

# IONIC RELATIONS OF MARINE ALGAE

## II.\* *GRIFFITHSIA*: IONIC FLUXES

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### Summary

Measurements were made of ionic fluxes in cells of the marine algae *G. monile* and *G. pulvinata*. The data for sodium and chloride fluxes appear to be consistent with the usually proposed model for the plant cell in which the vacuolar, cytoplasmic, and external compartments are in series, with the cytoplasm bounded on the inside by the tonoplast and on the outside by the plasmalemma. However, there are difficulties in explaining the results for potassium fluxes on this model. At the plasmalemma, the passive fluxes of potassium, sodium, and chloride were 50–380, 15, and 5 p-moles  $\text{cm}^{-2} \text{sec}^{-1}$  respectively. There was also an active chloride influx of 0–35 p-moles  $\text{cm}^{-2} \text{sec}^{-1}$  and an active sodium efflux of about 15 p-moles  $\text{cm}^{-2} \text{sec}^{-1}$ . The fluxes at the tonoplast, on the basis of the compartments-in-series model, were potassium 300–1000, sodium 10 p-moles  $\text{cm}^{-2} \text{sec}^{-1}$ , and chloride at least 30 p-moles  $\text{cm}^{-2} \text{sec}^{-1}$ . Electrochemical data (Findlay, Hope, and Williams 1969) indicate that there is an active flux of potassium across the tonoplast from the cytoplasm into the vacuole. The large fluxes of potassium and chloride at the tonoplast are difficult to reconcile with the electrical resistance of 5000  $\Omega\text{cm}^2$ , which suggests that a large part of the tonoplast potassium flux is exchange diffusion, or that vesicular movement of potassium and chloride ion pairs occurs. The influxes of  $\text{K}^+$ ,  $\text{Rb}^+$ ,  $\text{Cs}^+$ , and  $\text{Na}^+$  ions at the plasmalemma were measured and the following order of permeability obtained:  $P_{\text{K}} > P_{\text{Rb}} > P_{\text{Cs}} > P_{\text{Na}}$ . This order agrees with that obtained from measurements of depolarization of the plasmalemma potential.

### I. INTRODUCTION

The electrical properties of the membranes in several species of *Griffithsia* have been described in Part I of this series (Findlay, Hope, and Williams 1969). It was shown that the plasmalemma was highly selective for potassium compared with sodium and chloride, with values of the permeability ratio  $\alpha$  ( $= P_{\text{Na}}/P_{\text{K}}$ ) in the range 0.002–0.02. For cells in an artificial seawater (ASW) with  $[\text{K}_o]$  10 mM and  $[\text{Na}_o]$  490 mM,  $\alpha$  had a mean value of about 0.004.  $\alpha$  was calculated from measurements of changes in the plasmalemma p.d. with changes in  $[\text{K}_o]$ . The electrochemical data suggested that the tonoplast, on the other hand, was preferentially permeable to chloride ions.

It was concluded from studies of the electrical properties of the membranes, together with measurements of vacuolar concentrations of  $\text{K}^+$ ,  $\text{Na}^+$ , and  $\text{Cl}^-$  that in

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cells of *Griffithsia* spp. there is probably active transport of potassium from the cytoplasm to the vacuole across the tonoplast. The data also indicated active transport inwards of chloride and outwards of sodium between vacuole and outside, but the actual location of these pumps at a particular membrane could not be determined.

This paper describes measurements of the fluxes of potassium, sodium, and chloride across the membranes of *G. pulvinata* and *G. monile*. These measurements were made to enable a more comprehensive picture to be drawn of the ionic relations of *Griffithsia* cells, and in particular to allow the location of the various ion pumps to be determined.

The results are discussed in the light of ionic relations of other marine algae which have been recently reviewed by Gutknecht and Dainty (1968).

## II. MATERIAL AND METHODS

### (a) Material

Strands of cells of *G. monile* and *G. pulvinata* were collected from intertidal rock platforms at Robe, S.A., and stored in seawater at 12°C with natural light of low intensity. Both individual cells and short strands were used in the flux experiments. The basic experimental medium was ASW comprising KCl, 10 mM; NaCl, 490 mM; CaCl<sub>2</sub>, 11.5 mM; MgSO<sub>4</sub>, 25 mM; MgCl<sub>2</sub>, 25 mM; NaHCO<sub>3</sub>, 2.5 mM, and is called 10K ASW in this paper.

Throughout this paper, the Roman numeral following the species name indicates the date of collection of the batches of cells as follows: I, 1.x.66; II, 29.xi.66; III, 20.iv.67; IV, 24.viii.67; V, 27.iv.68; VI, 27.viii.68; VII, 4.v.69; VIII, 19.vi.69. Batches I–VI are the same as those described by Findlay, Hope, and Williams (1969).

### (b) Flux Measurements

(i) *Influx*.—The fluxes of potassium, sodium, and chloride were measured using <sup>42</sup>K, <sup>22</sup>Na, and <sup>36</sup>Cl as tracers. In a few experiments <sup>86</sup>Rb or <sup>134</sup>Cs was added to an ASW in which 3 mM RbCl or CsCl replaced an equal amount of KCl. The influx across the plasmalemma was estimated from the uptake of radioactivity after a 20–60-min period in labelled ASW, followed by a rinse for about 1 min in inactive ASW. The rinse was designed to remove extracellular activity. The time course of influx of K<sup>+</sup> over long periods was measured, *in vivo*, by the following method. A filament of about six cells, held in a perforated Perspex tube, was immersed in radioactive ASW for a known time. The tube was then removed from the solution, and the external radioactivity washed away with a stream of ASW. During the washing period, the assembly was placed in a scintillation counter (the counter being covered with a thin aluminium shield), and the radioactivity during a 3-min wash period was determined. Elution curves were subsequently interpreted [e.g. Fig. 4(c)] as showing that between 1 and 3 min extracellular activity was negligibly low, and intracellular activity had not been removed in significant amounts. The cell assembly was then returned to radioactive ASW for a further uptake period, and the whole process repeated. At the end of the experiment, the counts on the scintillation counter were converted to total radioactivity in the cell by drying the cells on a planchet, counting them in a gas-flow counter, and comparing the counts with those from an aliquot of radioactive ASW of known specific activity. Where it is not feasible to count a single strand directly with the scintillation counter, such as with the weak  $\beta$ -emitter <sup>36</sup>Cl, uptake over long periods was followed by soaking many cells in radioactive ASW, and withdrawing samples of 8–10 cells at intervals and counting them, and taking a mean value for the sample.

(ii) *Efflux*.—The efflux from previously labelled cells or strands was followed by collecting radioactivity released into inactive ASW (about 1 ml) in which the cells were left for varying periods. At the end of the experiment the specific activity of the vacuolar sap was usually estimated by drying the whole cell and counting it, and using values for vacuolar ion concentrations measured

in cells from the same batch as the experimental cells. Potassium and sodium concentrations were determined by flame photometry, and chloride concentration by potentiometric titration. The values obtained showed little variation within a particular batch of cells.

(iii) *Estimation of Radioactivity in the Vacuole and Cytoplasm.*—For a more detailed analysis of the cell fluxes, knowledge of the rate of change of vacuolar sap radioactivity, as well as whole cell activity, is necessary. Cells were put into radioactive ASW, and at different times removed, rinsed briefly, and vacuolar sap samples taken quickly from each by inserting an empty glass microelectrode (tip diameter 20–50  $\mu\text{m}$ ) into the vacuole and sucking out a sap sample. A measured volume of sap was then taken up in a Micro-cap capillary. This sap sample, and the remainder of the cell and sap were counted separately. If the volume of the cells is known, it is then possible to calculate the radioactivity in the sap and cytoplasm for various times of loading, and hence draw conclusions about the fluxes at the plasmalemma and tonoplast.

(c) *Calculation of Fluxes*

The total radioactivity within the cell after it has been placed in radioactive solution should rise linearly at a rate given by:

$$dY/dt = S_o \cdot \phi_{oc} \cdot A, \quad (1)$$

where  $Y$  is total radioactivity,  $S_o$  is specific activity of the radioactive solution (e.g. counts  $\text{min}^{-1}$  mole $^{-1}$ ), and  $A$  the cell surface area. Hence  $\phi_{oc}$ , the influx at the plasmalemma, can be calculated from  $Y$  after the cell has been in radioactive solution for time  $t^*$ , provided  $t^*$  is small enough for  $S_c$  (the specific activity in the cytoplasm) to be negligible compared with  $S_o$ . After longer times, the rate of increase of tracer in the cell decreases due to "backflux". Usually,  $t^*$  was about 20 min for potassium and up to 60 min for sodium and chloride. This method will be referred to as the initial uptake calculation.

For potassium, the uptake has been found clearly to be linear for more than 30 min, and application of equation (1) yielded very nearly the same result for  $\phi_{oc}$  for uptake times of 5 or 10 min as for a 20-min period. Equation (1) is not dependent on any assumptions about what happens to the radioactivity when it has passed the plasmalemma.

During both long loading and elution the specific activity in the cell approaches that of the external medium with a single exponential time course except for an initial deviation due to the cytoplasm. The rate constant,  $k_L$ , for the exchange between vacuole and external solution, can be estimated in the following ways:

(i) In elution experiments:

$$k_L = -d(\ln Y)/dt = -2.303 d(\log_{10} Y)/dt, \quad (2)$$

i.e. the negative of the slope of the graph of  $\ln Y$  versus  $t$  after it has become linear.

(ii) In uptake experiments:

$$k_L \simeq -d[\ln(1 - S_v/S_o)]/dt, \quad (3)$$

where  $S_v$  is the specific activity in the vacuole. Except during the initial period when the specific activity of the cytoplasm is increasing, most of the radioactivity in the cell is in the vacuole, and a reasonable estimation of  $S_v$  can be obtained from the total cell radioactivity. For times when  $S_v \ll S_o$  equation (3) reduces to

$$k_L \simeq \Delta S_v/(S_o \Delta t). \quad (3a)$$

(iii) For a loading time  $t^*$ , where  $t^* < 1/k_L$ :

$$k_L \simeq -\ln(1 - S_v/S_o)/t^*, \quad (4)$$

and hence a measurement of vacuolar radioactivity (leading to  $S_v$ ) will yield an approximate value of  $k_L$ .

The flux of ions between medium and vacuole is given by:

$$\phi_v = k_L Q/A, \quad (5)$$

where  $A$  is the surface area of the cell and  $Q$  the quantity of ions in the vacuole. Also,  $Q = C_v V$ , where  $C_v$  is the vacuolar ion concentration and  $V$  the vacuolar volume.

A simple model of isotopic exchange of ions in a plant cell is one involving two compartments, vacuole and cytoplasm, in series, separated from each other by the tonoplast, and from the external medium by the plasmalemma. These compartments are assumed to have an even distribution of radioactivity and of chemical species and the exchange of radioactivity is assumed to be limited by the membranes. This model may be an oversimplification because chloroplasts or mitochondria might form important compartments and there may be limitation of diffusion within cytoplasm comparable with the resistance of the membranes to permeation (Ling 1965). The series model, however, is widely used and is in fact suggested by our electrical measurements reported earlier. Pallaghy and Scott (1969) have given the complete solution to the differential equations describing the changes in radioactivity in such a model system during the processes of loading or elution. When the rate constants for cytoplasm and vacuole exchange are sufficiently different, the separate fluxes and the quantities of ions in the two compartments may be calculated from the elution curve. Exact solutions were limited to the situation where the net flux of the ion species across either membrane was zero. We shall refer to this method as "elution curve analysis".

In the series model the apparent flux into or out of the vacuole is related to the separate fluxes at the two limiting membranes by:

$$\phi_v = \phi_{oc}\phi_{cv}/(\phi_{co} + \phi_{cv}) \quad (6)$$

(Pallaghy and Scott 1969). From equation (6)  $\phi_{cv}$  may be calculated if  $\phi_v$  is known from equation (5), and  $\phi_{oc}$  from an initial uptake experiment.  $\phi_{co} = \phi_{oc}$  in the assumed steady state (see Fig. 1, Findlay, Hope, and Williams 1969).

The results from direct measurements of the rise in radioactivity in both the sap and cytoplasm [see Section II(b) above] may also be used to calculate  $\phi_{oc}$ ,  $\phi_{cv}$ , and  $Q_c$  (the quantity of ions in the cytoplasm compartment). Thus, after the cytoplasm has reached a quasi-steady specific activity, we have

$$\begin{aligned} dY_v/dt &= A \cdot \phi_{cv}(S_c - S_v) \\ &\simeq A \cdot \phi_{cv} \cdot S_c \end{aligned} \quad (7)$$

for short times, with

$$S_c = S_o \phi_{oc}/(\phi_{co} + \phi_{cv}) \quad (8)$$

(MacRobbie 1964). If  $\phi_{oc}$  ( $= \phi_{co}$ ) is known,  $\phi_{cv}$  can be calculated. Finally, in the quasi-steady state,

$$\begin{aligned} Q_c &= Y_c/S_c \\ &= Y_c(\phi_{co} + \phi_{cv})/(S_c \phi_{oc}). \end{aligned} \quad (9)$$

### III. RESULTS

#### (a) Fluxes of Potassium

In cells in 10K ASW and in the light,  $\phi_{oc}$  calculated from the short-term uptake was in the range 50–380 p-moles  $\text{cm}^{-2} \text{sec}^{-1}$ . The results for batches of cells sampled at various seasons over 16 months are tabulated below (the number of cells in each sample is shown in parenthesis):

Batch	III	IV	V(a)	V(b)	V(c)	VI
$\phi_{oc}$ (p-moles $\text{cm}^{-2} \text{sec}^{-1}$ )	$54 \pm 11(3)$	$290 \pm 59(6)$	$81 \cdot 3 \pm 21(4)$	$378 \pm 32(12)$	$80 \pm 10(6)$	$250 \pm 29(10)$

The first five batches were of cells of *G. pulvinata*, and the last batch cells of *G. monile*. The smallest fluxes (batch III) were associated with long storage of collected material under the conditions described in Section II(a). The variation in cells of batch V was between separate clumps of cells measured over about 10 days. Within each clump the S.E.M. for 10 cells was usually about 10% of the mean.

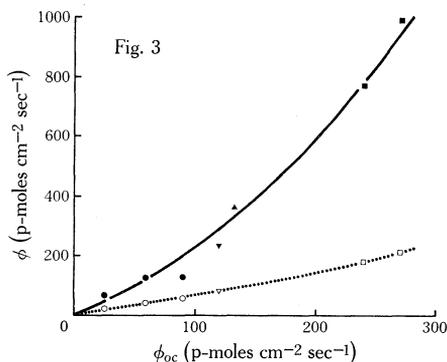
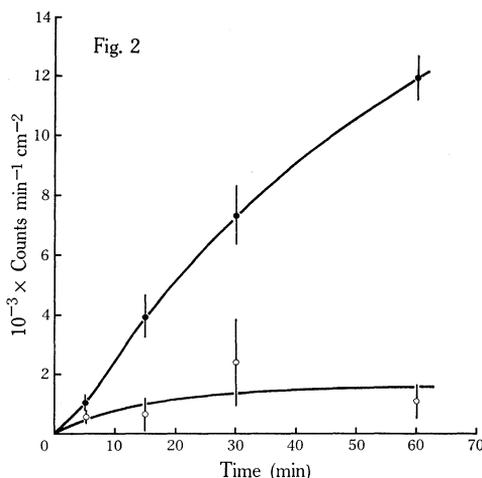
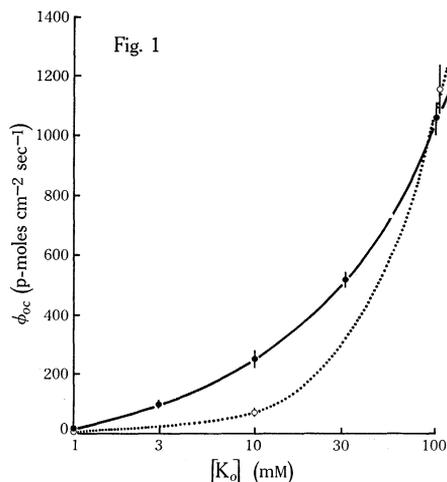


Fig. 1.—Influx of potassium at the plasma-lemma,  $\phi_{oc}$ , as a function of  $[K_o]$  for two levels of  $[Ca_o]$ , 11.5 mM (●) and 0.1 mM (○). Vertical bars indicate  $\pm$  one standard error of the mean (S.E.M.).

Fig. 2.—Time course of cytoplasmic (○) and vacuolar (●) radioactivity after the cells were placed in  $K^+$  10 ASW. The points are the means from a group of cells. Vertical bars represent  $\pm$  S.E.M.

Fig. 3.—Flux  $\phi_{ev}$  (closed symbols) of potassium on the basis of the series model and the flux  $\phi_v$  (open symbols) calculated from the rate of rise of vacuolar radioactivity, both plotted as a function of the flux  $\phi_{oc}$  of potassium. Values are means for several cells except for points ○ and ● which are single values. The data were obtained as follows: ●  $\phi_{oc}$ , the initial uptake rate; ○  $k_L$  for long loading times; ▼ initial uptake rate in 0–20 min; ▽ uptake rate in 0–900 min; ▲ elution curve analysis; ■, □ sap-cytoplasm separations;  $\phi_{oc}$  from 0–5, or 0–10 min,  $\phi_v$  for the period 15–30 min.

$\phi_{oc}$  (in cells of *G. monile* VI) was found to be strongly dependent on the potassium concentration in the ASW, and also upon calcium concentration, as shown in Figure 1.

Both the short-term and long-term uptakes of potassium in *G. pulvinata* V were unaffected by the following treatments: Dark conditions, CCCP (carbonyl cyanide *m*-chlorophenylhydrazone) 0.5 or 1  $\mu$ M, imidazole 0.1 or 1 mM, DIO-9 2.5 or 10  $\mu$ g/ml,

phlorizin 1 mM, oligomycin 2.5 or 5  $\mu\text{g}/\text{ml}$ , and DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] 1 or 10  $\mu\text{M}$ . Phlorizin at a concentration of 0.1 mM appeared to increase  $\phi_{oc}$  slightly.

Figure 2 shows the relation between the sap and cytoplasm activity in *G. monile* IV and time after cells were placed in radioactive ASW. From these data the quantities in Table 1 were calculated. These experiments, and others where the uptake was followed for up to 10 hr, showed that though the plasmalemma flux varied between batches of cells, the apparent vacuolar flux [equation (5)] was usually 0.8  $\phi_{oc}$ ; the tonoplast flux, calculated from equation (6), is also found to be related to  $\phi_{oc}$  and is about 3–4 times as great, in the experiments reported here—see Figure 3.

TABLE 1  
FLUXES AND QUANTITIES FOR POTASSIUM CALCULATED FROM  
SAP-CYTOPLASM SEPARATIONS

	Value		Equation
	30.viii.67	30.viii.68	
$\phi_{oc}$	270	240	1
$\phi_v$	212	183	3a,†5
$\phi_{cv}$	990	770	6
$Q_c$ ( $\mu\text{moles cm}^{-2}$ )*	0.24	0.76	9

\* Ion contents are calculated on the basis of unit area.

†  $\Delta t$  was the interval between 15 and 30 min.

When the elution of radioactivity was measured over a long period from cells of *G. monile* VI that had been soaked in  $\text{K}^*/\text{ASW}$  for about 15 hr, the total radioactivity in the cell changed with time as shown in Figures 4(a)–4(c). Following a rapid elution of radioactivity, the half-time of which is consistent with diffusion or ion exchange from the cell surface and wall, the remaining activity, which is taken to be intracellular, was exchanged with a time course that could be described as the sum of two exponential curves with different time constants (cf. Pallaghy and Scott 1969).

The long-term rate constants for both the loading and elution processes could be calculated for the same cell. For loading,  $k_L$  was calculated from equation (4), in which  $S_v$  was determined from the value  $Y_L$  as shown by the arrow in Figure 4(b).  $Y_L$ , the intercept of the extrapolation to zero time of the long-term elution curve, is sufficiently close to the radioactivity in the vacuole at the end of the loading time,  $t^*$ . The values of  $k_L$  so obtained should be reasonably accurate because the uptake curve for potassium is close to a single exponential (Fig. 5). When this value of  $k_L$  is close to that calculated from the elution curve using equation (2), a steady state can be assumed, and the results are suitable for elution analysis. The means and S.E.M. of the fluxes and ion contents of the compartments in *G. monile* VI determined by such an analysis for eight cells are given in the following tabulation:

$\phi_{oc}$ (p-moles $\text{cm}^{-2}$ $\text{sec}^{-1}$ )	$\phi_{cv}$ (p-moles $\text{cm}^{-2}$ $\text{sec}^{-1}$ )	$Q_c$ ( $\mu\text{moles cm}^{-2}$ )	$Q_{\text{total}}$ ( $\mu\text{moles cm}^{-2}$ )
134 $\pm$ 9	360 $\pm$ 40	1.8 $\pm$ 0.5	16.3 $\pm$ 1.8

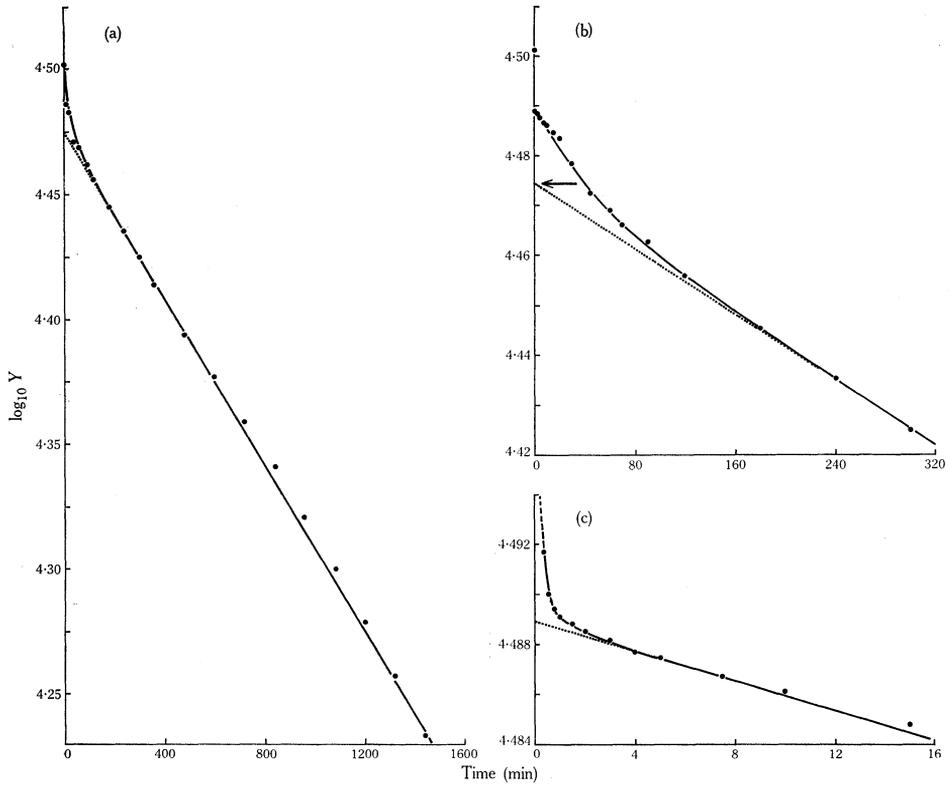


Fig. 4.—(a) Logarithm of total radioactivity,  $Y$ , plotted as a function of time, in a cell that had been soaked in  $K^*$  ASW for about 15 hr, and then eluted in ASW from time zero. (b) The same curve showing the elution in the first 320 min. The arrow refers to a value  $Y_L$  used for calculations in the text. The deviation of the curve from the extrapolation (---) indicates a medium-speed component of the efflux. (c) Elution curve for the same cell, for the first 16 min. The deviation of the curve from the extrapolation probably represents the component of efflux from the wall.

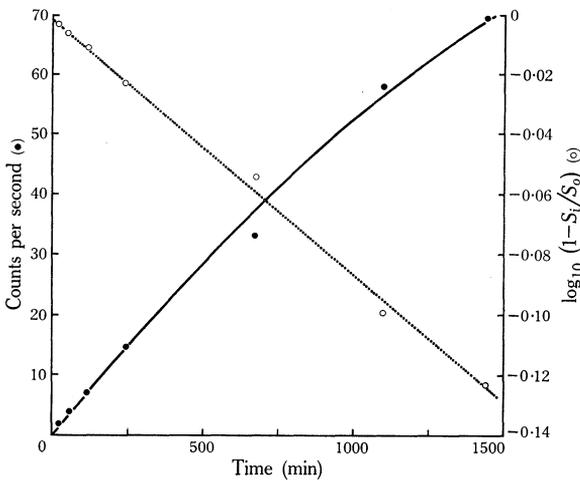


Fig. 5.—Uptake of radioactive potassium in a single cell of *G. pulvinata* plotted against time (●). The dotted curve is the function  $\log_{10}(1 - S_i/S_0)$  from the same data plotted against time (○).

Satisfactory agreement was found between the above calculated values and the observed potassium contents ( $15.6 \mu\text{moles cm}^{-2}$ ) of cells in this experiment, but the estimate of potassium content of the more quickly exchanging compartment is much in excess of the estimation in Table 1.

(b) *Sodium*

The influx of sodium at the plasmalemma was in the range  $12\text{--}20 \text{ p-moles cm}^{-2} \text{ sec}^{-1}$ . An elution curve for *G. pulvinata* V is shown in Figure 6(a) and a loading curve in Figure 6(b). In elution curves,  $k_L = 3.6 \times 10^{-6} \text{ sec}^{-1}$ , and in the uptake curve in Figure 6(b)  $k_L = 3.6 \times 10^{-6} \text{ sec}^{-1}$ . These values represent a vacuolar flux  $\phi_v$  of  $3\text{--}6$

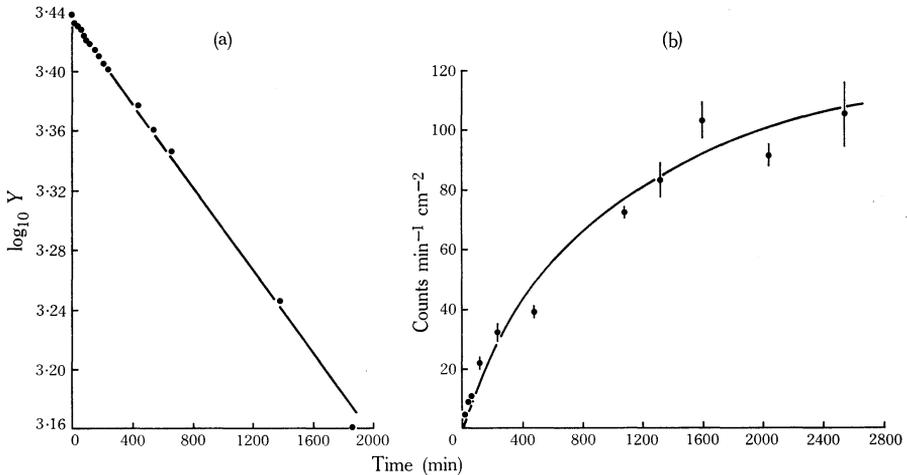


Fig. 6.—(a)  $\log_{10} Y$  plotted as a function of time, in a cell that had been loaded in  $\text{Na}^+$  ASW and then eluted in ASW from time zero. (b) The uptake of radioactive sodium into groups of cells, plotted as a function of time. Each point is a mean of six values. Vertical bars represent  $\pm$  S.E.M

$\text{p-moles cm}^{-2} \text{ sec}^{-1}$  [equation (5)]. In an experiment in which  $\phi_{oc}$  was  $17 \text{ p-moles cm}^{-2} \text{ sec}^{-1}$ ,  $\phi_v$  was calculated from  $k_L$  to be  $4.1 \text{ p-moles cm}^{-2} \text{ sec}^{-1}$ . Hence  $\phi_{cv}$  was estimated as  $5 \text{ p-moles cm}^{-2} \text{ sec}^{-1}$  [equation (6)]. In another experiment, *G. pulvinata* I, the rate of rise of sap activity suggested  $\phi_v$  was about  $6 \text{ p-moles cm}^{-2} \text{ sec}^{-1}$ . The initial rise in total cell activity corresponded to  $\phi_{oc} = 12 \pm 4(7) \text{ p-moles cm}^{-2} \text{ sec}^{-1}$ . From these values  $\phi_{cv} = 12 \text{ p-moles cm}^{-2} \text{ sec}^{-1}$ . The cytoplasmic radioactivity could not be estimated with any confidence.

(c) *Chloride*

The influx of chloride at the plasmalemma in cells in 10K ASW and in the light, calculated from the initial uptake, was in the range  $10\text{--}40 \text{ p-moles cm}^{-2} \text{ sec}^{-1}$ . The results are shown in Table 2. For long loading times with *G. pulvinata* V the activity rose with a single rate constant [Fig. 7(a)]. In the experiment that this figure illustrates, the apparent or vacuolar influx  $\phi_v$  was  $24.5 \text{ p-moles cm}^{-2} \text{ sec}^{-1}$ , while  $\phi_{oc}$  (from the initial uptake calculation) was  $22.0 \text{ p-moles cm}^{-2} \text{ sec}^{-1}$ . This result implies that

$\phi_{cv} \gg \phi_{oc}$ , i.e. that the plasmalemma is rate limiting for chloride, if the series model is adopted. If not,  $\phi_v$  is a lower limit for the tonoplast flux.

Figure 7(b) shows an elution curve for chloride. Like sodium, the chloride curves showed only a very small medium-speed component in the exchange and were not suitable for elution-curve analysis. The chloride curves also showed a continuing curvature of the long-term component, probably representing a steadily decreasing  $\phi_v$ .

Mean long-term rate constants over the period 0–1000 min corresponded to effluxes of 11, 4, and 4 p-moles  $\text{cm}^{-2} \text{sec}^{-1}$  in three cells of *G. pulvinata* V. Compared with the influxes calculated from the loading rate constants, a net influx was observed in two out of three of these cells.

TABLE 2  
CHLORIDE INFLUXES IN VARIOUS TREATMENTS

Pretreatment period 1–2 hr. Strands of 3–4 cells were used. The number of determinations is shown in parenthesis and fluxes are given as p-moles  $\text{cm}^{-2} \text{sec}^{-1}$

Species	Date	Experimental Conditions		
		Control (light)	Dark	DCMU
<i>G. pulvinata</i> I	6.x.66	42 ± 5 (9)	3.7 ± 0.4(9)	—
<i>G. pulvinata</i> III	20.vi.67	10 ± 1 (9)*	2.8 ± 0.7(4)	—
<i>G. pulvinata</i> III	22.vi.67	18.1 ± 1.8(4)	—	9.5 ± 1.1(4); 8.2 ± 0.02(4)†
<i>G. pulvinata</i> V	24.v.68	15.4 ± 2.5(6)	—	17.1 ± 3.4(6); 6.6 ± 0.7 (6)‡

\* This value is unexplainably low. On 21.vi.67 a control influx of 21 ± 3(9) was found.

† For DCMU concentrations of 3 and 10  $\mu\text{M}$  respectively.

‡ For DCMU concentrations of 1 and 10  $\mu\text{M}$  respectively.

There was a significant decrease in chloride influx into cells in the dark or when DCMU was added to ASW in the light. These findings are illustrated in Table 2. There appeared to be an inhibitory effect of oligomycin (2.5 or 5  $\mu\text{g}/\text{ml}$ ), but the following had no inhibitory effect: CCCP, 0.5, 1, or 3  $\mu\text{M}$  (10  $\mu\text{M}$  was inhibitory but also damaged cells visibly); phlorizin, 0.1 or 1 mM; imidazole, 0.1 or 1 mM; DIO-9, 2.5 or 10  $\mu\text{g}/\text{ml}$ .

#### (d) *Rubidium and Caesium*

The fluxes of these ions were measured to see if there was any relation between the influxes and the permeability series derived from the ability of the ions to depolarize the plasmalemma p.d. when added to ASW (Findlay, Hope, and Williams 1969). Strands of cells were placed in the labelled solutions without pretreatment. The time course of the uptake was followed for several hours. The initial slopes enabled  $\phi_{oc}$  to be calculated.

The influxes in six strands of cells of *G. pulvinata* III were  $\text{Rb}^+$ : 27, 37, 32;  $\text{Cs}^+$ : 3.0, 0.61, 0.96 p-moles  $\text{cm}^{-2} \text{sec}^{-1}$ .

## IV. DISCUSSION

(a) *Active and Passive Fluxes*

Earlier results with *Griffithsia* spp. suggested that potassium ions were almost in electrochemical equilibrium across the plasmalemma and hence a passive influx and efflux would be expected there, but that there was active transport of potassium from the cytoplasm to the vacuole, across the tonoplast. Also, part of the sodium efflux, and most of the chloride influx, between vacuole and outside, was expected to be active, but it was not possible to say at which membranes the transport occurred.

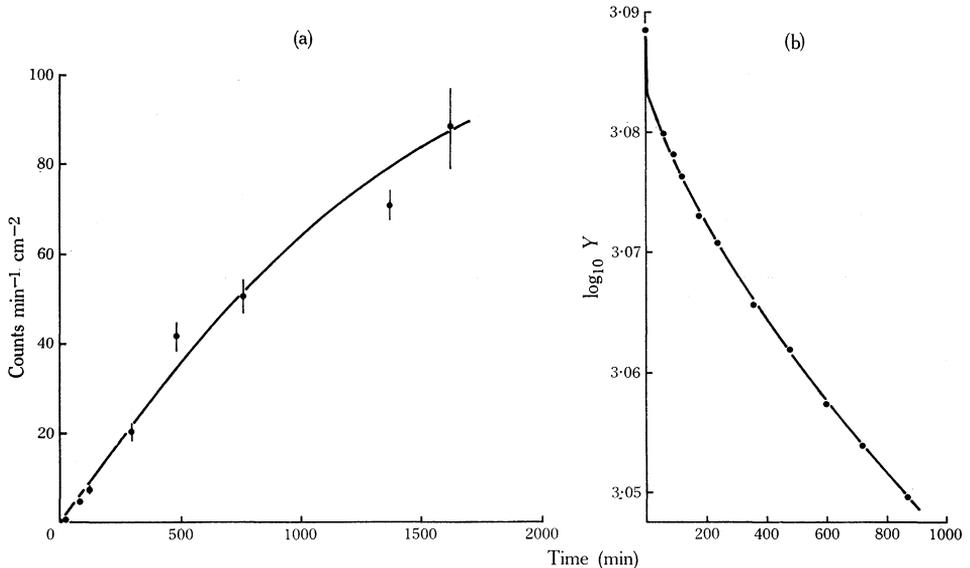


Fig. 7.—(a) Uptake of radioactive chloride into groups of cells, plotted as a function of time. Each point is a mean of six values. Vertical bars represent  $\pm$ S.E.M. (b)  $\log_{10} Y$  plotted as a function of time, in a cell that had been loaded in  $\text{Cl}^*$  ASW and then eluted in ASW from time zero.

The present results support most of these conclusions. The potassium fluxes at the plasmalemma were not influenced by darkness nor by many substances that affect active transport in other systems. The influx was strongly dependent on potassium concentration. The increase in influx with increased potassium concentrations in the ASW is in accordance with the corresponding increases in conductance of the plasmalemma found earlier.

The chloride influx, however, was lowered by darkness and DCMU, as found in *Nitella* (MacRobbie 1964), *Hydrodictyon* (Raven 1967), and *Chara* (Coster and Hope 1968). It is concluded that chloride inward transport is mediated by photosynthesis in *Griffithsia* also; further experiments are in progress to find more about the coupling of light energy and active transport in these algae. An active influx of about  $7\text{--}35$  p-moles  $\text{cm}^{-2} \text{sec}^{-1}$  is much higher than found in the freshwater coenocytes. A light-stimulated chloride influx has recently been observed in *Acetabularia* (Saddler,

personal communication). In *Chaetomorpha*, chloride fluxes appear to be passive (Dodd, Pitman, and West 1966) as they are in *Valonia* (Gutknecht 1966) unless it is perfused and short-circuited (Gutknecht 1967). The chloride influx in the dark may be an active one mediated by respiration in the dark, or alternatively part of an exchange diffusion system with an equal efflux. Such exchange diffusion is a satisfactory explanation of the dark influx in *Chara corallina* (Findlay *et al.* 1969).

The active chloride flux is probably located at the plasmalemma. If this is so, the large flux of chloride inferred for the tonoplast [Section III(c)] may be a passive one. For chloride ions to be in electrochemical equilibrium across the tonoplast with an observed electric potential of about 30 mV (vacuole positive to cytoplasm) the cytoplasmic activity would need to be 190 mM, compared with 620 mM in the vacuole. There is not enough information about the chloride content of the cytoplasm to decide whether this is reasonable or not.

It was suggested by Findlay, Hope, and Williams (1969) that, because the punch-through potential at the tonoplast was close to a possible value for the electrochemical equilibrium potential for chloride, the greatly increased permeability of the membrane was to chloride. While this may still be the case, the argument is probably incorrect, because there is no obvious reason why the punch-through potential should be determined by an electrochemical equilibrium potential; in fact at the plasmalemma in *Chara corallina*,  $\psi_{Cl}$  is further from the punch-through potential than is the equilibrium p.d. for the other ions.

The flux of potassium ions at the tonoplast was estimated on the series model to be 300–900 p-moles  $\text{cm}^{-2} \text{sec}^{-1}$ . It is not known how to divide this into active to passive flux. Even if any or all of the influx  $\phi_{cv}$  is active, the efflux  $\phi_{vc}$  should be passive, on the grounds of "downhill" movement. If this is so, then the larger passive flux occurs at the tonoplast which has a much smaller conductance than the plasmalemma. Even if the series model is not appropriate [see Sections IV(d) and IV(e)], the tonoplast flux has a lower limit of  $\phi_v$  and hence is still between 30 and 200 p-moles  $\text{cm}^{-2} \text{sec}^{-1}$  (Fig. 3). A way out of this dilemma is to propose that a large part of the tonoplast potassium flux is exchange diffusion or that vesicular (pinocytotic) movement of  $\text{K}^+$  and  $\text{Cl}^-$  ion pairs occurs at the tonoplast.

In the present experiments, the estimated tonoplast flux of potassium was found to be correlated with the plasmalemma flux of potassium. The results are considered further in Section IV(e) in the light of more detailed evidence recently published by MacRobbie (1969) regarding a vesicular or pinocytotic mechanism.

#### (b) Relation between Passive Fluxes and Conductance

If the fluxes of potassium are independent of each other, of the fluxes of other ions, and of water, then, at electrochemical equilibrium:

$$g_K = (F^2/RT)\phi_K, \quad (7)$$

where  $g_K$  is the partial conductance due to  $\text{K}^+$  and  $\phi_K$  is a unidirectional flux, either influx or efflux (Hodgkin 1951). We can apply this equation to the situation at the plasmalemma, where electric conductance and potassium fluxes have been measured on the same batch of cells (*G. monile* VI). Table 3 shows this comparison for various

values of  $[K_o]$  in ASW. Since the fluxes of the ions besides  $K^+$  are relatively small,  $g_K$  should be comparable with the total electric conductance of the plasmalemma,  $g_p$ . The large discrepancy found suggests that either the assumption of independence is wrong or that the values of flux or conductance are in error. A similar discrepancy is familiar in measurements in characean cells, as described in detail by Walker and Hope (1969).

TABLE 3  
PARTIAL CONDUCTANCE DUE TO  $K^+$ ,  $g_K$ , AND ELECTRIC CONDUCTANCE OF THE  
PLASMALEMMA  $g_p$ , IN *G. MONILE* VI

$[K_o]$ (mm)	$\phi_{oc}$ (p-moles $cm^{-2} sec^{-1}$ )	$g_K$ (m-mho $cm^{-2}$ )	$g_p^*$ (m-mho $cm^{-2}$ )	$g_p/g_K$
1	20	0.08	1.7	21
3	100	0.40	3.1	8
10	250	1.0	6.9	7
30	515	2.0	12	6
100	1055	4.2	14	3

\* From resistance values in Figure 4(c) of Findlay, Hope, and Williams (1969).

#### (c) *The Cation Permeability Series*

The influxes of  $K^+$ ,  $Rb^+$ ,  $Cs^+$ , and  $Na^+$  were all measured for the plasmalemma, in the present series of experiments. A suitable isotope of  $Li^+$  is not available. An order of permeability can be assembled from the fluxes, assuming they are passive. The evidence that this is so for potassium has been outlined. Sodium influx ought also to be passive because of the direction of the electrochemical gradient (inward for sodium), and because the electrical evidence showed that the resting potential was not established entirely by a potassium gradient but had a contribution from sodium. There is no reason to believe that  $Rb^+$  or  $Cs^+$  are actively transported at the plasmalemma. The relative permeability order from the fluxes was therefore  $P_K > P_{Rb} > P_{Cs} > P_{Na}$ . The first two inequalities follow from the relative fluxes when the respective concentrations in ASW were 3 mM. It is highly likely that the influx of  $Na^+$  from ASW with all  $Na^+$  except 3 mM replaced by, for example, choline<sup>+</sup>, would be less than the influx from 3 mM  $Cs^+$  ASW because the influx from 13 mM  $Na^+$  was only 0.1 p-moles  $cm^{-2} sec^{-1}$ , in cells of the same batch as used for the  $Rb^+$  and  $Cs^+$  experiments. The permeability has here been regarded as being proportional to  $\phi/C_o$  and we have ignored the minor effect of the p.d. variation in the various ASW's. Thus the permeability order from the influxes agrees with that from the depolarizing effects, which was  $P_K > P_{Rb} > P_{Cs} > P_{Na} > P_{Li}$ . The data do not allow a comparison to be made of the relative permeabilities except that  $P_{Na}/P_K (= \alpha)$  is about 0.0013 (fluxes) and 0.004 (electrical). The permeability ratio is influenced by two factors, relative equilibrium adsorption with the membrane, and by the relative mobilities, as stressed by Diamond and Wright (1969). These factors have not been separately assessed in biological systems.

#### (d) *The Model with Series Compartments*

Electrical measurements with *Griffithsia* spp. clearly indicated a model for the cell in which the vacuole was separated from the cytoplasm by a membrane (the

tonoplast) with a resistance of about  $5000 \Omega\text{cm}^2$  and a p.d. of 25–35 mV across it, and the cytoplasm from the external medium by a membrane (the plasmalemma) with a resistance of about  $200 \Omega\text{cm}^2$  and  $-85$  mV across it, with respect to 10 mM K ASW. Strong evidence in favour of the model was that the plasmalemma p.d. and resistance varied promptly with changes in the potassium concentration in the ASW. There is, however, some contrary evidence which makes the series interpretation not entirely satisfactory. Much smaller, though significant, changes in tonoplast p.d. occurred at the same time as changes in plasmalemma p.d., and the resistance of the tonoplast changed markedly, in the opposite direction to the changes in the plasmalemma, when  $[\text{K}_o]$  was altered. Nevertheless, at present we do not see a satisfactory alternative model for the electrical results. One of us (Findlay, unpublished data) has made a further study of another *Griffithsia* species, *G. flabelliformis* VIII, in which cells are rather transparent, and it is possible to see clearly the position of the electrode tip either in the cytoplasm or in the vacuole. The measurements of p.d. and resistance are very similar to those for the other species of *Griffithsia*, and support a model with the two membranes in series, each with the permeability properties already described.

Elution curve analysis, based on the series model, has been performed on many of the results for potassium isotope exchange [e.g. see tabulation in Section III(a) above]. Agreement between parameters calculated from this, and ones estimated by independent means, for example the total cellular content of potassium, shows that the results are not inconsistent with the model. Many other models may, of course, give consistency as well. The cytoplasmic potassium content ( $1.8 \pm 0.5 \mu\text{moles cm}^{-2}$ ) is most variable and exceeds the other estimates (Table 1) that also depend on the series interpretation. If the mean of these estimates is taken as  $1 \mu\text{mole cm}^{-2}$ , then with a mean  $\text{K}^+$  concentration of 330 mM, the value needed to give the observed plasmalemma p.d. (Findlay, Hope, and Williams 1969) and one not very different from that observed by an inserted  $\text{K}^+$ -selective microelectrode (Vorobiev 1967), suggests that the cytoplasm would need to be  $30 \mu\text{m}$  thick. However, electron micrographs of *Griffithsia* cells (N.G. Marinos, unpublished results) show in section a cytoplasm thickness of about  $5 \mu\text{m}$ . Even allowing for some shrinking of the cytoplasm during fixation, the estimates of  $Q_c$  for  $\text{K}^+$  are on the high side.

#### (e) *Modifications to the Series Model*

MacRobbie (1969) has presented some evidence from the behaviour of chloride fluxes in *Nitella* that is puzzling when considered against the series model. A model in which the chloroplasts, rather than the whole of the cytoplasm, form the compartment exchanging with medium speed ( $\approx 1$  hr), was rejected. A mechanism was favoured in which there is a direct path for chloride ions from the plasmalemma, or just inside it, into the vacuole, with little exchange with the cytoplasm *en route*.

Thus, vacuolar chloride builds up quicker than predicted by the series model, as observed, and there is the possibility of explaining the correlation between rate of arrival of activity in the vacuole and the plasmalemma influx (MacRobbie 1964, also observed in *Chara* by Coster and Hope 1968). The "direct" flux is suggested as being composed of vesicles which trap chloride of high specific activity and travel across the cytoplasm discharging through the tonoplast.

There are some indications in our results of similar behaviour in *Griffithsia* to that which led MacRobbie to the hypothesis discussed above for *Nitella*. There is little

indication of the expected lag in the build-up of vacuolar radioactivity (Fig. 2). Also, the apparent cytoplasmic rate constant for  $K^+$  exchange,  $k_c$ , obtained from the proportion of cytoplasmic to total radioactivity at short times (MacRobbie 1964) is

	Artificial sea water		Cytoplasm		Vacuole
			Plasmalemma	Tonoplast	
Concentrations (mM)					
K	10		(300)		550
Na	490				50
Cl	573				620
Fluxes ( $\mu\text{-moles cm}^{-2} \text{sec}^{-1}$ )					
K	50-380				(30-200)
Na	15				(5)
Cl	5				(30)
Potentials (mV)					
			$\phi_{co}$ -85	$\phi_{vc}$ +35	
Resistances ( $\Omega\text{cm}^2$ )			200	5000	

Fig. 8.—Diagram summarizing the measured or inferred fluxes of potassium, sodium, and chloride at the plasmalemma and tonoplast in *Griffithsia*, in the light. Ionic concentrations, resistance, and p.d. from Findlay, Hope, and Walker (1969) are also shown. The heavy arrows indicate active transport, the light arrows passive fluxes either following the electrochemical gradient, or part of an exchange diffusion system. The values of fluxes shown in brackets are for  $\phi_v$ , calculated as described in the text, and are lower limits for the tonoplast fluxes. The dotted lines indicate a possible component of ion transport through the cytoplasm by vesiculation.

greater than  $k_c$  calculated from elution curves. The accuracy of the  $k_c$  from sap-cytoplasmic separations is, however, poor. Finally, a correlation between  $\phi_v$  and  $\phi_{oc}$  is observed for  $K^+$  (Fig. 3).

Thus, it is possible that such a vesicular flux may be operating on potassium or other ions, in *Griffithsia*, but further work is necessary to assess its likelihood. If the vesicular flux were present then (1) we would have overestimated  $\phi_{cv}$  by assuming that tracer reaching the vacuole had mixed with cytoplasmic ions, and (2), the discrepancy between passive flux and conductance at the plasmalemma (Table 3) might be greater, but only if the direct flux is associated with pinocytosis starting with the external (cell wall) solution. If vesiculation starts just inside the plasmalemma, nearly all of  $\phi_{oc}$  may still be passive and contribute to the electric conductance.

Since the vesicular influx is expected to be balanced at the tonoplast by an equal efflux of ions that must diffuse through the cytoplasm it is expected that conclusions from elution analysis should be valid.

Figure 8 summarizes the fluxes found in *Griffithsia*, in the light, with cells in K10 ASW. The figures in brackets are those depending on a calculation using the series model. In the dark, the chief change in fluxes is a reduction in the chloride influx to about 5 p-moles  $\text{cm}^{-2} \text{sec}^{-1}$ . The dotted circles refer to one possible means of explaining (i) a chloride influx in the dark, by means of a coupled, exchange carrier in which the influx and efflux are equal, and (ii), a large flux of potassium at the tonoplast, not contributing to the electric conductance. Again, an exchange carrier is suggested.

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#### VI. REFERENCES

- COSTER, H. G. L., and HOPE, A. B. (1968).—Ionic relations of cells of *Chara australis*. XI. Chloride fluxes. *Aust. J. biol. Sci.* **21**, 243.
- DIAMOND, J. M., and WRIGHT, E. M. (1969).—The physical basis of ion and non-electrolyte selectivity. *A. Rev. Physiol.* **31**, 581.
- DODD, W. A., PITMAN, M. G., and WEST, K. R. (1966).—Sodium and potassium transport in the marine alga *Chaetomorpha darwinii*. *Aust. J. biol. Sci.* **19**, 341.
- FINDLAY, G. P., HOPE, A. B., PITMAN, M. G., SMITH, F. A., and WALKER, N. A. (1969).—Ionic fluxes in cells of *Chara corallina*. *Biochim. Biophys. Acta* **183** (3), 565.
- FINDLAY, G. P., HOPE, A. B., and WILLIAMS, E. J. (1969).—Ionic relations of marine algae. I. *Griffithsia*: Membrane electrical properties. *Aust. J. biol. Sci.* **22**, 1163.
- GUTKNECHT, J. (1966).—Sodium, potassium and chloride transport and membrane potentials in *Valonia ventricosa*. *Biol. Bull. mar. biol. Lab., Woods Hole* **130**, 331.
- GUTKNECHT, J. (1967).—Ion fluxes and short-circuit current in internally perfused cells of *Valonia ventricosa*. *J. gen. Physiol.* **50**, 1821.
- GUTKNECHT, J., and DAINTY, J. (1968).—Ionic relations of marine algae. *A. Rev. Oceanogr. Biol.* **6**, 163.
- HODGKIN, A. L. (1951).—The ionic basis of electrical activity in nerve and muscle. *Biol. Rev.* **26**, 339.
- LING, G. N. (1965).—The physical state of water in the living cell. In "Forms of Water in Biologic Systems". *Ann. N.Y. Acad. Sci.* **125** (2), 401.

- MACROBBIE, E. A. C. (1964).—Factors affecting the fluxes of potassium and chloride ions in *Nitella translucens*. *J. gen. Physiol.* **47**, 859.
- MACROBBIE, E. A. C. (1969).—Ion fluxes to the vacuole of *Nitella translucens*. *J. exp. Bot.* **20**, 236.
- PALLAGHY, C. K., and SCOTT, B. I. H. (1969).—The electrochemical state of cells of broad bean roots. II. Potassium kinetics in excised root tissue. *Aust. J. biol. Sci.* **22**, 585.
- RAVEN, J. A. (1967).—Light stimulation of active transport in *Hydrodictyon africanum*. *J. gen. Physiol.* **50**, 1627.
- VOROBIEV, L. N. (1967).—Potassium ion activity in the cytoplasm and the vacuole of cells of *Chara* and *Griffithsia*. *Nature, Lond.* **216**, 1325.
- WALKER, N. A., and HOPE, A. B. (1969).—Membrane fluxes and electric conductance in characean cells. *Aust. J. biol. Sci.* **22**, 1179.