IONIC RELATIONS OF MARINE ALGAE

III.* *CHAETOMORPHA*: MEMBRANE ELECTRICAL PROPERTIES AND CHLORIDE FLUXES

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Abstract

In the large coenocytic cell of the marine green alga *Chaetomorpha darwinii*, the electric potential difference, ψ_{vo}, between the vacuole and the outside seawater can have either of two distinct states, a positive, and more usual state, with $\psi_{vo} = +5$ mV, and a negative state with $\psi_{vo} = -29$ mV. The p.d. across the plasmalemma of the cell was approximately -72 mV, and the difference between the positive and negative states occurred at the tonoplast with $\psi_{vc} = +77$ mV or $+43$ mV respectively. In the change from the positive state to the negative state, the electrical resistance of the plasmalemma increased from 510 to 750 Ω cm^2, and the resistance of the tonoplast increased from 4900 to 7100 Ω cm^2.

The plasmalemma depolarized when the external potassium concentration was increased, and when the external chloride concentration was decreased. The results indicate permeability ratios $\alpha = P_{Na}/P_K$ of 0·01–0·1, and $\delta = P_{Cl}/P_K$ of 0·05–0·1.

In cells with ψ_{vo} in the positive state, chloride was usually near electrochemical equilibrium between vacuole and outside. At the plasmalemma the influx and efflux of chloride were usually in the range 80–260 pmoles cm^{-2} sec^{-1}, and at the tonoplast about 50–230 pmoles cm^{-2} sec^{-1}. These fluxes, together with the potassium and sodium fluxes of 100 and 150 pmoles cm^{-2} sec^{-1} at the plasmalemma, and 100 and 4 pmoles cm^{-2} sec^{-1} at the tonoplast (Dodd, Pitman, and West 1966), are too high, particularly at the tonoplast, to be compatible with the electrical resistances of the membranes. It is concluded that an appreciable fraction of each of the ionic fluxes does not occur by passive diffusion, but possibly exchange diffusion. Such fluxes will not contribute to the electrical resistance.

It is argued that the change in ψ_{vc} between positive and negative states is caused by a decrease in the permeability of the tonoplast to chloride ions.

I. INTRODUCTION

Dodd, Pitman, and West (1966) have measured membrane potentials and fluxes of potassium and sodium in cells of the marine alga *Chaetomorpha darwinii* (Hooker) Kuetzing, a marine member of the Cladophorales. They found that the cell

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contained relatively high concentrations of potassium (540 mM) and low concentrations of sodium (25 mM), and suggested that the discrimination for potassium was due to active transport of potassium inwards and sodium outwards at the plasmalemma. Fluxes of potassium across both plasmalemma and tonoplast were about 100 pmoles cm^{-2} sec^{-1}. No measurements were made of chloride fluxes, but the chloride concentration in the vacuole (600 mM) was somewhat higher than that in seawater. With respect to the outside the potential in the vacuole was about +10 mV and the potential in the cytoplasm was thought to be −32 mV, though there was some uncertainty about this value.

The present paper describes further measurements of the electrical properties of the cell membranes in *Chaetomorpha* and gives estimates of the fluxes of chloride across plasmalemma and tonoplast. A more complete description of ion transport processes in the cell is given.

II. Material and Methods

(*a*) *Plant Material*

Strands of cells of *C. darwinii* were collected from the edge of an intertidal rock platform at Robe, S.A., from a similar location on Kangaroo Island, S.A., and from a shallow beach at Port Elliot, S.A. The cells were stored in aerated seawater at 12°C in natural light of low intensity. In the experiments either single cells or short strands of four to five cells were used, usually within 2–3 weeks after their collection. The temperature was 23°C and the experimental solution was an artificial seawater normally comprising 490 mM NaCl, 10 mM KCl, 11·5 mM $CaCl_2$, 25 mM $MgCl_2$, 25 mM $MgSO_4$, and 2·5 mM $NaHCO_3$. This solution will be referred to as ASW. In some experiments the composition of the bathing solution was varied, as described below.

(*b*) *Estimation of Vacuolar Ionic Concentration*

Vacuolar sap was collected and analysed for potassium, sodium, and chloride as described by Findlay, Hope, and Williams (1969) for cells of *Griffithsia*.

(*c*) *Electrical Measurements*

The methods used in measuring membrane p.d. and resistance were similar to those used for *Griffithsia* cells as described previously (Findlay, Hope, and Williams 1969).

It was usually not possible to record the potential in the cytoplasm for longer than a few minutes before the tip of the inserted microelectrode either became sealed (in which case the recorded p.d. went to near zero) or broke into the vacuole. We have not been able to discover any consistently reliable way to insert an electrode into the cytoplasm. Rarely was a careful insertion just through the cell wall successful for more than a few seconds. The most successful measurements were obtained by inserting the microelectrode into a region where there was an accumulation of cytoplasm—either near another inserted electrode, or in a region where an electrode had previously been inserted and subsequently withdrawn. While there is no certainty that measurements of potential made in this way represent the potential of the cytoplasmic phase as a whole, insertions in different places in the one cell usually gave the same result.

(*d*) *Measurement of Chloride Fluxes*

Fluxes of chloride at the plasmalemma (ϕ_{oc}, ϕ_{co}) and tonoplast (ϕ_{cv}, ϕ_{vc}) were measured either by efflux analysis, or by comparison of uptake of radioactive isotope to cytoplasm and vacuole. These methods for estimating fluxes have been described previously (Findlay, Hope, and Williams 1970).

The long-term fluxes, ϕ_{ov} and ϕ_{vo}, were determined using the equations

$$\phi_{ov} = \Delta Y_v/(\Delta t.S_o) \text{ with } S_v \simeq 0$$

and

$$\phi_{vo} = \Delta Y_o/(\Delta t.S_v) \text{ with } S_o \simeq 0,$$

where ΔY_v and ΔY_o are the changes in amount of radioactivity in either the vacuole or outside, in time Δt; S_o and S_v are the specific activities outside the cell and in the vacuole respectively.

In the situation where S_v, during a long-term uptake of radioactivity, becomes an appreciable fraction of S_o, ϕ_{ov} cannot be calculated directly using the equation above. Instead, ΔY_v, for a small Δt, was calculated from the equation for uptake, viz:

$$1-S_v/S_o = \exp(-k_L t),$$

where k_L is the rate constant for exchange of radioactivity between the vacuole and the outside. From the value of S_v for large Δt, k_L is determined, and its value then put into the equation to calculate S_v and Y_v for small Δt, when $S_v \ll S_o$; ϕ_{ov} is then calculated as above. The rate constant for exchange of the cytoplasmic phase we have called k_c.

When the specific activity, S_c, in the cytoplasm has reached a quasi-steady level (MacRobbie 1964) and is approximately constant, then

$$\phi_{ov} \simeq \phi_{oc}.\phi_{cv}/(\phi_{co}+\phi_{cv}),$$

and

$$\phi_{vo} \simeq \phi_{co}.\phi_{vc}/(\phi_{co}+\phi_{cv}).$$

When $\phi_{oc} = \phi_{co}$ and $\phi_{cv} = \phi_{vc}$, then $\phi_{ov} = \phi_{vo}$.

III. Results

(a) *Ionic Composition of the Vacuole*

Measurements of the vacuolar concentrations of potassium, sodium, and chloride, made on different batches of cells over a 3-yr period gave the following values: potassium, $532\pm10(9)$ mM; sodium, $56\pm12(9)$ mM; chloride, $619\pm15(7)$ mM. These values were obtained by first calculating a mean value for each batch, and then taking the mean of these mean values, with their S.E.M. The numbers in brackets show the number of batches. Within a particular batch of cells the variability in values of potassium and sodium was usually smaller than between batches.

(b) *Electrical Measurements*

(i) *Potential Differences* ψ_{vo}, ψ_{co}, ψ_{vc} *with Cells in ASW*

The potential difference between the vacuole and the outside, ψ_{vo}, was usually in the range 0 to +15 mV. In a few cells, particularly those collected from Kangaroo I., ψ_{vo} was negative, about −25 to −40 mV.

When an electrode was inserted into the vacuole ψ_{vo} usually had a positive value often followed up to 30 min later by a swing to a negative value, and then a return to the positive value. Such changes sometimes occurred more than once during an experiment lasting up to 8 hr. When ψ_{vo} was negative at the time of insertion it tended to stay that way, although in a few cells where ψ_{vo} was initially negative it eventually became positive. In one cell ψ_{vo}, which was initially positive, later developed a series of oscillations between the two states, and eventually went into the negative state (see Fig. 1).

The results of measurements of ψ_{vo} in 43 cells with ASW as external medium are shown in Figure 2. For each cell a mean ψ_{vo} was estimated within 1 hr and before any changes in external media were made. The histogram shows the distribution of values of ψ_{vo}. Twelve cells contributed two values, one for the positive state and one for the negative state. There appears to be a separation, at −12 mV, of values of ψ_{vo} for the two states. The mean value of ψ_{vo} for the positive state was $5 \cdot 2 \pm 0 \cdot 9$ mV (35) and for the negative state $-29 \cdot 0 \pm 2 \cdot 2$ mV (20).

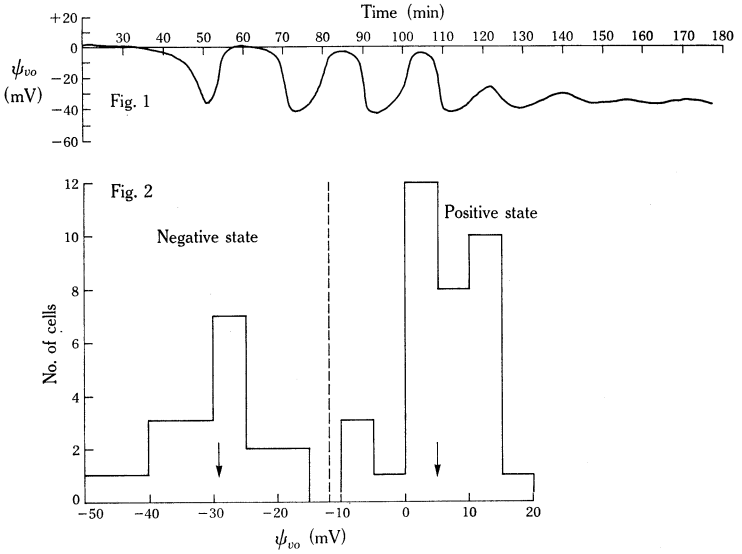


Fig. 1.—Oscillatory behaviour of ψ_{vo} for a cell initially in the positive state. Microelectrode inserted at $t = 0$.

Fig. 2.—Frequency distribution of ψ_{vo} in 43 cells showing the two states of ψ_{vo}. Arrows indicate mean values for the two states.

The p.d. across the plasmalemma, ψ_{co}, was approximately −72 mV regardless of whether the cell was in its positive or negative state. In a group of five cells in the positive state in which ψ_{co} and ψ_{vc} were measured simultaneously, $\psi_{vo} = +3 \pm 3$ mV, $\psi_{co} = -72 \pm 3$ mV, and $\psi_{vc} = +75 \pm 6$ mV. In another group of six cells, in the negative state, $\psi_{vo} = -26 \pm 3$ mV, $\psi_{co} = -72 \pm 1$ mV, $\psi_{vc} = +46 \pm 3$ mV. Thus the difference between positive and negative states occurs at the tonoplast.

(ii) *Electrical Resistances of Cells in ASW*

In 25 cells in the positive state, the total electrical resistance between the vacuole and outside, R_{vo}, was $4 \cdot 01 \pm 0 \cdot 6$ kΩ cm² (25). In three of these cells the mean electrical resistance of the plasmalemma, R_{co}, was $0 \cdot 5 \pm 0 \cdot 2$ kΩ cm² and of the tonoplast $4 \cdot 9 \pm 1 \cdot 1$ kΩ cm².

(iii) *Effect of Changes in* $[K_o]$ *and* $[Cl_o]$

Changes in $[K_o]$ were accompanied by concomitant changes in $[Na_o]$, to keep $[K_o + Na_o]$ constant. Changes in $[Cl_o]$ were produced by replacing sodium chloride with an equivalent concentration of sodium benzene sulphonate.

Changes in $[K_o]$ caused changes in p.d. and resistance at the plasmalemma and tonoplast. Figure 3(*a*) shows the time course of ψ_{vo} following changes in $[K_o]$ from 10 mM to either 1 or 100 mM. It can be seen that the response of ψ_{vo} to the decrease in $[K_o]$ from 10 mM is different from the response to the increase in $[K_o]$. Other measurements showed that the second phase of hyperpolarization which occurs when $[K_o]$ is decreased is largely a change in p.d. across the tonoplast.

Figure 3(*b*) shows the relationship between ψ_{co}, ψ_{vc}, ψ_{vo}, and $[K_o]$. The measurements were made using ASW (i.e. $[K_o] = 10$ mM) as the starting solution, changing to the next potassium concentration, then changing back to ASW before the next change was made. The points were obtained within 5–10 min of the change in $[K_o]$.

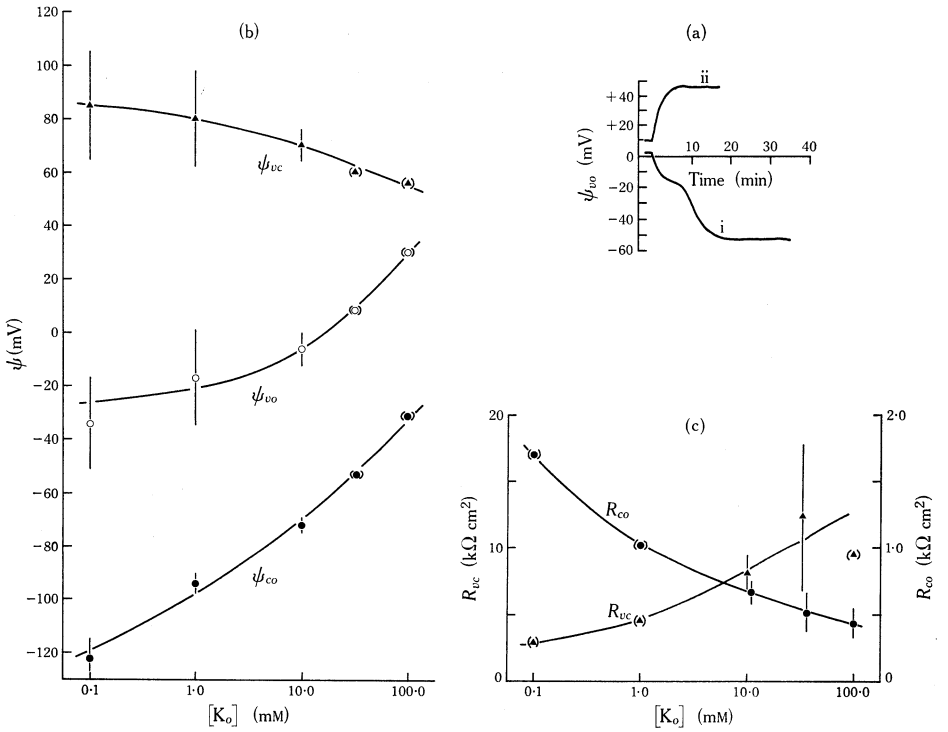


Fig. 3.—(*a*) Responses of ψ_{vo} following a change in $[K_o]$ (i) from 10 to 1 mM, (ii) from 10 to 100 mM. (*b*) Effect of changing $[K_o]$ on the potentials ψ_{vc}, ψ_{vo}, and ψ_{co}. (*c*) Effect of changing $[K_o]$ on the resistances R_{vc} and R_{co}. Brackets around points indicate single values. Bars show ± 1 S.E.M.

Figure 3(*c*) shows how R_{co} and R_{vc} vary with $[K_o]$. The essential conclusion from this data is that R_{co} increases as $[K_o]$ decreases, while R_{vc} shows the opposite tendency. There is usually a considerable amount of variability in results for R_{vc}, particularly as R_{vc} also varies with time after $[K_o]$ is changed.

Changes in $[Cl_o]$ cause changes in ψ_{co}, but not in ψ_{vc}. Figure 4(*a*) shows ψ_{co} and ψ_{vc} as a function of $[K_o]$, with $[Cl_o] = 573$ and 191 mM. Figure 4(*b*) shows ψ_{vo} as a function of $[Cl_o]$ in the range 57·3–573 mM, with $[K_o] = 10$ mM.

(iv) *Effects of CCCP*

The addition to ASW of the uncoupler CCCP (carbonyl cyanide *m*-chlorophenyl hydrazone) at a concentration of 4–5 μM, causes changes in the p.d. and resistance of both plasmalemma and tonoplast. At the plasmalemma, the addition of CCCP appears to increase R_{co}, while slightly depolarizing ψ_{co}, within 10–15 min. In one cell ψ_{co} was depolarized by about 5 mV, and R_{co} changed from 0·42 to 2·12 kΩ cm^2 within 30 min, compared with R_{vc} which changed from 17·3 to 4·5 kΩ cm^2 in the same period. At the tonoplast in two cells where ψ_{vo} was positive, R_{vo} was 3·3 and 2·42 kΩ cm^2 in ASW. After the addition of 5 μM CCCP, R_{vo} decreased within a few minutes and then slowly increased over a period of 2 hr to 9·9 and 12·4 kΩ cm^2 respectively.

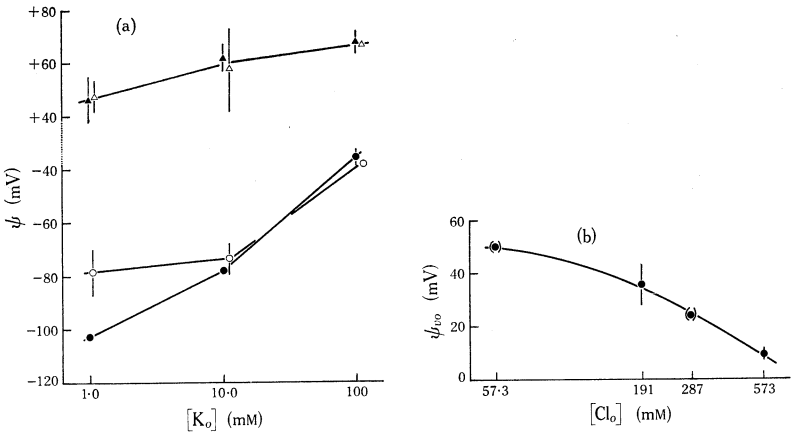


Fig. 4.—(*a*) Effect of changing [K$_o$] on ψ_{co} (circles) and on ψ_{vc} (triangles) for two values of [Cl$_o$], 573 mM (●,▲) and 191 mM (○,△). (*b*) Effect of changing [Cl$_o$] on ψ_{vo} when [K$_o$] = 10 mM. The cells were originally in the positive state. Brackets around points indicate single values. Bars show ± 1 S.E.M.

(*c*) *Fluxes of Chloride*

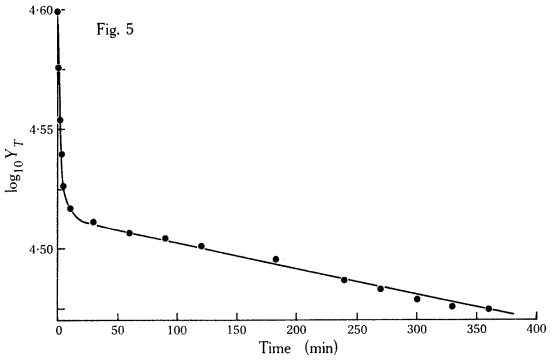
(i) *Exchange over Long Periods*

After 24 hr in labelled ASW the specific activity of the vacuolar sap in four separate cells rose to 52, 39, 32, and 31% of S_o respectively. These values show that there are large fluxes of chloride into and out of *Chaetomorpha* cells. For these four cells ϕ_{ov} was 190, 170, 145, and 115 pmoles cm^{-2} sec^{-1} respectively.

Figure 5 shows that after a cell had been labelled in ^{36}Cl ASW, and then transferred to ASW the radioactivity in the cell fell exponentially after an initial 10–15 min. These results show that free space and cytoplasm exchange rapidly—within 10–15 min—but that the bulk of the chloride in the cell is in the one compartment, the vacuole. In this example ϕ_{vo} was 230 pmoles cm^{-2} sec^{-1} and the rate constant k_L was $4{\cdot}8 \times 10^{-6}$ sec^{-1}. Usually ϕ_{ov} was equal to ϕ_{vo} but in some cases (cf. Table 3) ϕ_{vo} was larger than ϕ_{ov}, i.e. there was a net efflux of chloride.

(ii) *Estimation of Fluxes from Sap Separation*

Figure 6 shows the time course of uptake of ^{36}Cl to the whole cell and to the vacuole. The two curves have been dotted in for the first 10 min, as the data were not sufficiently detailed to provide an accurate time course over that period. The values are the means of batches of 7–10 single cells. The cells were rinsed for 30–60 sec



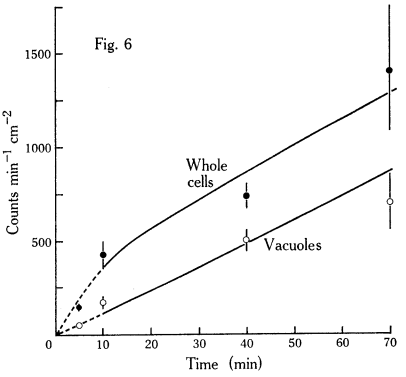


Fig. 5.—Time course of change in total radioactivity (^{36}Cl) in the cell (Y_T).

Fig. 6.—Time course of uptake of ^{36}Cl to the whole cell and the vacuole. Bars show ± 1 S.E.M.

in unlabelled ASW before separation of the sap sample. This period was based on other measurements which showed that 99·7% of the exchange with isolated walls free of cytoplasm was complete in 30 sec of rinsing.

The results show that in the first 10 min there was a rapid increase in total radioactivity in the cell but a less rapid increase in the vacuole. The rate of uptake to the whole cell over the first 5–10 min gives an estimate of ϕ_{oc}. During the later

TABLE 1

VALUES OF ϕ_{oc}, ϕ_{cv}, Q_c, AND k_c FOR CHLORIDE ESTIMATED FROM SAP-SEPARATION EXPERIMENTS FOR TWO GROUPS OF CELLS*

Cell Group	ϕ_{oc} (pmoles cm^{-2} sec^{-1})	ϕ_{cv} (pmoles cm^{-2} sec^{-1})	Q_c (nmoles cm^{-2})	k_c (sec^{-1})
1	85	155	75	$3{\cdot}2\times10^{-3}$
2	130	50	65	$2{\cdot}8\times10^{-3}$

* For methods of calculation see Findlay, Hope, and Williams (1970).

stages of uptake to the whole cell, ϕ_{ov} was about the same as the rate of uptake to the cytoplasm. Values of fluxes and cytoplasmic content estimated from this and similar experiments are set out in Table 1; it was assumed in making these calculations that the net flux was zero because in similar cells ϕ_{ov} and ϕ_{vo} were about equal.

(iii) *Efflux after Short Labelling Periods*

Although the exchange of ^{36}Cl with the cytoplasmic phase is rapid, it is possible to separate the components due to wall and cytoplasm and so estimate the individual fluxes. The times for half-exchange for these components were about 20 sec and 3·7 min respectively. Figures 7(*a*) and 7(*b*) show the results of one such experiment in which the uptake was for 15·5 min and S_v/S_o was only 0·005.

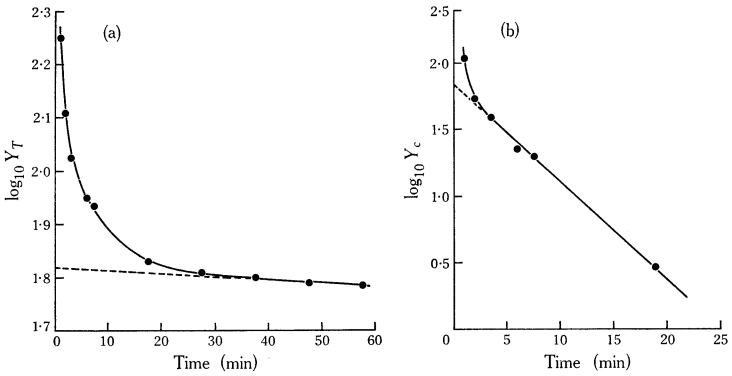


Fig. 7.—(*a*) The time course of Y_T in a cell. (*b*) Change in Y_c, the radioactivity in the cytoplasmic phase estimated as the difference between Y_T and the dotted line in Figure 7(*a*).

Table 2 gives estimates of fluxes and content of the cytoplasmic phase (Q_c) obtained from efflux analysis of cells loaded for varied lengths of time. These values show that there was a net efflux from the cells. But nevertheless the results were consistent with

TABLE 2

VALUES OF ϕ_{ov}, ϕ_{oc}, ϕ_{co}, ϕ_{cv}, ϕ_{vc}, Q_c, AND k_c FOR CHLORIDE ESTIMATED FROM EFFLUX ANALYSIS OF EXCHANGE AFTER SHORT PERIODS OF UPTAKE

One single strand of about five cells used for each determination

Uptake Time (min)	ϕ_{ov}	ϕ_{oc}	ϕ_{co}	ϕ_{cv}	ϕ_{vc}	Q_c (nmoles cm^{-2})	k_c (sec^{-1})
		(pmoles cm^{-2} sec^{-1})					
4·5	85	80	200	110	230	70	$4·7 \times 10^{-3}$
12·5	52	75	185	120	230	85	$3·3 \times 10^{-3}$
15·5	46	100	220	90	210	100	$3·1 \times 10^{-3}$
84	32	140	260	70	190	105	$3·1 \times 10^{-3}$
99	31	90	240	110	260	110	$3·1 \times 10^{-3}$

estimates made from sap separation (Table 1). For example Q_c was about 70–110 nmoles cm^{-2} and k_c was about $3·5 \times 10^{-3}$ sec^{-1}. The flux ϕ_{oc} was in the range 80–150 pmoles cm^{-2} sec^{-1} and ϕ_{cv} about 100 pmoles cm^{-2} sec^{-1}.

(iv) *Effect of Darkness and CCCP*

Table 3 gives some further estimates of ϕ_{ov}, ϕ_{oc}, and ϕ_{cv} in light and dark. In general, short-term uptake was the same for light and dark but ϕ_{ov} was reduced by about half. Hence it appears that ϕ_{oc} is not dependent on light but that ϕ_{cv} is. No effect of light on ϕ_{co} was found in efflux experiments.

TABLE 3

EFFECT OF DARKNESS ON CHLORIDE FLUXES

ϕ_{ov}, ϕ_{vo} measured from long-term experiments, ϕ_{oc} from short-term experiments. Eight groups of cells. Numbers in parenthesis indicate number of replications

Cell Group	Experimental Conditions	ϕ_{ov}	ϕ_{vo} (pmoles cm^{-2} sec^{-1})	ϕ_{oc}	ϕ_{cv}	Q_c (nmoles cm^{-2})	k_c (sec^{-1})
			Sap-separation experiments				
1*	Light	360		1000	560	190	$8{\cdot}2\times10^{-3}$
	Dark	80		650	90	90	$8{\cdot}0\times10^{-3}$
			Short-term uptake experiments				
2	Light (10 min)			235±55(8)			
	Dark			255±25(7)			
3	Light (10 min)			230±30(8)			
	Dark			205±45(8)			
			Long-term uptake experiments				
4	Light (1 hr)	385±25(10)					
	Dark	190±25(10)					
5	Light (1 hr)	475±60(3)	390±40(3)				
	Dark	260±3(3)	410±100(3)				
6*	Light (3 hr)	344					
	Dark	220					
7	Light (24 hr)	155±15(4)					
	Dark	50±5(4)					
			Efflux experiments				
8†	Light		587±283(3)				
	Dark		247±113(3)				

* Single values obtained from the slope of an uptake curve, each point on the curve being the mean of several cells.

† The large S.E.M. is caused by a wide range in the individual values. However, the mean of the dark flux as a percentage of the light flux in the three cells was 43±4%.

Effects of CCCP on ϕ_{ov} are shown in Table 4. A concentration of 5–10 μM CCCP reduced ϕ_{ov} and ϕ_{vo} by 80–90%. Short-term influx was also inhibited and it is inferred that ϕ_{oc} is sensitive to the inhibitor. We cannot tell from these results

if ϕ_{cv} was also affected. Figure 8 illustrates the inhibition of ϕ_{vo} by CCCP and its slow recovery when CCCP was removed.

TABLE 4

EFFECT OF CCCP ON CHLORIDE FLUXES

Four groups of cells. Numbers in parenthesis indicate number of replications

Cell Group	Treatments	ϕ_{ov}	ϕ_{oc} (pmoles cm^{-2} sec^{-1})	ϕ_{vo}	Influx (as % of controls)
1*	ASW	103			
	+3 μM CCCP	23			22
2*	ASW	90			
	+3 μM CCCP	18			20
3	ASW	385±25(8)			
	+1 μM CCCP	450±40(8)			117
	+5 μM CCCP	205±25(8)			53
	+10 μM CCCP	40±15(8)			10
	ASW		230±30(8)		
	+1 μM CCCP		210±40(8)		92
	+5 μM CCCP		120±15(8)		53
	+10 μM CCCP		55±15(8)		24
4*	ASW	110		140	
	+6 μM CCCP			10	7

* Single values obtained from the slope of an influx or efflux curve, each point on the curve being the mean of several cells.

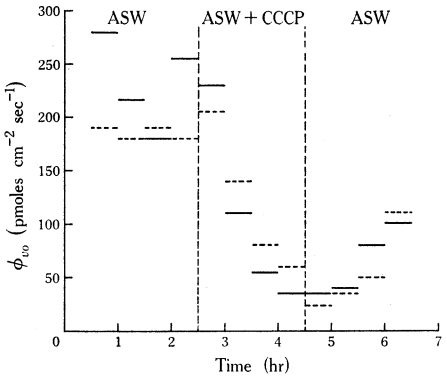


Fig. 8.—Inhibition of ϕ_{vo} by 7 μM CCCP and its recovery when CCCP was removed; solid and dotted lines show the results for two strands of cells.

(*d*) *Fluxes of Potassium and Sodium*

Fluxes of potassium and sodium have also been estimated. The values were similar to those found by Dodd, Pitman, and West (1966). We have made the following further observations:

(1) Dark conditions and DCMU [3(3,4-dichlorophenyl)-1,1-dimethylurea, an inhibitor of photosynthetic electron flow] lowered the influx of potassium at the plasmalemma.

(2) CCCP at 4–10 μM lowered the influx of potassium. The effects are tabulated below:

Flux	Experimental Conditions			
	Light	Dark	Light+ DCMU (1·5 μM)	Dark+ CCCP (7 μM)
ϕ_{ov} (pmoles cm^{-2} sec^{-1})	310	104	170	37–51

IV. Discussion

(a) *Membrane Potential Differences*

The present results show that ψ_{vo} can be in one of two states; the positive state (+5 mV), or the negative state (−30 mV) which is usually not stable. In both states ψ_{co} was −72 mV and hence ψ_{vc} was either +77 or +42 mV. There is a clear correlation between ψ_{vc} and R_{vc}; where $\psi_{vc} = +77$ mV, $R_{vc} = 4{\cdot}1$ kΩcm^2, and where $\psi_{vc} = +42$ mV, $R_{vc} = 7{\cdot}1$ kΩcm^2. Dodd, Pitman, and West (1966) suggested that ψ_{co} was −32 mV, although Walker was quoted as having found $\psi_{co} = -75$ mV. It seems clear that the value of −32 mV was in fact a measure of ψ_{vo} with the cell in its negative state.

(b) *Fluxes and Electrical Properties of Membranes*

Ideally it should be possible to calculate the electrical resistance of the cell membranes from the diffusion fluxes which are dependent on the electrochemical potential gradient. In practice there is uncertainty about the proportion of the flux that is independent passive diffusion and about the relation between this flux, concentrations, and the membrane potential.

As a basis for discussion we assume that ions move by independent passive diffusion. In spite of the arbitary assumption involved in the derivation of the Goldman equation for ion flux, it is not unreasonable to use it [equation (1)] in the calculation of what is expected from independent passive diffusion. If results differ widely from its predictions one may conclude that other mechanisms of ion movement are involved.

The Goldman equation for flux of ion j is:

$$\phi_j = -\frac{P_j z_j \boldsymbol{F}\psi_{io}}{\boldsymbol{RT}} \cdot \frac{[c_j^o - c_j^i \exp(z_j \boldsymbol{F}\psi_{io}/\boldsymbol{RT})]}{1-\exp(z_j \boldsymbol{F}\psi_{io}/\boldsymbol{RT})}, \tag{1}$$

where ϕ_j is the net flux, P_j is the permeability (cm sec^{-1}), z_j the valency, ψ_{io} the membrane potential, and $c_j^{o,i}$ the concentrations of j outside and inside. This flux equation can be substituted into other equations involving fluxes to give a relationship between concentration and potential. For example,

$$\psi_{io} = \frac{\boldsymbol{RT}}{\boldsymbol{F}} \ln \frac{[\mathrm{K}_o]+\alpha[\mathrm{Na}_o]+\delta[\mathrm{Cl}_i]}{[\mathrm{K}_i]+\alpha[\mathrm{Na}_i]+\delta[\mathrm{Cl}_o]}, \tag{2}$$

where $\alpha = P_{\mathrm{Na}}/P_{\mathrm{K}}$ and $\delta = P_{\mathrm{Cl}}/P_{\mathrm{K}}$.

In the following, the electrical measurements are discussed in terms of the fluxes, using the Goldman equations, and an attempt is made to reconcile observed resistances with values calculated from fluxes. Where the discrepancies are very

large, a partition of the total ion flux into (1) active transport or exchange diffusion (not dependent on potential and not contributing to resistance) and (2) passive diffusive fluxes, is suggested.

(i) *Plasmalemma*

The electrical properties of the plasmalemma in *C. darwinii* are very much like those in *Griffithsia* (Findlay, Hope, and Williams 1969, 1970). The potential ψ_{co} changes with $[K_o]$ but is little affected by $[Na_o]$ and so P_{Na}/P_K $(= \alpha)$ must be small. In order to fit the data of Figure 3(*b*), using equation (2), α for *C. darwinii* must be 0·001–0·01. Unlike in *Griffithsia* the response of ψ_{co} to changes in $[Cl_o]$ shows that δ must be appreciable. The data in Figure 4(*a*) are best fitted if δ is in the range 0·05–0·1.

For the purpose of calculation the value of the fluxes of potassium and chloride at the plasmalemma are taken as 200 pmoles cm^{-2} sec^{-1} and of sodium as 100 pmoles cm^{-2} sec^{-1}. In practice the flux of chloride ranged from 50 to 1000 pmoles cm^{-2} sec^{-1} with values usually about 200–300 pmoles cm^{-2} sec^{-1}. A wide range of values for potassium fluxes was also found by Dodd, Pitman, and West (1966).

The cytoplasm of *C. darwinii* is about 7 μm thick, giving a volume of 0·7 μl cm^{-2}; of this volume 30–50% is occupied by the chloroplasts (cf. electron micrographs from Dodd, Pitman, and West 1966) and consequently the volume of free cytoplasm may be only 0·35 μl cm^{-2}.

We have assumed that all the potassium in the cytoplasm is available for exchange and is in a volume 0·35 μl. This makes $[K_c]$, the cytoplasmic concentration, about 400 mM which is in reasonable agreement with the estimate of $[K_c]$ of 350 mM from the data of Figure 3(*b*) using equation (2). Similarly we estimate $[Na_c]$ to be about 50 mM compared with 35 mM proposed by Dodd *et al.* The amount of chloride in the cytoplasm (100 nmoles cm^{-2}) would lead to a concentration of 140 mM, but it is suggested that the chloride is in fact largely in the chloroplasts at a concentration of about 250 mM and in the rest of the cytoplasm at about 35 mM. This distribution of chloride between plastids and cytoplasm would be like that found for *Chara corallina* (Coster and Hope 1968) and *Tolypella intricata* (Larkum 1968). Assuming this low value of 35 mM for $[Cl_c]$, chloride in the cytoplasm would be very nearly in electrochemical equilibrium with the outside solution and with the vacuole when $\psi_{vo} = +5$ mV.

The electrochemical equilibrium potentials of potassium, sodium, and chloride, calculated using the Nernst equation, are respectively $\psi_K = -96$, $\psi_{Na} = +58$, $\psi_{Cl} = -74$ mV compared with the observed value of -72 mV for ψ_{co}. At the plasmalemma there must be an active influx of potassium and efflux of sodium. If we assume that the efflux of potassium and influx of sodium are both passive and independent, the Ussing equation gives the passive components of the influx of potassium and efflux of sodium as 85 and 0·4 pmoles cm^{-2} sec^{-1} respectively. These fluxes subtracted from the totals, give the active components as: influx of potassium 115 pmoles cm^{-2} sec^{-1} and efflux of sodium 100 pmoles cm^{-2} sec^{-1}.

Substitution of these values of passive fluxes into equation (1) gives $P_K = 2{\cdot}82 \times 10^{-6}$ cm sec^{-1} and $P_{Na} = 6{\cdot}7 \times 10^{-8}$ cm sec^{-1} from which a value of α of 0·025 is obtained. This value is not unreasonably larger than the value of 0·01 calculated from the data of Figure 3(*b*).

The value of δ estimated from the data of Figures 3(*b*) and 4(*a*) was at the most 0·1. If the fluxes of chloride across the plasmalemma were entirely passive and determined by potential and concentration as in equation (1), then P_{Cl} would be $2{\cdot}0\times10^{-6}$, giving $\delta = 0{\cdot}7$. This value is too large to agree with that for δ calculated from electrical data. If δ were taken as 0·1, then P_{Cl} would be $0{\cdot}28\times10^{-6}$ cm sec^{-1}, and the passive flux of chloride would be only 30 pmoles cm^{-2} sec^{-1}. The remainder of the flux would not be a passive independent flux; a possible mechanism would be a Cl–Cl exchange diffusion of 170 pmoles cm^{-2} sec^{-1}.

The values of passive fluxes can be used to calculate separate ion conductances from the equation:

$$g_j = F\partial\phi_j/\partial\psi \simeq F\Delta\phi_j/\Delta\psi, \tag{3}$$

where ϕ_j as a function of ψ is given by equation (1). The total conductance ($= g_K+g_{Na}+g_{Cl}$) was 0·98 mmhos cm^{-2} giving a calculated resistance for the plasmalemma of 1020 Ω cm^2. The observed value was 510–750 Ω cm^2. An increase in potassium flux from 200 to 360 pmoles cm^{-2} sec^{-1} would reduce the calculated resistance to the observed value and this value for potassium flux is certainly possible in view of the wide range in fluxes.

(ii) *Tonoplast*

Fluxes of chloride and potassium at the tonoplast were large and showed the same wide range as at the plasmalemma; the sodium flux was small and about 5–10 pmoles cm^{-2} sec^{-1}, as found by Dodd *et al.* The values of electrochemical activity of both potassium and sodium in cytoplasm and vacuole indicate that there is active transport of potassium and sodium into the vacuole. If all the flux of potassium out of the vacuole were passive then from equation (1), P_K would be $1{\cdot}2\times10^{-7}$ cm sec^{-1} which gives a passive flux of potassium into the vacuole of 15 pmoles cm^{-2} sec^{-1}; thus the active component into the vacuole would be 185 out of a total of 200 pmoles cm^{-2} sec^{-1}. Similarly, P_{Na} would be 6×10^{-8} cm sec^{-1}, with an active influx of 9·5 out of 10 pmoles cm^{-2} sec^{-1}. At the tonoplast, chloride appears to be in electrochemical equilibrium, and P_{Cl} is then $1{\cdot}56\times10^{-6}$ cm sec^{-1} for a flux of 200 pmoles cm^{-2} sec^{-1}. Using these values to calculate conductance terms from equation (3), we get $g_K = 0{\cdot}25$, $g_{Na} = 0{\cdot}013$ and $g_{Cl} = 0{\cdot}57$ mmhos cm^{-2} and the total resistance at the tonoplast is then 1·2 kΩ cm^2. This calculated resistance is lower than the observed value of R_{vc} (3·6 kΩ cm^2 when $\psi_{vc} = -77$ mV), implying that only a proportion of the total flux is contributing to the conductance.

The electrical and flux data could be in agreement if there were exchange diffusion components of the fluxes of potassium and of chloride. A similar proposal was made about the nature of potassium transport in *Griffithsia* (Findlay, Hope, and Williams 1970).

The fluxes and resistance of the tonoplast would be compatible if P_{Cl} were reduced to about $0{\cdot}8\times10^{-6}$ cm sec^{-1} and P_K (and P_{Na}) were in the range $2\text{–}3\times10^{-8}$ cm sec^{-1}. In this case only about 50% of the chloride flux and 20% of potassium flux would be passive.

In the change from the positive to the negative state the resistance of the tonoplast increases, hence the permeability to one or more ions must decrease, thus the potential difference must move away from the "effective" electrochemical

equilibrium potential of the ion(s). On the scheme shown in Figure 9 only a decrease in P_{Cl} at the tonoplast could be responsible for the change from positive to negative state.

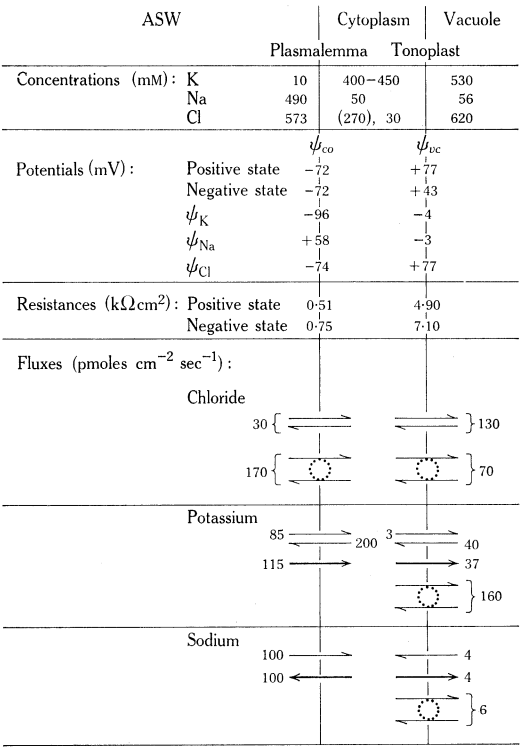


Fig. 9.—Diagram summarizing the measured or inferred fluxes of chloride, sodium, and potassium at the plasmalemma and tonoplast in *C. darwinii*, in the light. Ionic concentrations, and electrical resistance, and potential differences are also shown. The data for chloride in the cytoplasm are given as the estimated concentration in chloroplasts (270 mM) and remainder of cytoplasm (30 mM). The heavy arrows indicate active transport, the light arrows passive fluxes either following the electrochemical gradient or as part of an exchange diffusion system.

(*c*) *Summary of Results and Effects of Dark and CCCP*

Figure 9 summarizes the electrical and flux measurements in the light, as discussed above. Addition of CCCP tended to increase the resistance of both membranes although R_{vc} decreases initially (within a few minutes) but then increases to a value greater than at the start. This inhibitor reduced ϕ_{ov} and ϕ_{vo} for chloride but it was not clear whether this effect was at the plasmalemma or tonoplast or both. As there seems to be no active transport of chloride, it would appear that CCCP affects both P_{Cl} and the component due to non-independent diffusion.

Change from light to dark also reduced fluxes of chloride and potassium. For chloride, this reduction appeared to take place at the tonoplast, and was an effect on both independent and non-independent diffusion.

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