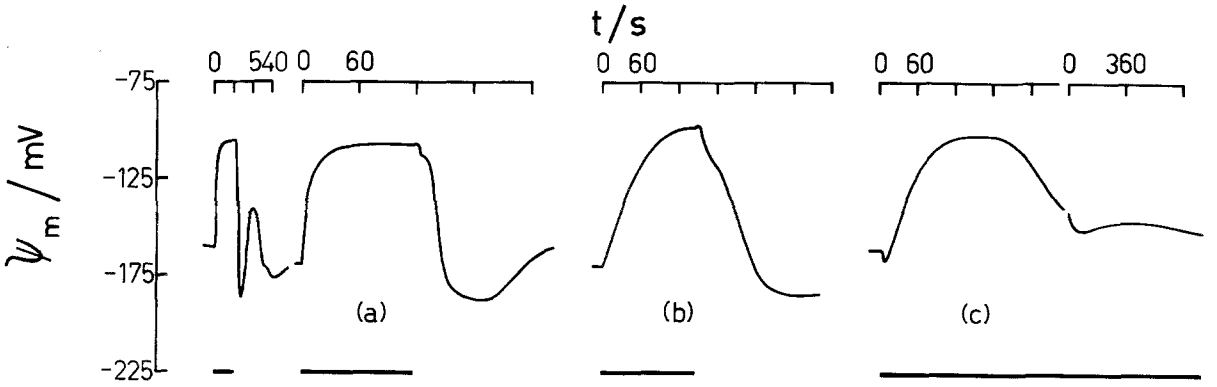


**Fig. 2.** A representative set of data showing the response of $\psi_m$ to light-on and light-off. $[K^+]_o = 0.1$ mM. Curves are tracings of the original pen-recordings

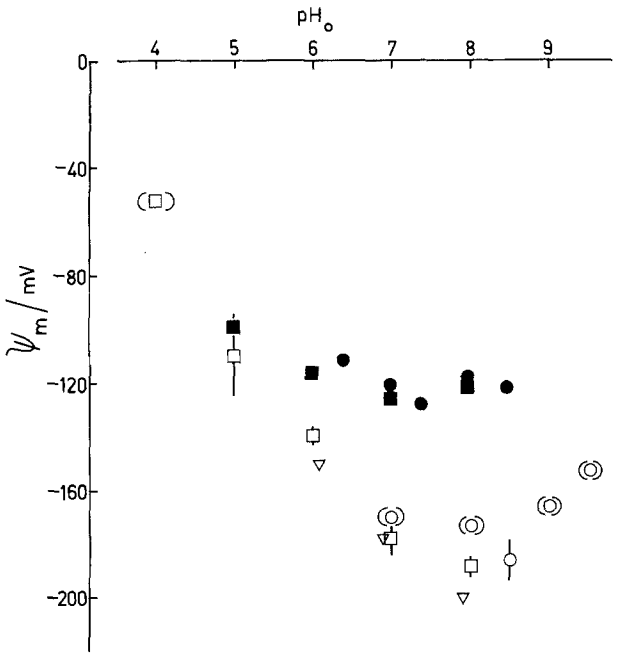


**Fig. 3.** Data from two separate batches of cells showing $\psi_m$ as a function of external pH in the light (○, □) and in the dark (●, ■). The external solutions were buffered, and $[K^+]_o$ was kept constant at 0.1 mM. Also shown (▽) are corresponding data from Table 3 of De Michelis, Raven and Jayasuriya (1969). Points in brackets are single observations. Vertical bars show SEM

of $\psi_m$ showed a characteristic point of inflection, followed by a more rapid rise to a steady level. In most cells this steady level was maintained for at least 10 min.

The rapid increase or "switching" in $g_m$ occurs when $\psi_m$ moves above a threshold level. This level is a function of $[K^+]_o$, as shown in Fig. 5. The threshold level of $\psi_m$ was taken as the value of $\psi_m$ at which $g_m$ started to rise, as shown by the arrows in Fig. 4. In two cells with $pH_o=8.5$, the change in membrane conductance in the dark as a result of switching (calculated as the difference between the minimum value of $g_m$ attained after light-off, and its maximum value after switching) was 0.081 and 0.062 S m$^{-2}$ for $[K^+]_o=3.0$ mM, and 0.157 and 0.182 S m$^{-2}$ for $[K^+]_o=10.0$ mM. The half-time for switching of $g_m$ was about 30 sec for both $K^+$ concentrations.

In cells where switching had occurred $\psi_m$ and $g_m$ at light-on responded in one of two ways. After the initial short-term transient in $\psi_m$ lasting some seconds, $g_m$ almost invariably rose to a value greater than the prior dark value, with a peak at about 100 sec before decreasing either to the original light level, or to a level near the dark level. In the first case $\psi_m$ returned to its prior light level; in the second case, $\psi_m$ rarely

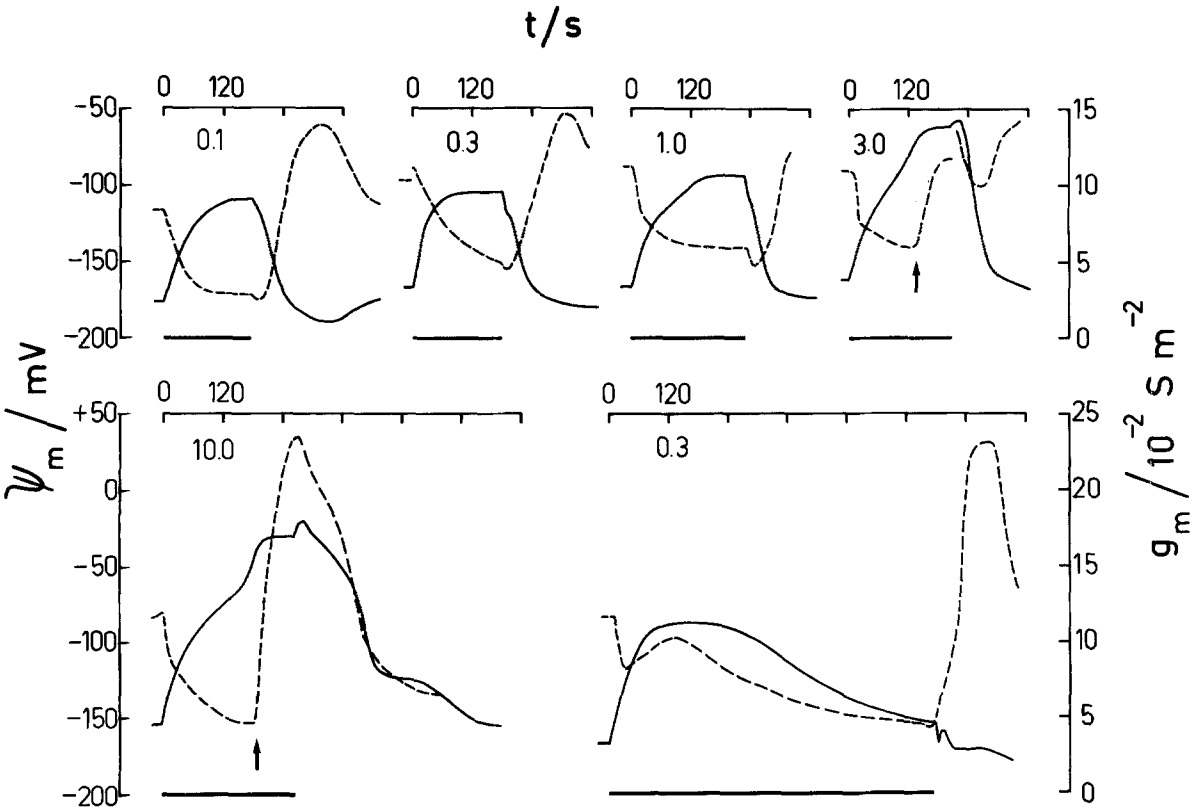


**Fig. 4.** Light-off and light-on responses of $\psi_m$ (continuous curve) and $g_m$ (dashed curve) in one cell as a function of $[K^+]_o$. The external medium was HPW 5 K 0.1–10.0. The vertical arrows show the start of switching of $g_m$. Bars show periods of darkness. Curves of $\psi_m$ are tracings from the original pen-recordings. Curves of $g_m$ are drawn through estimates of $g_m$ made every 15 to 30 sec

became more that 20 mV more negative than the dark level. Figure 6 shows a sequence of light-off and light-on responses for increasing $[K^+]_o$. For $[K^+]_o$ up to 1.0 mM, $\psi_m$ showed the first type of response, and for $[K^+]_o > 1.0$ mM, the second type of response.

*(ii) $\psi_m$ as a Function of $[K^+]_o$ in the Dark; Hodgkin-Katz Equation Fitted to the Data.* In cells where the membrane was hyperpolarized in the light, $\psi_m$ in the light was much less sensitive to changes in $[K^+]_o$ than was its steady value after short periods of darkness. For most cells it is possible to fit a Hodgkin-Katz (1949) equation to the data of $\psi_m$ as a function of $[K^+]_o$ in the dark, but not in the light. The equation used was $\psi_m = 58 \log ([K^+]_o + \alpha[Na^+]_o)/[C_i]$ where $\alpha = P_{Na}/P_K$, the permeabilities of the membrane to $Na^+$ and $K^+$, respectively, and $[C_i]$ is an internal concentration term. $[C_i]$ was estimated approximately by extrapolating the data for high $[K^+]_o$ with a slope of 58 mV per 10-fold change in $[K^+]_o$, and determining the value of $[K^+]_o$ for which $\psi_m = 0$. $\alpha$ was adjusted to give as close a fit as possible, and then small adjustments were made in $[C_i]$ and $\alpha$ re-adjusted, and so on. Figure 7(*a*) shows data from one cell, where $[K^+ + Na^+]_o$ was kept constant at either 1.5 or 10.5 mM. For six separate batches of cells, totalling 11 cells in all, $\alpha = 0.09 \pm 0.02$ and $[C_i] = 46 \pm 8$ mM.

In one particular batch of three cells, buffered at pH 8.5, the data for $\psi_m$ in the dark lay on two separate curves (*see* Fig. 7*b*). Only the upper curve could be fitted by a Hodgkin-Katz equation, with $\alpha = 0.05$ and $C_i = 50$ mM. In one of these cells, the points lay on the upper curve for all values of $[K^+]_o$; in another cell the points lay on the lower curve. However, with $[K^+]_o = 10$ mM, a short depolarizing current caused $\psi_m$ to move to a less negative value lying on the upper curve. In the third cell, values of $\psi_m$ lay on the lower curve for $[K^+]_o$ up to 1.0 mM, and on the upper curve for $[K^+]_o = 3.0$ and 10.0 mM.

### *(e) Voltage-clamp Experiments*

In some preliminary experiments, $\psi_m$ was clamped at its light level and the current required to maintain $\psi_m$ at this level during light-off and light-on was measured. At light-off, there was an inward current with an initial peak followed by a decrease to a steady level. Following light-on there was a transient outward current (*see* Fig. 8).

### *(f) Transients in $\psi_m$ and $g_m$*

The transient responses of $\psi_m$ and $g_m$ to light-on and light-off have been described in sections (*b*) and (*d*). A variety of responses can also be initiated both in light and dark by the passage across the membrane of externally applied current $I_m$, and by changes in various constituents of the external bathing medium, particularly $[K^+]_o$ and $pH_o$. Some of these responses are illustrated in Fig. 9. Figure 9(*a*) shows a standard response to light-on and light-off, with $[K^+]_o = 1.0$ mM. In Fig. 9(*b*), $\psi_m$ at light-off was steady at

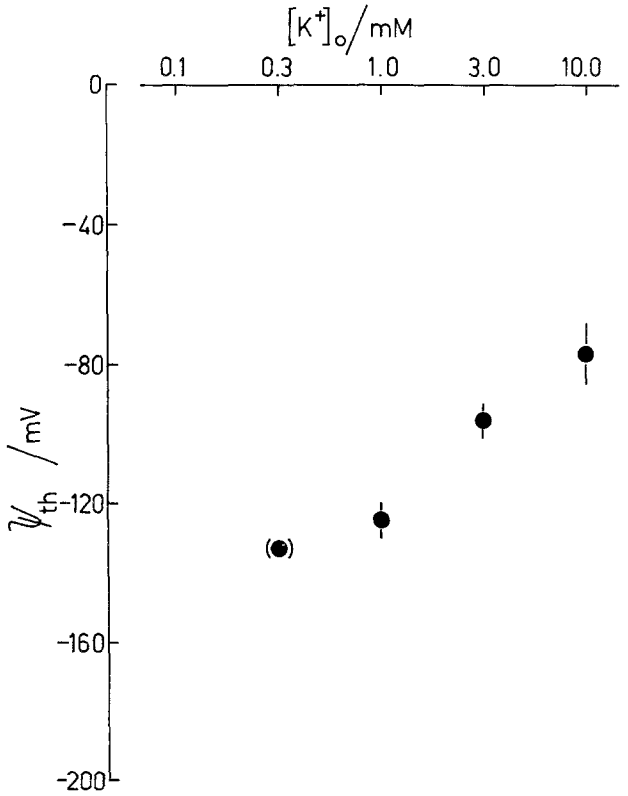


**Fig. 5.** Membrane PD, $\psi_{th}$, at which $g_m$ switches from a low to a high value, as a function of $[K^+]_o$. Vertical bars are SEM and the point in brackets is a single observation. Further details in the text

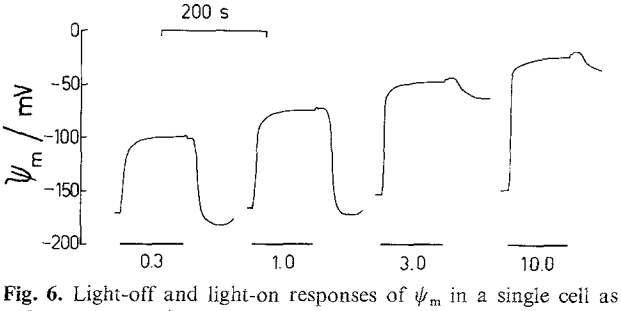


**Fig. 6.** Light-off and light-on responses of $\psi_m$ in a single cell as a function of $[K^+]_o$. Number near each curve shows $[K^+]_o$/mM. For $[K^+]_o$ up to 1.0 mM, the external solution was HPW 1, K 0.1–1.0, and for $[K^+]_o$ 3.0, 10.0 mM, HPW 3, K 3.0, 10.0. Bars show periods of darkness. Curves are tracings of the original pen-recordings

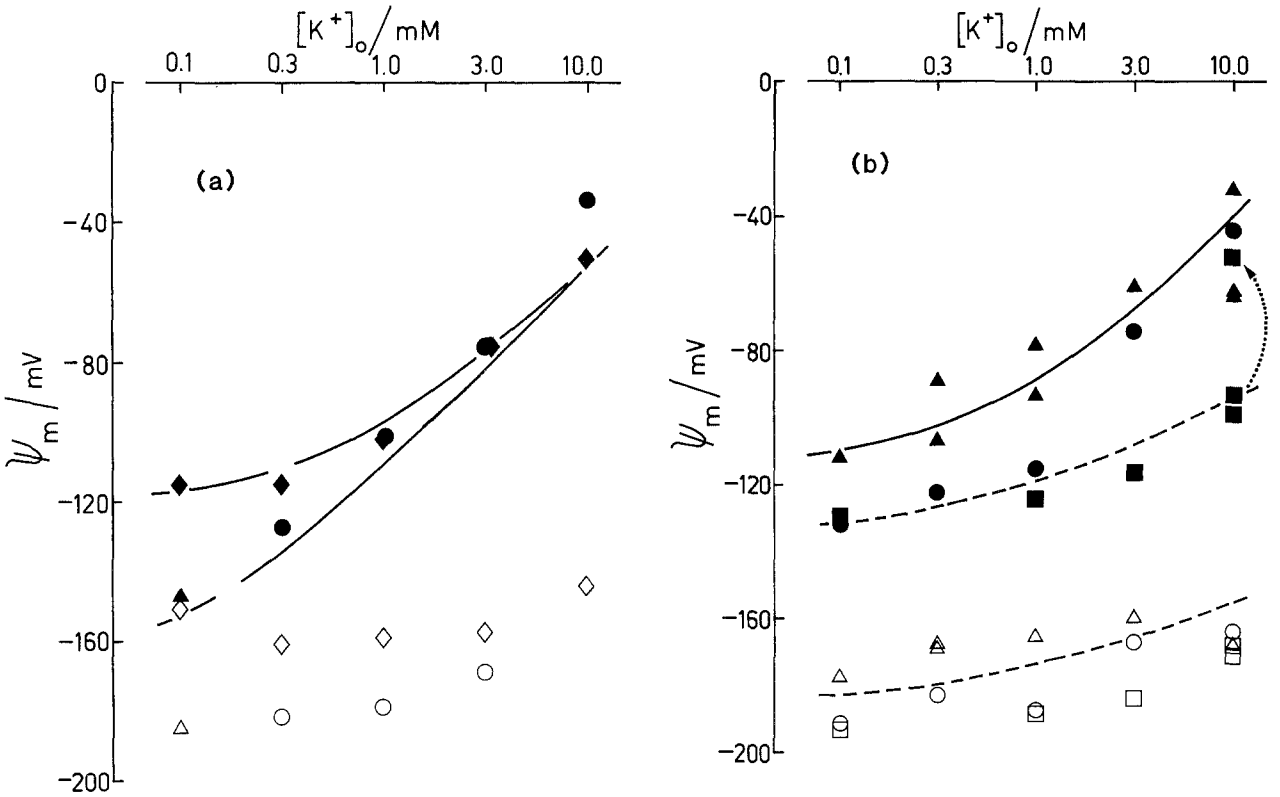


**Fig. 7.** (*a*). $\psi_m$ in the light (open symbols) and in the dark (closed symbols) as a function of $[K^+]_o$; (◇, ◆) HPW 4, K 0.1 to 10.0, (○, ●) Na 0.5 Ca 0.1 $HCO_3$ 0.4 Cl 1.3 K 0.3 to 10.0; (△, ▲) HPW 2, K 0.1. The continuous curves are theoretical ones fitted from the Hodgkin-Katz equation with $[C^+]_i=75$, $\alpha=0.06$. (*b*) $\psi_m$ in the light (open sympols) and in the dark (closed symbols) as a function of $[K^+]_o$ for three cells in a buffered external solution, HPW 5 HEPES 4, pH 8.5. The values of $\psi_m$ in the dark lie on the upper curve when the membrane has switched to its high-$g_m$ state. The continuous curve is a theroretical one fitted from the Hodgkin-Katz equation with $[C^+]_i=50$, $\alpha=0.05$; details are given in the text. The dashed curves were fitted by eye. The arrow shows the change in $\psi_m$ when a short pulse of depolarizing current was applied across the membrane, as illustrated in Fig. 9(*e*)

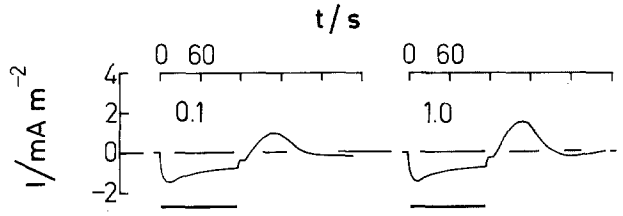


**Fig. 8.** Membrane current $I_m$ with $\psi_m$ voltage-clamped at the hyperpolarized level in the light, for a light-off, light-on sequence. External solutions were HPW 2, K 0.1, 1.0. Numbers near the curves show $[K^+]_o$/mM. Bars show periods of darkness. Curves are tracings of the original pen-recordings

−55 mV. Two stepwise increases in hyperpolarizing current produced steps in $\psi_m$. On the third step, $\psi_m$, after an initial step, then changed to −190 mV with a time course similar to that at light-on as shown in Fig. 9(*a*). After $I_m$ was reduced to zero, $\psi_m$ remained at −150 mV. A subsequent hyperpolarizing current of 4 mA m$^{-2}$ changed $\psi_m$ to −195 mV, and a period of darkness caused $\psi_m$ to become more negative by about 20 mV. When the hyperpolarizing current was reduced to zero, in the light, $\psi_m$ returned to about −150 mV.

In some cells in the light and in high $[K^+]_o$, the membrane was not hyperpolarized. Pulses of hyperpolarizing current of short duration and sufficient magnitude applied to these cells caused a change in $\psi_m$ from the unhyperpolarized to a hyperpolarized level, with a characteristic time course and corresponding decrease in $g_m$; but at the end of the pulse, $\psi_m$ returned to its original level (*see* Fig. 9*c*).

While stepwise increasing hyperpolarizing currents produced similar changes in $\psi_m$ in both light and dark, the responses of the membrane to the removal of the current differed. This is shown in Fig. 9(*d*). In the dark, the complete removal of the current caused $\psi_m$ to return to the original unhyperpolarized level of ~ −75 mV, whereas in the light $\psi_m$ remained at the hyperpolarized level. In cells where

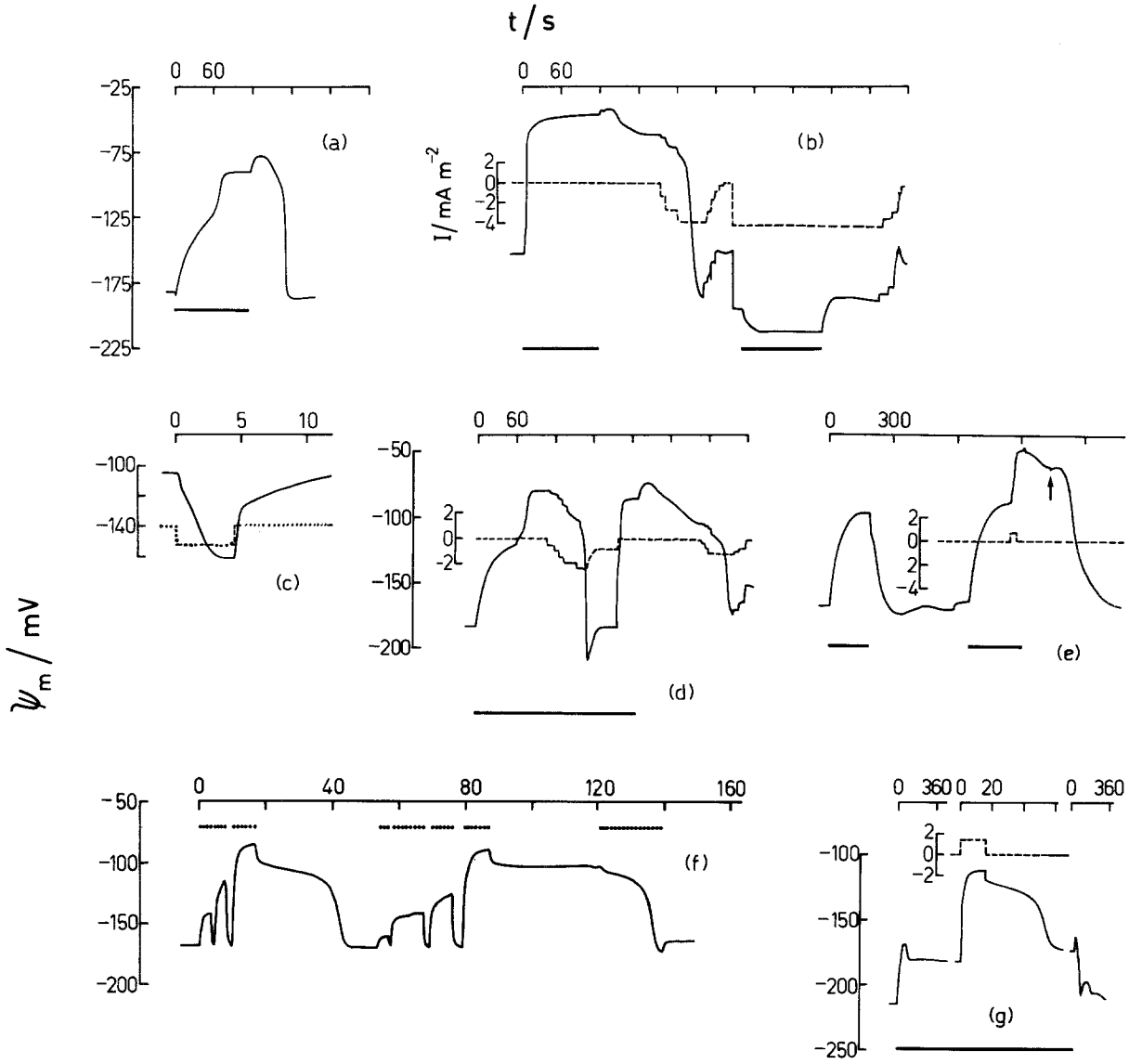


**Fig. 9.** Transient responses of $\psi_m$ to light-off, light-on and pulses of applied electric current. The bathing medium for each cell was (*a*) HPW 2, K 1.0; (*b*) HPW 3, K 3.0; (*c*) HPW 1, K 1.0; (*d*) HPW 2, K 1.0; (*e*) HPW 5, K 10.0; (*f*) HPW 1, K 1.0; (*g*) HPW 2, K 1.0. Bars show periods of darkness. Dashed lines show calibrated applied current, dotted lines uncalibrated applied current. The curves are tracings of the original pen-recordings. Further details are in the text

$\psi_m$ at light-off changed to a partially hyperpolarized level (*see d(ii)* of Results), a short pulse of depolarizing current caused $g_m$ to increase and $\psi_m$ to change to the unhyperpolarized level. This is shown in the second response of Fig. 9(*e*). After the current pulse $\psi_m$ was $-50$ mV and at light-on $\psi_m$ became more negative by about 15 mV. An increase in the light intensity caused $\psi_m$ to change to approximately the original level of $\sim -175$ mV.

In cells where $[K^+]_o \geqq 1.0$ mM, depolarizing current pulses produced the responses in $\psi_m$ shown in Fig. 9(*f*). In the first group of responses, the third depolarizing pulse has changed $\psi_m$ from $-170$ to $\sim -100$ mV from which it recovered to 170 mV with a time course showing a characteristic "shoulder". The final pulse of the next group of pulses has again shifted $\psi_m$ to $\sim -100$ mV, where it remained until a hyperpolarizing pulse caused a change to $-170$ mV with time course similar to the previous recovery. In the few cells where $\psi_m$ in the dark was at an intermediate hyperpolarized level with $[K^+]_o =$ 1.0 mM, depolarizing current pulses produced similar responses to those shown in Fig. 9(*f*) (*see* Fig. 9*g*).

A decrease in $pH_o$ from 8.5 to 6.5 with $[K^+]_o =$

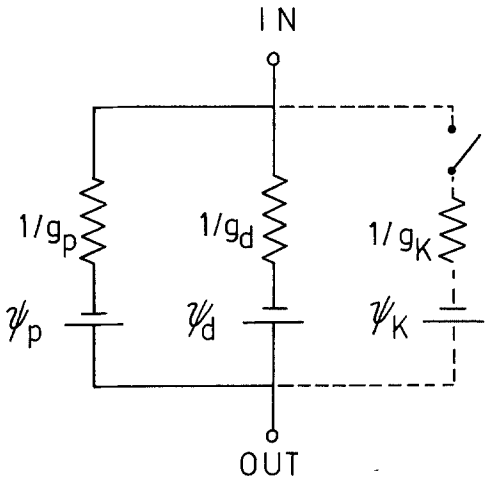


**Fig. 10.** Electrical analog of the *Hydrodictyon* membrane; before switching in $g_K$ shown by continuous lines, and after switching the with addition of the dashed lines, with the switch closed.

1.0 mM often caused $\psi_m$ to change from a hyperpolarized level to the unhyperpolarized level with a time course quite similar to that produced by light-off. Occasionally a change in $[K^+]_o$ to a high value like 1.0 mM, also produced a similar transient in $\psi_m$.

## Discussion

### *Electrical Model of the Membrane*

There is now reasonable evidence that the active efflux of $H^+$ is a major transport process across many plant, fungal and bacterial membranes and that the electrical properties of these membranes are probably dominated by the characteristics of the pump itself (Spanswick, 1981). When the $H^+$ pump is operating $\psi_m$ and $g_m$ will be determined mainly by the nature of the $H^+$ transport; when it is not, $\psi_m$ and $g_m$ will be determined by the diffusive properties of the membrane. The simplest electrical model is shown in Fig. 10. In this series-conductance model the membrane conductance $g_m$ is given by:

$$g_m = g_p + g_d \tag{1}$$

where $g_p$ is the conductance of the electrogenic pump and $g_d$ is the diffusive conductance. We may write:

$$\psi_m = \psi_d + \{g_p/(g_d+g_p)\}(\psi_P - \psi_d) \tag{2}$$

where $\psi_p$ is the PD the membrane would have if the pump were operating and $g_d=0$, and $\psi_d$ the PD when the pump is not operating and $g_p=0$.

The experiments described in this paper provide information about both pump and diffusive properties of the *Hydrodictyon* membrane and it will be shown that the series conductance model, with some elaboration of the diffusive components to allow for switching of $g_K$, provides a satisfactory account of membrane electrophysiological behavior in *Hydrodictyon*.

### *The Electrogenic Pump*

It will be assumed that $\psi_m$ is the membrane PD between the cytoplasm and outside, across the plasmalemma alone, although it has been found that in fitting the Hodgkin-Katz equation to the data relating $\psi_m$ to $[K^+]_o$ in the dark the internal concentration $[C^+]_i(=[K^+]_i+\alpha[Na^+]_i)$ occasionally needed to be as low as 25 mM. It seems unlikely that this concentration is realistic for the cytoplasm (Wyn-Jones, Brady & Speirs, 1979) although it could apply to the vacuole as low vacuolar concentrations have been measured by Smith, Raven and Jayasuriya (1978) and in the present investigations.

A demonstration of an electrogenic pump relies to a considerable extent on the observation that the membrane PD is more negative than the possible Nernst potentials for the ions in the system. For the vacuole $\psi_K$ lies between $-100$ and $-150$ mV, and $\psi_{Na}$ between $-10$ and $-64$ mV. Cytoplasmic concentrations were not measured, but if we take $[K^+]_{cyt.}=93$ mM and $[Na^+]_{cyt.}=57$ mM as given by Raven (1967), then for $[K^+]_o=0.1$ mM and $[Na^+]_o=1.0$ mM, $\psi_K=-172$ mV and $\psi_{Na}=-99$ mV. Anions such as $Cl^-$ and $HCO_3^-$ will probably have positive Nernst potentials in the vacuole and cytoplasm. De Michelis, Raven and Jayasuriya (1979) have measured the pH of vacuole and cytoplasm in *Hydrodictyon africanum* as a function of $pH_o$. Their measurements of intracellular PD as a function of $pH_o$ are similar to the results reported in this paper (*see* Fig. 3) and assuming that their estimates of internal pH are appropriate for the cells used in the experiments reported in this paper we have for $pH_o=7.9$, the cytoplasmic pH at 7.6, with $\psi_H=-17$ mV, and the vacuolar pH at 5.7 with $\psi_H=-128$ mV. In cells where $pH_o \geqq 8.0$, $\psi_m$ had values more negative than $\psi_K$, $\psi_{Na}$, $\psi_H$ and $\psi_{Cl}$ and in these cells the conclusion that there is electrogenic transport seems to be unequivocal, regardless of whether $\psi_m$ is the PD between cytoplasm or vacuole and the outside. When $pH_o=5.0$–$6.0$, $\psi_m$ was not always more negative than $\psi_K$. However, a value of $\psi_m$ lying within the possible range of Nernst potentials does not rule out the possibility of an electrogenic transport, but the presence of such a transport would have to be established by other means. The evidence that $\psi_m$ is unaffected by changes in $[HCO_3^-]_o$, when the external pH is kept constant is probably sufficient

to rule out the possibility of an electrogenic $HCO_3^-$ transport. There may, however, be a nonelectrogenic transport of $HCO_3^-$, although Walker, Smith and Cathers (1980) have argued that in *Chara corallina* assimilation by diffusion of $CO_2$ is possible, notwithstanding the generally held view that there is inward transport of $HCO_3^-$ that may be electrogenic (Lucas, 1977; Walker & Smith, 1977).

Changes in $[Cl^-]_o$ had no appreciable effect on $\psi_m$ (Findlay, *unpublished data*), except in one batch of cells which depolarized when external $Cl^-$ was replaced by $SO_4^{2-}$, but only after $\psi_m$ had been hyperpolarized by an increase in $[K^+]_o$ from 0.1 to 1.0 mM. Rybová, Janacek and Slavikova (1977) have also found a hyperpolarizing effect of high $[K^+]_o$ on $\psi_m$ in *Hydrodictyon reticulatum,* but without the $Cl^-$ effect. Thus $Cl^-$ does not in general appear to be involved in an electrogenic transport in *Hydrodictyon africanum* and its role in the one batch of cells is unclear.

Of the cations $K^+$, $Na^+$ and $H^+$, Raven (1967) has shown that the efflux of $K^+$ is essentially passive. This leaves us with the effluxes of $Na^+$ and $H^+$. As the evidence from other plant, fungal and bacterial systems (*see* Spanswick, 1981) points strongly in the direction of an electrogenic $H^+$ transport it is most likely that in *Hydrodictyon*, also, the electrogenic transport is predominantly of $H^+$ outwards, although at present there does not seem to be any evidence which would definitely rule out an electrogenic transport of $Na^+$.

In terms of the electrical analog shown in Fig. 10, the $H^+$ pump will be characterized when $g_p$ and $\psi_p$ known. In most cells $g_m$, after short periods of darkness has decreased and $\psi_m$ behaves predominantly as a $K^+$ diffusion potential, implying that the pump components probably no longer appear to any appreciable extent in the circuit. Hence $g_{dD}=g_{mD}$, where $g_{dD}$, $g_{mD}$ are the diffusive and total membrane conductances in the dark, respectively. From Eq. (1) we have $g_p+g_{dL}=g_{mL}$ where $g_{dL}$, $g_{mL}$ are diffusive and total membrane conductances in the light. Thus $g_{mL}-g_{mD}=g_p+(g_{dL}-g_{dD})$. If it is assumed that $g_{dL}=g_{dD}$, then $g_p=g_{mL}-g_{mD}$ and the data in Fig. 1 show that for $[K^+]_o=0.1$ mM, $g_p$, calculated from the difference between the two curves, ranges from 0.079 S m$^{-2}$ for $\psi_m=-150$ mV, to 0.037 S m$^{-2}$ for $\psi_m=-225$ mV. Another set of data, where the mean value of $\psi_m$ in the light was $-213$ mV gave $g_p=0.052$ S m$^{-2}$. As $\psi_m$ in the light is about 50 to 75 mV more negative than $\psi_m$ in the dark it is only where $g_{dD}$ (assumed equal to $g_{dL}$) is small compared with $g_{mL}$ or relatively independent of $\psi_m$, that the change in $g_m$ at light-off will give a measure of $g_p$. It is worth noting here that in *Hydrodictyon*, $g_m$ in the light and in the dark is low compared with cells of other plant genera, where $g_m$ is often in the range 0.3 to 1.0 S m$^{-2}$; for example in *Riccia fluitans* (Felle & Bentrup, 1976).

Under conditions where the diffusive conductance is relatively independent of $\psi_m$ and again with the assumption that $g_{dD}=g_{dL}$, $\psi_p$ can be calculated from Eq. (2). In three cells, with $[K^+]_o=0.1$ mM, $\psi_p=247\pm21$ mV, a value reasonably close to that expected for an $H^+$ efflux pump working with a $2H^+$/ATP stoichiometry (Walker & Smith, 1975; De Michelis, Raven & Jayasuriya, 1979).

If the assumption that $g_{dD}=g_{dL}$ is invalid it is only possible to set an upper limit for $g_p$; $g_p=g_{mL}$ if $g_{dL}=0$. Furthermore it will not be possible to calculate $\psi_p$.

Any changes in $g_d$ resulting from changes in $\psi_m$ should be eliminated if $\psi_m$ is clamped at a constant level at light-off. The voltage-clamp data of Fig. 8 show that to hold $\psi_m$ constant at light-off it is necessary, within 10–15 sec, to provide a membrane current of 1.6 mA m$^{-2}$ (16.58 nmol m$^{-2}$ sec$^{-1}$). This current should represent the current provided in the light by the $H^+$ pump if the pump had stopped completely at this time and provided $g_{dL}=g_{dD}$. Subsequently the clamp current declines to a steady value of about 0.8 mA m$^{-2}$. This change could represent a recommencement of the pump, or the presence of a residual pump activity in the dark together with a decrease in $g_d$; either would tend to hyperpolarize the membrane and diminish the required clamp current. Certainly in some cells (*see* Figure 9*b*) $\psi_m$ at light-off changed to a level distinctly below the expected diffusive level suggesting that the $H^+$ pump had not stopped entirely.

The voltage-clamp data also show that at light-on there is a pronounced transient outward current most probably arising from a temporary enhancement of the pump. Further evidence for pump activity immediately after light-on comes from Fig. 4, which shows a transient increase in $g_m$ above the steady level in the light, almost certainly caused by an increase in $g_p$, rather than $g_d$, because at the same time $\psi_m$ is becoming more negative.

### *Diffusive Components*

At light-off $g_m$ initially decreases, but at the same time $\psi_m$ becomes less negative. When $\psi_m$ moves over a threshold level, $\psi_{th}$, $g_m$ rises to a new level, higher than the original level in the light. Thus when this occurs the steady level of $g_m$ in the dark is higher than the steady level in the light. We need to be aware that $g_m$ has undergone two distinct changes – the initial decrease followed by an increase (*see*

Fig. 4). A comparison of Fig. 5 with Fig. 7(*a*) shows that as well as being a function of $[K^+]_o$, $\psi_{th}$ also lies at more negative values that the steady dark level of $\psi_m$ for all $[K^+]_o > 0.3$ mM. The simplest interpretation of these results is that at light-off first the pump rate and consequently $g_p$ decreases and following this $g_d$ increases. The reasonable fit of the Hodgkin-Katz equation to the data after 2–4 min of darkness strongly suggests that at this time the measured parameters are those for the diffusive components of the membrane; the membrane being appreciably more permeable to $K^+$ than to $Na^+$, with $\alpha$ $(=P_{Na}/P_K) \simeq$ 0.09. This implies that the prior increase in $g_d$ (by at least 4× when $[K^+]_o = 10$ mM) is caused by an increase or "switching" in the $K^+$ conductance, $g_K$. Felle and Bentrup (1976) have found similar behavior in *Riccia fluitans*.

### *Transient Responses of the Membrane*

*Response to Light-off and Light-on.* The broad features of the transient responses of $\psi_m$ and $g_m$ to light-off, light-on, to changes in light intensity, and to the passage of applied electric current across the membrane (Fig. 9) can now be seen to result from the following aspects of behavior of the pump and diffusive pathways in the membrane; (a) at light-off the pump rate diminishes, and within 2–4 min $g_p$ has usually become very small compared with $g_d$; (b) at light-on the pump rate, and consequently $g_p$, increases and is temporarily enhanced above its steady rate in the light; (c) switching occurs in $g_K$ when $\psi_m$ passes a threshold level, $\psi_{th}$; (d) $\psi_{th}$ is a function of $[K^+]_o$; (e) the switching in $g_K$ is not instantaneous.

There are two types of response of the hyperpolarized membrane to light-off. In the most common, (Fig. 9*a*, *b*, *d*), as the pump rate diminishes, and $g_p$ decreases, $\psi_m$ becomes less negative, and $g_K$ rises when $\psi_m$ moves through $\psi_{th}$; $\psi_m$ then changes to a level determined predominantly by the diffusion of $K^+$. The total membrane conductance $g_m$ thus initially declines, and then increases (*see* Fig. 4; $[K^+]_o = 3.0$, 10.0 mM). In the other type of response (Fig. 9*e*), $\psi_m$ first becomes less negative and $g_m$ decreases, but the pump rate apparently does not decline sufficiently to move $\psi_m$ through $\psi_{th}$, and $\psi_m$ approaches a steady hyperpolarized level which varies with $[K^+]_o$ as shown in Fig. 7(*b*), but cannot be fitted by the Hodgkin-Katz equation. At light-on $\psi_m$ simply returns to the light level. Where $\psi_m$ is determined by diffusion, with $g_K$ switched to its higher level, the membrane responds to light-on in one of two ways. Apart from an initial transient, $\psi_m$ shows a slow initial change preceding an acceleration of $\psi_m$ with time, and a characteristic "slipping" of $\psi_m$ to the original more negative light level. Here, the pump activity initiated by light-on has been sufficient to hyperploarize the membrane to the extent that $\psi_m$ goes through $\psi_{th}$; thus $g_K$, and consequently $g_m$ decreases, and consequently $dg_m/dt$ increases. Eventually the pump rate declines, with the $H^+$ transport sufficient to hold the membrane at its hyperpolarized level (Fig. 9*a*). Similar behavior in *Chara* has been described by Hope (1965) and in *Hydrodictyon reticulatum* by Metlička and Rybová (1967). In the other response to light-on, which often occurs when $[K^+]_o \geqq 3.0$ mM, $\psi_m$ becomes more negative by less than 20–25 mV, and $g_m$ remains high (Fig. 9*b*). In this case, the high value of $g_K$ has prevented the peak pump current from producing a change in $\psi_m$ sufficient to move it through $\psi_{th}$, and thus the diffusive pathways continue largely to determine the membrane properties.

*Response to Applied Electric Current.* The responses of the membrane to applied electric current appear to arise almost solely from changes in the diffusive components of the membrane. The applied current moves $\psi_m$ through $\psi_{th}$, switching in $g_K$ is initiated, and the values of $\psi_m$ and $g_m$ after the current is removed depend largely on the extent of the change in $g_K$ while the current was applied. This is particularly apparent in Fig. 9(*f*) where the third of the first group of depolarizing current pulses has partially switched $g_K$ on, but when the current is removed, although $\psi_m$ is near $\psi_K$ the pump current is sufficient to return $\psi_m$ to its original level. However, the fourth of the second group of pulses has produced an increase in $g_K$ sufficient to counteract the effect of the pump when the applied current pulse is removed, and thus $\psi_m$ remains near $\psi_K$. Switching of $g_K$ is not noticeably light-dependent as shown by the responses of the membrane to current pulses in light (Fig. 9*c*, *f*) and dark (Fig. 9*g*).

The response of the membrane to hyperpolarizing current pulses depends on the extent to which $g_K$ switches off, and the magnitude of the pump current. In fact, hyperpolarizing current acts as an augmentation of the pump current. In Fig. 9*b*, the application of hyperpolarizing pulses of increasing intensity to a cell in the light eventually moved $\psi_m$ below $\psi_{th}$, $g_K$ switched off, and $\psi_m$ moved to $\sim -195$ mV. The current was then decreased in steps, and eventually removed altogether with the remaining pump current sufficient to keep the membrane hyperpolarized. Another pulse of hyperpolarizing current which returned $\psi_m$ to $-195$ mV, caused an extra 20-mV hyperpolarization of the membrane at light-off, as a result of a decrease in $g_m$ – presumably a decrease in $g_p$. In a cell in the dark (Fig. 9*d*), a stepwise increasing hyperpolarizing current caused the membrane to hyper-

polarize as in Fig. 9(*b*), but the pump current alone was not sufficient to maintain $\psi_m$ at the hyperpolarized level, and $\psi_m$ returned to its original value of $\sim -90$ mV, when the applied current was removed. Later, with the same cell in the light, the pump current alone was sufficient to hold $\psi_m$ at its more negative level after $g_K$ had switched off.

*Other Stimuli.* At the time shown by the arrow in Fig. 9(*e*) the light intensity was increased. Prior to this time, a depolarizing pulse of current had caused $g_K$ to switch on, but at light-on the resultant pump current was insufficient to cause $\psi_m$ to go to its original level of $-165$ mV and $\psi_m$ steadied at $\sim -70$ mV. The subsequent hyperpolarization of the membrane when the light intensity was increased is easily accounted for if it is assumed that the pump current increased and that $\psi_m$ moved through $\psi_{th}$ with a resultant decrease in $g_K$. An alternative explanation, that the pump rate did not change but $g_m$ decreased seems unlikely.

Changes in appropriate constituents of the external solution which move $\psi_m$ through $\psi_{th}$ also cause switching in $g_K$. In some cells a decrease in $pH_o$ from 8.5 to 6 was sufficient to cause $\psi_m$ to go from $-180$ to $-120$ mV. An increase in $[K^+]_o$ often achieved the same result.

## Conclusions

The important aspects of the electrophysiology of the *Hydrodictyon* membrane are summarized in Fig. 10. There is an electrogenic $H^+$ efflux pump, represented by the electromotive force $\psi_p$ in series with the pump resistance, $1/g_p$. Across the membrane, the diffusive component of the membrane is in parallel with the pump component. The passive conductance has two components, $g_d$ and $g_d+g_K$, depending on whether $\psi_m$ is more or less negative than a threshold value $\psi_{th}$, and switches from one to the other when $\psi_m$ moves through $\psi_{th}$. On the assumption that $g_d$ is the same in light and dark, estimates of various parameters can be made with $pH_o=8.5$ and $[K^+]_o=0.1$ mM; $\psi_p \simeq -250$ mV, $\psi_d \simeq -110$ mV, $g_p \simeq 0.05$ S m$^{-2}$ and $g_d \simeq 0.03$ S m$^{-2}$. When $[K^+]_o=10$ mM, $\psi_K \simeq -40$ mV and $g_K \simeq 0.18$ S m$^{-2}$. The pump conductance is diminished to a low value after short periods of darkness, although it is not known if $\psi_p$ is a function of light intensity. The switching of $g_K$ can occur in light and dark, and is largely determined by the level of $\psi_m$.

I thank my colleagues, N.A. Walker, F.A. Smith, A.B. Hope and E.A.C. MacRobbie for critical comments on the manuscript. Dr. MacRobbie kindly provided me with laboratory space in the Botany School, University of Cambridge, for some of the experimental work.

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Received 5 January 1982