IONIC RELATIONS OF MARINE ALGAE

I. *GRIFFITHSIA*: MEMBRANE ELECTRICAL PROPERTIES

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Summary

Several species of *Griffithsia* were studied as part of an investigation into the ionic movements and membrane properties in marine algal cells. Vacuolar potassium concentration was normally 500–600 mM, that of sodium 30–90 mM, and of chloride 600–650 mM.

It was found that the potential difference (p.d.) across the plasmalemma responded quickly to changes in external potassium concentration, $[K_o]$, with $([K_o]+[Na_o])$ kept constant. The plasmalemma depolarized when $[K_o]$ was increased. Calculations made from these results suggested a constant permeability ratio $\alpha = P_{Na}/P_K$ of 0·002–0·006 and a constant cytoplasmic concentration parameter, $([K_c]+\alpha[Na_c])$, of 300–350 mM in several sets of experiments, as $[K_o]$ was varied. In other experiments P_{Na}/P_K may have increased from 0·002 to 0·02, and the cytoplasmic concentration parameter from 260 to 390 mM, as $[K_o]$ was increased from 1 to 100 mM.

When substitution of Rb^+, Cs^+, Na^+, and Li^+ ions were made for some of the external K^+, p.d. changes occurred which enabled the relative permeabilities of these ions to be determined. The permeability order was either $P_K > P_{Rb} > P_{Cs} > P_{Na} > P_{Li}$ or $P_{Rb} > P_K > P_{Cs} > P_{Na} > P_{Li}$.

The electric resistance of the plasmalemma in an artificial seawater (ASW), corresponding in $[K_o]$ and $[Na_o]$ to seawater, was 150–260 Ω cm². That of the tonoplast was 4000–5500 Ω cm² in the same ASW. The resistance of the plasmalemma decreased as $[K_o]$ increased, but that of the tonoplast increased. A model involving constant P_K and P_{Na} (as $[K_o]$ was varied) was only an approximation to the data on the resistance of the plasmalemma.

It was concluded from application of the Nernst criterion to the data for p.d. and ion concentrations that the vacuolar contents were maintained at the observed levels by active transport of potassium inwards at the tonoplast, and active transport of sodium outwards and of chloride inwards at sites to be determined. Potassium ions were not far from equilibrium across the plasmalemma; here the specificity in favour of potassium over sodium in passive permeation was unusually high.

I. INTRODUCTION

During the last 10 years there has been a considerable increase in published data dealing with ionic relations of plant cells, in particular the large coenocytic cells of the Characeae. Some of this work, which has been reviewed by Dainty (1962) and Scott (1967), has dealt with the electrical properties of the plasmalemma and

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tonoplast, and fluxes of ions across these membranes. The main purpose of these investigations has been to discover which ions are actively transported, either into or out of the cell, and the links between these active ionic fluxes and the metabolism of the cell. Rapid progress has been made recently towards getting all the necessary electrochemical data for such species as *Chara corallina*, *Nitella translucens*, and *Hydrodictyon africanum*.

Although the large single cells of the marine algae *Valonia* and *Halicystis* have been studied for many years, it is only in the last few years that investigations along the lines discussed above have been started. Recently, experiments which examine the electrophysiological properties of membranes in the giant cells of the marine algae *Valonia ventricosa* (Gutknecht 1966, 1967) and *Chaetomorpha darwinii* (Dodd, Pitman, and West 1966) have been described. However, Gutknecht (1966) was unable to measure the electrical potential difference across both the plasmalemma and the tonoplast in any one cell, and Dodd, Pitman, and West (1966) could only measure the potential in the cytoplasm for short periods. In neither case was it possible to determine the electrical resistance of the two membranes separately. Such measurements are necessary if one is to attempt reasonably comprehensive studies of the ionic relations of these cells.

The large coenocytic cells of the red alga *Griffithsia* are suitable for measurements of electrical properties of both membranes and ionic fluxes across them. This paper describes the electrical properties of the plasmalemma and tonoplast, and a succeeding paper will present the results of measurements of ionic fluxes in the cell.

II. Materials and Methods

(*a*) *Material*

The species mostly used is undescribed, but the provisional name supplied by H. B. S. Womersley is *Griffithsia pulvinata* Baldock, and it will be described in a forthcoming publication by R. N. Baldock. Two other species, *G. monile* Harvey and *G. teges* Harvey, were used. Material of these species is filed in the Algal Herbarium, Department of Botany, University of Adelaide, under No. A 32,711 (*G. pulvinata*), A 32,712 (*G. monile*), and A 32,713 (*G. teges*).

Cells of these three species of *Griffithsia* were collected at low tide from intertidal rock platforms at Robe, S.A., stored in aerated seawater at a temperature of about 12°C, and illuminated with daylight at low intensity. Experiments were done with cells stored for periods ranging from a few days to 2 or 3 months. The plants consist of branching filaments and either single cells or a number of cells in a filament were used.

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.

(*b*) *Cell Surface Area*

The most appropriate expression to calculate the surface area of the cells seemed to be that for a prolate spheroid, viz.:

$$A = 2\pi[b^2 + (ab.\arcsin\varepsilon)/\varepsilon],$$

where A is the surface area, $\varepsilon = [(a^2-b^2)/a^2]^{\frac{1}{2}}$, a is the length of the semi-major axis, and b the length of the semi-minor axis. Almost all *Griffithsia* cells are radially symmetrical about the major axis, but tend to have their maximum width about one-third of the distance from the end of the cell. Typical cells have $2a$ = 2–3 mm and $2b$ = 1–2 mm, with surface areas 0·05–0·2 cm^2.

The accuracy of estimates of surface area made on the basis of a prolate spheroid was checked by drawing, with a camera lucida, an enlarged image of the circumference of a section of

the cell containing the major axis and calculating the area of the cell from this image by a numerical integration. The image of the cell was divided into 50 parallel segments perpendicular to the major axis, and the numerical integration performed on the basis that each segment was a right section of a cone. Results from several cells showed that the area calculated by numerical integration differed by less than 10% from that calculated on the basis of a prolate spheroid.

(c) *Electrical Measurements*

Standard electrophysiological methods were employed to measure the electrical potential difference (p.d.) across the plasmalemma and the tonoplast, and the electrical resistance of both of these membranes. A filament of cells was held in a perspex chamber, and irrigated with an appropriate artificial seawater (ASW). The standard ASW used, which approximated to ordinary seawater, comprised 490 mM NaCl, 10 mM KCl, 11·5 mM $CaCl_2$, 25 mM $MgCl_2$, 25 mM $MgSO_4$, 1 mM NaBr, and 2·5 mM $NaHCO_3$, and is called 10K ASW in this paper. Other ASW solutions with different potassium concentrations are written as 1K ASW, 3K ASW, etc.

In all the experiments described in this paper, the concentration of ($K^+ + Na^+$) in the external solution ($[K_o]+[Na_o]$)* was kept constant at 500 mM, and consequently any change in $[K_o]$ was accompanied by an opposite change in $[Na_o]$. This procedure was adopted to eliminate any changes in p.d. caused by a Donnan system in the cell wall (Hope and Walker 1961).

Glass microelectrodes, filled with 3M KCl and of tip diameter 1–2 μm, were inserted into the central region of one of the cells of the filament, to measure the potential of the vacuole. Occasionally, gushing of cell sap into the electrode during the insertion occurred and this was taken as evidence that the tonoplast was broken by the electrode, and that the tip of the electrode was in the vacuole. A second electrode inserted into the vacuole was used to pass an electric current from the cell to the outside medium.

The p.d. across the plasmalemma was measured with a microelectrode of tip diameter less than 2 μm which was carefully inserted a short distance through the cell wall and into the cytoplasm. Such an insertion into the cytoplasm was considered to be successful if the potential reached a steady value within 2–5 min after insertion. Occasionally, the potential reached a steady value within a few seconds of the insertion. The p.d. across the tonoplast was either measured directly between the cytoplasmic and vacuolar electrodes, or obtained later by subtracting the cytoplasmic from the vacuolar p.d., each being measured against the outside reference. The external reference electrode consisted of a thin plastic tube filled with 3M KCl in 3% agar, connected to a calomel half-cell. Similar tubes connected the intracellular microelectrodes to calomel half-cells. A current generator was connected to the appropriate vacuolar microelectrode via a silver chloride–silver wire.

(d) *Chemical Analysis of Vacuolar Sap*

The concentrations of sodium and potassium were measured by flame-photometry and the concentration of chloride measured by a standard electrometric titration method. The cell was removed from seawater or ASW, blotted gently, and then rinsed for 15 sec in 0·33M $Ca(NO_3)_2$ solution to remove, by exchange, sodium and potassium ions from the cell wall. The cell was again blotted, put on a lightly greased plate, and sliced open with a new razor blade. A 1-μl sample of sap, free of cytoplasmic contamination, was taken and diluted with distilled water.

III. Results

(a) *Ionic Composition of the Vacuole*

Analyses have been made of the potassium, sodium, and chloride content of the vacuolar sap from cells of *G. pulvinata* and *G. monile*, collected at different times during 1966–68. The cells were transferred to ASW several days before analysis. This treatment was the same for cells used for electrical measurements. The results from

* In this paper, square brackets are used to denote concentrations of ions in m-mole/litre.

seven groups of cells are shown in Table 1. The vacuolar concentrations of potassium and sodium as a function of time after transfer of cells of *G. pulvinata* V from seawater

TABLE 1

VACUOLAR CONCENTRATIONS OF POTASSIUM, SODIUM, AND CHLORIDE IN *GRIFFITHSIA* CELLS IN 10K ASW

Analyses were made within 2–7 days after transferring the cells from seawater to 10K ASW. Values are means ± standard error of the mean (numbers of cells in each batch given in parentheses)

Species	Vacuolar Concentrations (mM)		
	K^+	Na^+	Cl^-
G. pulvinata I	—	—	651±11 (5)
G. pulvinata II	556± 5 (5)	41± 6 (5)	—
G. pulvinata III	565±22 (5)	60± 7 (5)	—
G. pulvinata IV	530±10 (6)	96±15 (6)	—
G. monile IV	546± 7 (6)	63± 4 (6)	—
G. pulvinata V	535±15 (6)	30± 4 (6)	606±19 (4)
G. monile VI*	401±22 (8)	47± 5 (8)	—

* In a later collection of this species (26.xi.68), the vacuolar concentrations of K^+, Na^+, and Cl^- in cells in seawater were 585±13, 31±2, and 626±6 mM respectively.

to 10K ASW are shown in Figure 1. In similar measurements with *G. pulvinata* III, cells were transferred from seawater to 3K, 10K, and 30K ASW, and the vacuolar concentrations of K^+ and Na^+ determined as a function of time. From 1 to 6 days the K^+ concentration remained constant to within 10% and the Na^+ concentration to within 15%.

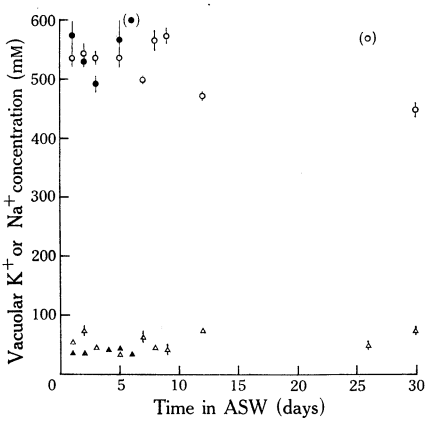

Fig. 1.—The vacuolar concentrations of potassium (○, ●) and sodium (△, ▲) in cells of *G. pulvinata* III (closed symbols) and *G. pulvinata* V (open symbols) plotted against time after transfer from seawater to 10K ASW. Vertical bars represent ± the standard error of the mean (SEM). Parentheses indicate single values.

(*b*) *Ionic Composition of the Cytoplasm*

So far, we have been unable to measure directly potassium and sodium concentrations in the cytoplasmic phase because the volume of the cytoplasm is very small, and it is difficult to prevent contamination from the vacuolar sap. Attempts to

replace the vacuolar sap by a perfusion technique and thence determine ionic content of the remaining cytoplasm (Kishimoto and Tazawa 1965; Coster and Hope 1968) have not been successful.

(*c*) *Electric Potential Difference across the Plasmalemma and Tonoplast*

The p.d. across both plasmalemma and tonoplast has been examined as a function of external potassium concentration, $[K_o]$, for each of the three species of *Griffithsia*. The results are shown in Figure 2. For *G. teges* II [Fig. 2(*a*)] and *G. pulvinata*

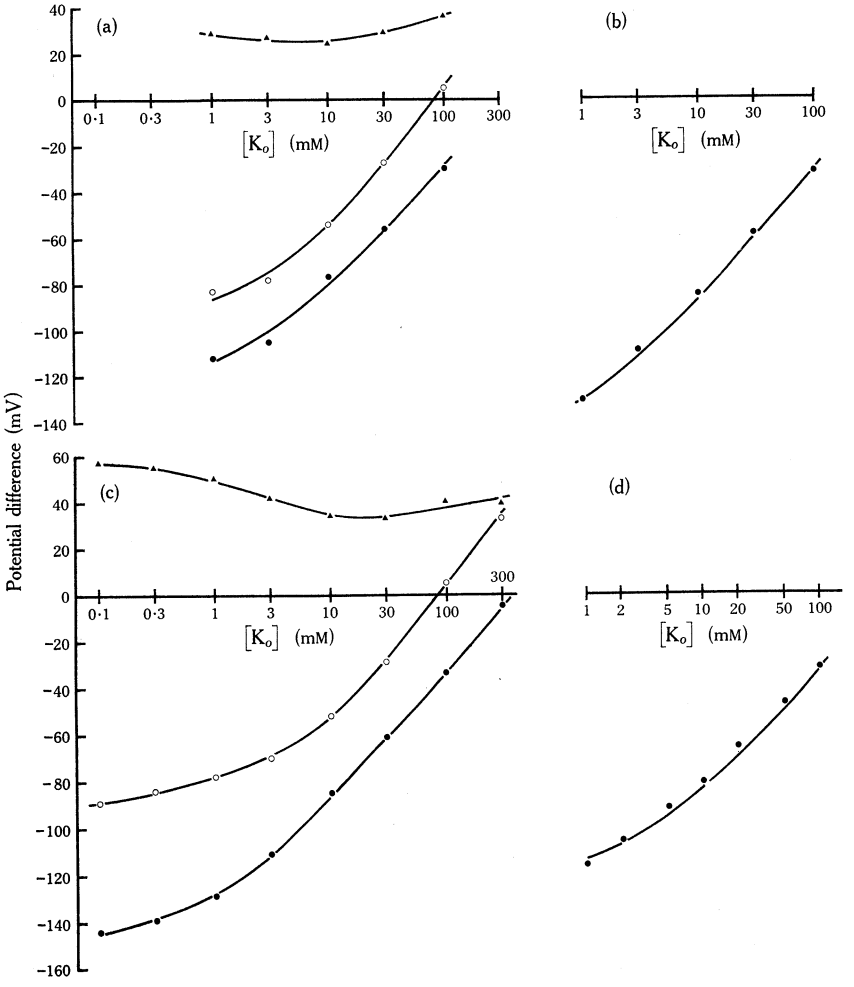

Fig. 2.—Ψ_{vo} (○), Ψ_{co} (●), and Ψ_{vc} (▲) plotted against $[K_o]$ for the three different species of *Griffithsia*. (*a*) *G. teges* II, (*b*) *G. monile* VI, (*c*) *G. pulvinata* V, (*d*) *G. pulvinata* II. The curves through Ψ_{vo} and Ψ_{vc} were drawn by eye, while the curves for Ψ_{co} are theoretical ones as described in the text. For all points the SEM is less than 3 mV.

II [Fig. 2(*d*)] the experiments were performed by taking ASW with $[K_o] = 10$ mM as control solution, changing to the next concentration (either greater or smaller), then

changing back to $[K_o]$ = 10 mM before the next change was made. The points plotted in Figures 2(*a*) and 2(*d*) represent values of p.d. after any initial transient had disappeared, usually within 10–15 min of the change in $[K_o]$, except for $[K_o]$ = 100 mM, where the value of the p.d. was usually taken within 5 min of the change of $[K_o]$, and the solution was removed soon after. Increasingly longer times of exposure of the cell to 100 mM K^+ (and to a lesser extent 30 mM K^+) caused the membrane potential to take correspondingly longer times to return to the control value after the cell had been transferred back to 10 mM K^+. For *G. pulvinata* V [Fig. 2(*c*)] and *G. monile* VI [Fig. 2(*b*)] the solution changes were made in order of increasing (or decreasing) $[K_o]$ and without returning to the 10 mM K^+ solution as an intermediate step; the starting point was 10 mM K^+ as for the first two batches. In Figure 2(*c*), for 8 of the 10 cells of *G. pulvinata* V the concentration range extended from 0·1 to 300 mM K^+. Ψ'_{co} (p.d. between cytoplasm and solution) invariably reached a steady level within a minute of solution change, but initial transients were occasionally observed for Ψ'_{vo} (p.d. between vacuole and solution). Hysteresis effects were small or non-existent with Ψ'_{co} but rather more pronounced with Ψ'_{vo}.

In Figure 2 the curves for Ψ'_{vo} and Ψ'_{vc} were drawn by eye whereas the curves for Ψ'_{co} are fitted curves based on the equation:

$$\Psi'_{co} = 58 \log_{10}([K_o]+\alpha[Na_o])/([K_c]+\alpha[Na_c]), \qquad (1)$$

where α is the permeability coefficient ratio P_{Na}/P_K and $[K_c]$ and $[Na_c]$ are the cytoplasmic K^+ and Na^+ concentrations (Hodgkin and Katz 1949). Both α and $([K_c]+\alpha[Na_c])(= C_c)$ are assumed constant over the concentration range, and the values of these parameters which give the best fit are shown in the following tabulation:

Parameter	*G. teges* II	*G. pulvinata* II	
		Experiment 1	Experiment 2
C_c (mM)	300	350	300
α	0·005	0·005–0·006	0·002

Strictly speaking, activity, and not concentration, terms should be used in equation (1), but we have assumed that the activity coefficients in cytoplasm and seawater are about the same and will therefore cancel out in the equation.

TABLE 2

PARAMETERS α AND C_c CALCULATED FROM EQUATION 1 AS DESCRIBED IN TEXT

Parameter	*G. pulvinata* II (Experiment 1)							*G. pulvinata* II (Experiment 2)				
$[K_o]$ (mM)	1	2	5	10	20	50	100	1	3	10	30	100
Ψ'_{co}	−116	−106	−92	−81	−66	−47	−32	−126	−107	−81	−58	−32
α	0·0038	0·0043	0·0070	0·0027	0·013	0·075		0·0016	0·002	0·007	0·020	
C_c (mM)	266	273	338	284	350	384		260	294	333	394	

It can be seen that for *G. teges* II, *G. pulvinata* V, and *G. monile* VI a reasonable fit was obtained. However, a good fit was not possible with *G. pulvinata* II [Fig. 2(*d*)]. Here a more appropriate model was one in which α and C_c increased as $[K_o]$ increased. The constants α and C_c, as shown in Table 2, were calculated by solving equation (1)

for successive pairs of values of $[K_o]$ and Ψ_{co}. Two sets of values, calculated from the results of two experiments for *G. pulvinata* II, are shown in Table 2.

Figure 3 shows the effect on Ψ_{co} of lowering the calcium level in ASW from its normal value of 11·5 mM to 0·1 mM. The cells were transferred from normal ASW

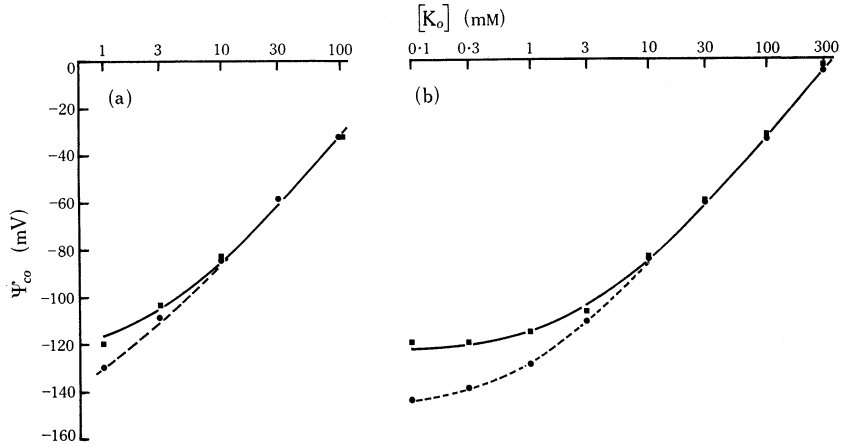

Fig. 3.—Effect of low $[Ca_o]$ (0·1 mM) on Ψ_{co} versus $[K_o]$ in *G. monile* VI (*a*) and *G. pulvinata* V (*b*). The continuous curves are for $[Ca_o]$ = 0·1 mM, and for comparison, the dotted curves are for $[Ca_o]$ = 11·5 mM (normal ASW), the same experiments as in Figures 2(*b*) and 2(*c*) respectively.

to low-calcium ASW for about 10 min before solution changes, in which $[K_o]$ was increased or decreased. Points obtained with low calcium and $[K_o]$ = 0·1 mM are not very accurate, as fluctuations in the potential were observed under these conditions. The reasons for such behaviour are unknown. A fitted curve based on equation (1) can be drawn with α and C_c constant, but with a higher value of α. The results are shown in the following tabulation:

Parameter	*G. pulvinata* V		*G. monile* VI	
	Normal Ca	Low Ca	Normal Ca	Low Ca
C_c (mM)	350	350	350	350
α	0·002	0·0054	0·002	0·005

The cells were exposed to low-calcium ASW for about 30–40 min, and after the changes in $[K_o]$ had been completed were transferred back to normal ASW. Ψ_{co} then returned to its original value within a few minutes.

(*d*) *Resistance of the Plasmalemma and Tonoplast*

The following tabulation shows values of the area specific resistance of the tonoplast (R_{vo}) and the plasmalemma (R_{co}) for cells in 10K ASW:

	G. teges II	*G. pulvinata* II	*G. pulvinata* V	*G. monile* VI
R_{co} (Ω cm^2)	265 (1)	193±17 (5)	190±40 (8)	148±13 (5)
R_{vo} (Ω cm^2)	5400 (1)	5140±36 (5)	5100±800 (8)	4000 (1)

The measurements were made at least 20 min after the electrodes had been inserted into the cell, and when the vacuolar potential had reached a reasonably steady level.

The values shown in the above tabulation represent resistances at the resting potentials of the two membranes. They were obtained by taking the mean of the two resistance values calculated from the currents needed to produce a depolarizing and a hyperpolarizing change in the p.d. The excursion in p.d. was no more than about 5–10 mV at the tonoplast, or 1–2 mV at the plasmalemma.

The resistance of the tonoplast is much larger than the resistance of the plasmalemma. The ratio of tonoplast to plasmalemma resistance was never less than about 10 and could be as high as 60. Thus no great error is made in taking, as we have done in a number of instances, the total resistance from vacuole to outside as a measure of the tonoplast resistance.

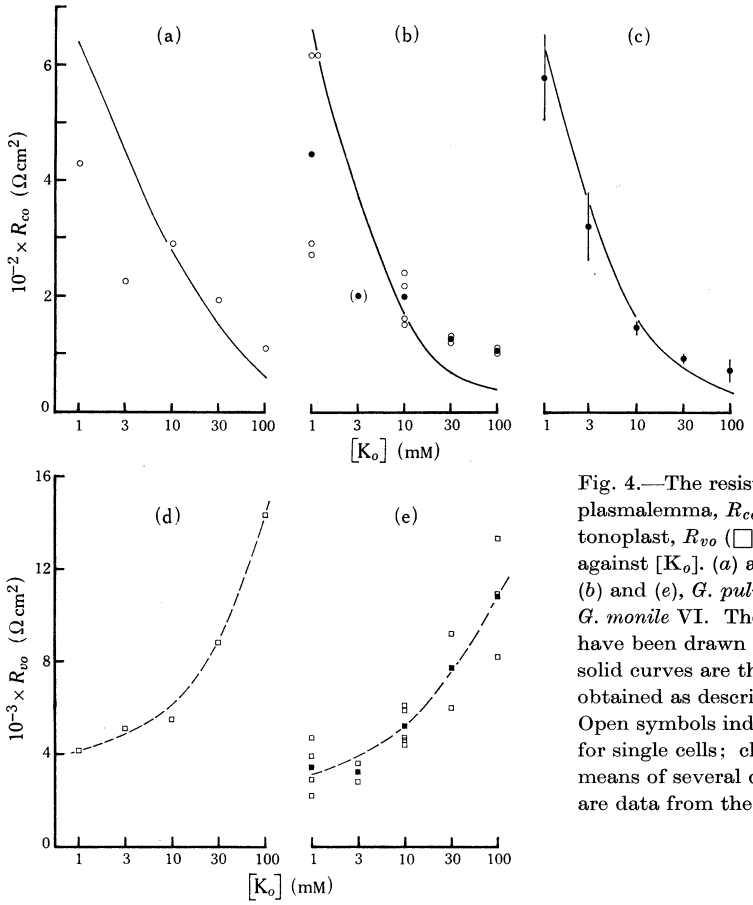

Fig. 4.—The resistance of the plasmalemma, R_{co} (○, ●), and tonoplast, R_{vo} (□, ■), plotted against [K$_o$]. (*a*) and (*d*), *G. teges* II; (*b*) and (*e*), *G. pulvinata* II; (*c*), *G. monile* VI. The broken curves have been drawn by eye. The solid curves are theoretical ones obtained as described in the text. Open symbols indicate estimates for single cells; closed symbols are means of several cells. (*a*) and (*d*) are data from the same cell.

The resistance of the plasmalemma as a function of [K$_o$] for *G. teges* II, *G. pulvinata* II, and *G. monile* VI is shown in Figures 4(*a*), 4(*b*), and 4(*c*). The values of resistance were usually obtained 5–10 min after the change in [K$_o$]. It can be seen that

the resistance of the plasmalemma decreases with increasing $[K_o]$. The solid lines are curves of best fit, drawn from the expression for membrane resistance:

$$R_m = \mathbf{R}T[(1/C_o)-(1/C_c)]/[P_K\mathbf{F}^2\ln(C_c/C_o)], \quad (2)$$

where $C_o = ([K_o]+\alpha[Na_o])$, $C_c = ([K_c]+\alpha[Na_c])$, and $\alpha = P_{Na}/P_K$ (Hope and Walker 1961). The values of C_c and α are those determined, for the particular batch of cells, from the behaviour of Ψ_{co} as a function of $[K_o]$; see Section III(*c*). The curves of best fit were obtained by adjusting P_K, the only remaining parameter.

Figures 4(*d*) and 4(*e*) show the variation of the total resistance (plasmalemma + tonoplast) with $[K_o]$ for *G. teges* II and *G. pulvinata* II. This is a close approximation to the tonoplast resistance. The behaviour of the resistance of the tonoplast with changing $[K_o]$ is not as consistent as that of the plasmalemma, as there are transients in resistance which follow a change in $[K_o]$ (see Fig. 5). In Figures 4(*d*) and 4(*e*),

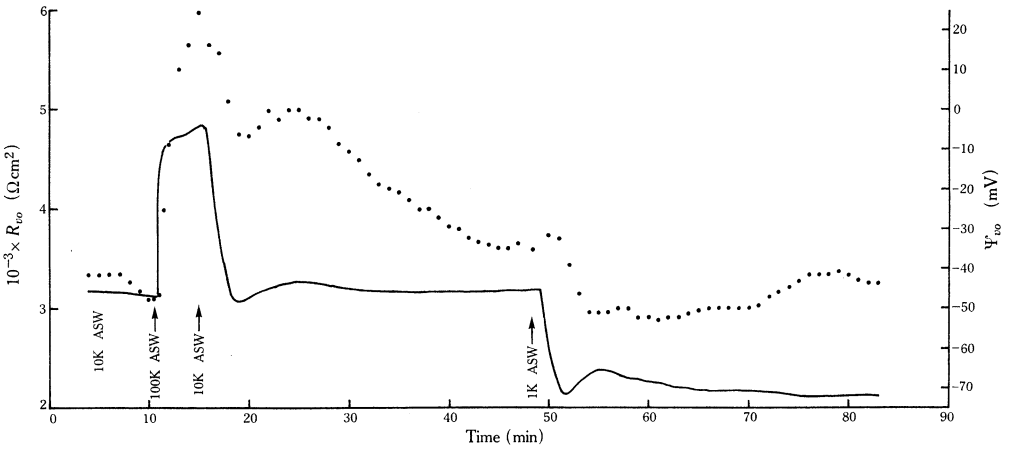

Fig. 5.—Time response of Ψ_{vo} (continuous curve) and R_{vo} (dotted curve) in a cell of *G. pulvinata* I, to changes in $[K_o]$ made at the times indicated.

$[K_o]$ was changed from the standard 10K ASW to a new level, and thence back to 10K ASW before a further change was made, and so on. The values of resistance shown in the graph are the maximum resistances reached in the new solutions, usually 5–10 min after the change in $[K_o]$. A complete set of measurements was not made in *G. monile*, but available evidence suggests that the behaviour of the tonoplast resistance for $[K_o]$ in the range 1·0–10·0 mM is not as consistent as in *G. teges* and *G. pulvinata*. However, for changes to solutions with $[K_o]$ greater than 10 mM, the resistance increases as $[K_o]$ increases.

(*e*) *The* Ψ–J *Characteristics of the Membranes*

Figure 6(*a*) shows Ψ_{co} as a function of J, the current density across the plasmalemma, for a cell of *G. monile* VI in 1K, 10K, and 100K ASW. Similar, but not so complete, results have been obtained from *G. pulvinata*. Figure 6(*b*) shows Ψ_{vc} plotted

against J in *G. pulvinata* V for the same range of $[K_o]$ as above. It can be seen that for large depolarizing currents the differential resistance $(\partial\Psi/\partial J)$ of the tonoplast approaches a low value, possibly zero. This behaviour is similar to the "punch-

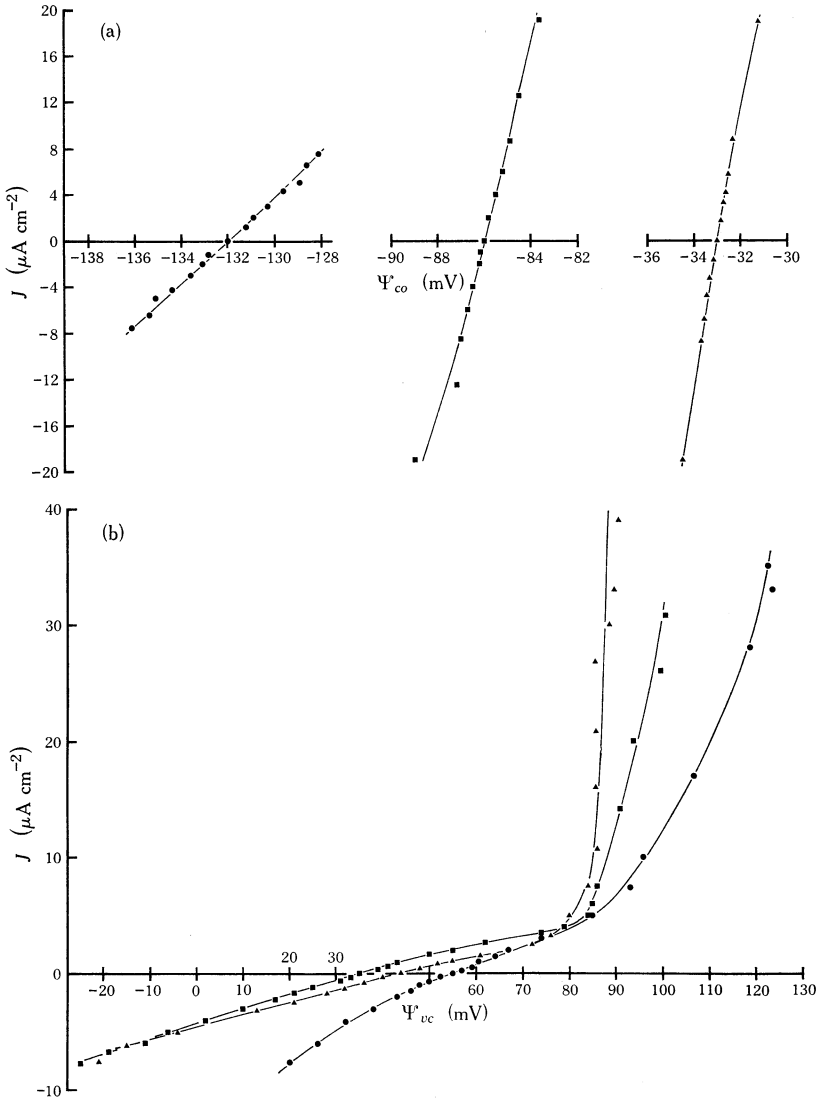

Fig. 6.—Ψ_{co} in a cell of *G. monile* VI (*a*) and Ψ_{vc} in a cell of *G. pulvinata* V (*b*) both as functions of J, the applied current density, with $[K_o] = 1$ (●), 10 (■), and 100 mM (▲).

through" phenomenon observed in both *Chara corallina* (Coster 1965) and *Nitella translucens* (Williams and Bradley 1968), though for these cells the punch-through occurs with hyperpolarizing current.

Figure 7 shows the types of response in Ψ_{vo} occurring at and near punch-through. The shapes of these responses require that some consistent criterion be adopted for measuring the voltage response. In the present work the value of the voltage response was measured after the large initial transient was complete, and about 5 sec after the initiation of the pulse.

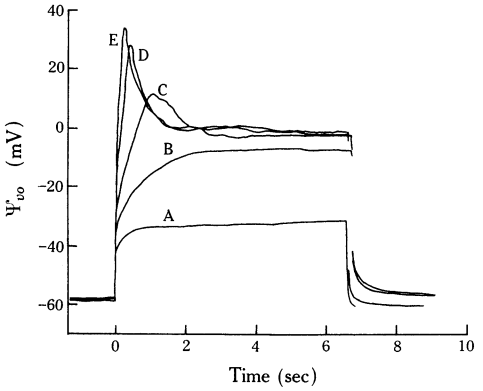

Fig. 7.—Response of Ψ_{vo}, in a cell of *G. pulvinata* V, to pulses of applied current of increasing intensity. The current densities (μA cm^{-2}) were, in curve *A* 7·0, *B* 10·0, *C* 12·2, *D* 17·0, and *E* 19·7.

(*f*) *Relative Permeability of the Plasmalemma to Potassium, Rubidium, Lithium, and Caesium*

To compare the permeability of the plasmalemma to other cations with reference to potassium, 70% of the potassium in 10K ASW was replaced by either Rb^+, Li^+, or Cs^+ [e.g. 10K ASW changed to (3K+7Rb) ASW] and the change in either Ψ_{vo} or Ψ_{co} observed. As the changes in external solution from 10K ASW only lasted from 3 to 5 min, it was assumed that most of the change observed in Ψ_{vo} was in fact change in Ψ_{co} and that C_c could be assumed constant. The results from three cells of *G. pulvinata* IV are shown in Table 3.

TABLE 3

RELATIVE PERMEABILITIES OF THE PLASMALEMMA OF *G. PULVINATA* II, CALCULATED FROM CHANGES IN Ψ_{vo}

Values are means ± SEM (numbers of cells in each batch given in parentheses)

Cell No.	Parameter*	Solution Changed from 10K ASW to:			
		3K+7Na	3K+7Rb	3K+7Cs	3K+7Li
1	$\Delta\Psi_{vo}$	−19·2±0·6 (7)	+1·4±1·8 (7)	−4·7±1·2 (6)	—
	P_X/P_K	0·006	1·11	0·69	—
1A†	$\Delta\Psi_{vo}$	−23·7±0·4 (4)	−7·7, −8·7	−15·0, −14·2	—
	P_X/P_K	0·003	0·55	0·28	—
2	$\Delta\Psi_{vo}$	−14·3±0·7 (8)	+0·5±0·8 (7)	−5·5±2·2 (7)	—
	P_X/P_K	0·012	1·05	0·55	—
3	$\Delta\Psi_{vo}$	−25, −25	−8·5, −8·5	−19, −18·5	−25·8, −26·8
	P_X/P_K	0·002	0·55	0·17	negative

* $\Delta\Psi_{vo}$ is the p.d. in the test solution minus the p.d. in 10K ASW.

† A later series using cell 1.

The ratios of permeabilities with respect to potassium have been calculated as follows. By changing from 10K to (3K+7Na) ASW, α can be calculated from the relation

$$\Delta\Psi_{co} = 58 \log_{10}(3+497\alpha)/(10+490\alpha),$$

where $\Delta\Psi_{co}$ is the change in Ψ_{co} observed. This value of α can then be put into the expression for $\Delta\Psi_{co}$ when 10K ASW is, for example, changed to (3K+7Rb) ASW, viz.:

$$\Delta\Psi_{co} = 58 \log_{10}(3+7\beta+490\alpha)/(10+490\alpha),$$

where $\beta = P_{Rb}/P_K$. For the three cells (Table 3) we found the following permeability series: cell 1, $P_{Rb} > P_K > P_{Cs} > P_{Na}$; cell 1A, $P_K > P_{Rb} > P_{Cs} > P_{Na}$; cell 2, $P_{Rb} > P_K > P_{Cs} > P_{Na}$; cell 3, $P_K > P_{Rb} > P_{Cs} > P_{Na} > P_{Li}$. In cell 3, the negative value for P_{Li}/P_K effectively means that P_{Li} is very much smaller than P_K, and that only a small error in experimental measurement can easily lead to a value of $\Delta\Psi_{co}$ slightly too large.

IV. Discussion

The first conclusion to be drawn from the experiments described in this paper is that the electrophysiological properties of the three species of *Griffithsia*—*G. teges*, *G. monile*, and *G. pulvinata*—are surprisingly similar, considering the morphological differences, particularly between *G. teges* and the other two.

Our measurements of ionic content of the vacuoles of *G. monile* and *G. pulvinata* show that the cells accumulate potassium and largely exclude sodium ions, behaviour similar to the marine algae *Chaetomorpha darwinii* (Dodd, Pitman, and West 1966) and *Valonia ventricosa* (Gutknecht 1966), and also similar to practically all animal cells, e.g. squid giant axon (Hodgkin 1958). The vacuolar concentrations of potassium and sodium in *G. pulvinata* have remained fairly similar in batches collected over a period of 2 years. However, the vacuolar concentration of potassium in *G. monile* VI is distinctly lower than in *G. monile* IV. A later collection of *G. monile* had the higher value. Seasonal fluctuations are possible.

Before we can draw conclusions about transport of ions in the cells it is necessary to determine whether the cells are in a steady state with regard to a particular ion, i.e. that the net flux of those ions is zero. The results in Figure 1 show that in cells transferred from seawater to an artificial seawater (10K ASW), which was made to approximate seawater as far as the major ions are concerned, there is very little change in vacuolar ($K^+ + Na^+$) concentration during nearly 3 weeks. Thus we can conclude from the data shown in Figure 1 that the cells remain in an approximately steady state for potassium and sodium. The same situation is also true for cells transferred from seawater to either 3K or 30K ASW. These particular results have no immediate significance for the present paper, but establish that cells in 3K or 30K ASW over a period of a few days are in a steady state, information that is required when measurements are made of fluxes of potassium under these conditions. The results of such flux measurements will be described in a subsequent paper.

The net flux of sodium and potassium ions from the vacuole is about zero. Therefore the Nernst equation (MacRobbie and Dainty 1958) can be applied to determine the electrochemical equilibrium potential for the two ions, and this value

may be compared with the observed p.d. to determine whether the ions are passively distributed or actively transported. The appropriate calculations are shown in the tabulation that follows:

Species	Concentration in Vacuole and Outside (mM)						Observed Ψ_{vo} (mV)	Calculated Nernst Potential (mV)		
	$[K_v]$	$[Na_v]$	$[Cl_v]$	$[K_o]$	$[Na_o]$	$[Cl_o]$		Ψ_K	Ψ_{Na}	Ψ_{Cl}
G. pulvinata V	535	30	606	10	490	573	−52	−100	+70	+1·5
G. monile VI	401	47	—	10	490	573	−54	−93	+59	—

It is clear that the electrochemical gradients for both sodium and potassium are not zero, and hence there must be active transport of both these ions, potassium transported inwards, and sodium outwards. For chloride there is not sufficient data to decide definitely whether its net flux is zero, but it is not very large compared with the unidirectional fluxes (Findlay, Hope, and Williams, unpublished data). Chloride is included tentatively in the above tabulation for this reason. It is apparent from this tabulation that chloride is far from electrochemical equilibrium, and hence is almost certainly actively transported inwards.

The relationship between the p.d. across the plasmalemma and the concentration of K^+, Na^+, and Cl^- in the external solution can be reasonably accounted for by assuming that the plasmalemma is specifically very permeable to potassium compared with the other ions. This specificity is a very striking feature of *Griffithsia* cells. Most plant and animal cell membranes show some specificity in their permeability to potassium as against sodium with $\alpha = 0{\cdot}01$–$0{\cdot}1$, but none seem to be as specific for potassium as *Griffithsia*, where α can be as low as $0{\cdot}002$.

We have used equation (1) to estimate α and C_c, assuming that α and C_c are constant over the range of values of $[K_o]$. It is also assumed that P_{Cl}/P_K is very small, as replacement of 75% of the external chloride by the large anion benzene sulphonate had practically no effect on the p.d. across the plasmalemma (Findlay, Hope, and Williams, unpublished data). The calculated values of C_c range from 300 to 350 mM, and the potassium concentration in the cytoplasm should be close to the values of C_c because α is very small. Vorobiev (1967), in this laboratory, using a potassium-sensitive electrode inserted into the cytoplasm of *G. pulvinata* III, obtained a value of 153 mM for potassium activity. If we assume that the activity coefficient, γ, in the cytoplasm is the same as in seawater, i.e. $0{\cdot}68$, then $[K_c] = 225$ mM, a value somewhat lower than our estimate. Two explanations of the difference are possible. Firstly, there may have been an actual difference in $[K_c]$ between the cells used by Vorobiev and the cells we used, although this is unlikely as the variation in p.d. of the plasmalemma and tonoplast, and the vacuolar concentrations in and between various batches of cells, was small. Secondly, the activity coefficient in the cytoplasm may be lower than in seawater. A value of $0{\cdot}43$ for γ would be needed to give a value of 350 mM for $[K_c]$.

Although the simple diffusional model (with constant α and C_c) of the plasmalemma p.d. as a function of $[K_o]$ gives a reasonable fit to most of the data, for one batch of cells of *G. pulvinata* II, a better fit was obtained by allowing C_c and α to increase with increasing external potassium concentration (see Table 2). In the range 1–100 mM of $[K_o]$, α increased about 10-fold, and C_c by about $1{\cdot}5$-fold. A

change in C_c with increasing $[K_o]$ is not unreasonable, for as the volume of the cytoplasm is small a net influx of potassium, as $[K_o]$ is increased, could change $[K_c]$. For instance, a net flux of 50 p-mole cm^{-2} sec^{-1} into the cytoplasm (assumed about 5 μm thick) for 5 min would cause a change in $[K_c]$ of 15 mM. We have not pursued this line of argument further, because other models would also fit the data, e.g. constant C_c and varying α. More information is required about P_K and P_{Na} separately, as well as independent determinations of C_c, before these problems can be resolved.

The extremely low value for α means that, for $[K_o] = 10$ mM or greater, equation (1) approximates to the Nernst equation for potassium, $\Psi_{co} = 58\log_{10}([K_o]/[K_i])$, implying that potassium is near electrochemical equilibrium across the plasmalemma. We conclude, therefore, that the active inward pump of potassium between the vacuole and the seawater is located not at the plasmalemma but at the tonoplast.

On the simplest model, with α and C_c not functions of $[K_o]$, it is seen that lowering the external calcium concentration increases α. As lowering $[Ca_o]$ also decreases the resistance of the plasmalemma (Findlay, Hope, and Williams, unpublished data), P_{Na} must increase.

Perhaps the most striking feature of these *Griffithsia* cells is the very low resistance of the outer cytoplasmic membrane compared with the inner one—in complete contrast to freshwater plant cells such as *Chara corallina* (Findlay and Hope 1964), *Nitella translucens* (Spanswick and Costerton 1967), and root epidermal cells (Greenham 1966). On the other hand, MacRobbie and Dainty (1958) have concluded from flux data that in the brackish-water characean, *Nitellopsis obtusa*, the resistance of the plasmalemma is less than that of the tonoplast, although more recent measurements (Findlay, unpublished data) indicated that this is not always so.

We have used the Goldman constant-field model to describe the resistance of the plasmalemma. The solid curves in Figure 4 show the behaviour predicted from equation (2). For constant C_c and α, the theoretical curves do not fit the experimental points over the whole range of values of $[K_o]$, although the deviation of the theoretical from the experimental is not great. Better agreement could be obtained by allowing P_K to decrease by a factor of about 2 over the range $[K_o] = 1$–100 mM. This implies an increase in α. It is interesting to note here that if α increases with $[K_o]$ there is closer agreement with the data on Ψ_{co} versus $[K_o]$ from equation (1). Estimates of P_K and P_{Na} from the above analysis are:

	G. pulvinata II	*G. monile* VI	*G. teges* II
$10^5 \times P_K$ (cm sec^{-1})	3·4	4·0	2·52
$10^7 \times P_{Na}$ (cm sec^{-1})	0·68	0·8	1·26

The properties of the tonoplast are more difficult to work out, largely because it has not been possible to control the ionic composition of either of the two phases which this membrane separates. Changes taking place at the tonoplast consequent on changes in the ionic composition of the external medium presumably reflect changes in the ionic composition in the cytoplasm. The changes in resistance and p.d. of the tonoplast with changes in $[K_o]$ appear to be in the opposite direction from those expected by comparison with the plasmalemma. This may indicate that the tonoplast is more permeable to sodium than to potassium, and that changes in K^+

and Na^+ concentrations outside cause parallel changes in K^+ and Na^+ concentrations in the cytoplasm. On this basis, of course, it would be more appropriate to use a model for the plasmalemma in which both C_c and α increase with increasing $[K_o]$. It is clear that there are many problems associated with the tonoplast in *Griffithsia*.

The Ψ versus J characteristics of the tonoplast show rectification effects. Furthermore, it is not possible to depolarize the tonoplast to values more positive than about +90 to +120 mV. This effect is similar to the punch-through phenomenon described by Coster (1965) and Williams and Bradley (1968) for *Chara corallina* and *Nitella translucens* respectively, but in these cells punch-through occurred at the plasmalemma and for large hyperpolarizations (−350 mV) of that membrane. In the punch-through condition the differential membrane resistance approaches zero and the membrane permeability must increase and give rise to an increase in ionic fluxes. In *C. corallina*, Coster and Hope (1968) have shown that at punch-through there is a greatly increased chloride efflux across the plasmalemma. Although at present we have no direct evidence relating to punch-through in *Griffithsia*, some simple calculations show that a greatly increased permeability of the tonoplast to chloride ions, but not potassium or sodium, may be involved. A large increase in membrane permeability to one ion should effectively "clamp" the membrane p.d. near the electrochemical equilibrium potential for that ion, and hence from the Nernst equation the expected cytoplasmic concentration of the ion can be calculated. In a cell of *G. pulvinata* V [Fig. 6(*b*)] the punch-through potentials were approximately +125 mV ($[K_o]$ = 1 mM), +105 mV ($[K_o]$ = 10 mM), and +90 mV ($[K_o]$ = 100 mM). The vacuolar concentrations were 535 mM K^+, 30 mM Na^+, and 606 mM Cl^-. Hence for $[K_o]$ = 1, 10, and 100 mM, we get $[Na_c]$ = 4,335, 1,938, and 1,065 mM, $[K_c]$ = 70,200, 34,600, and 19,000 mM, and $[Cl_c] = 4 \cdot 2, 9 \cdot 4$, and $17 \cdot 0$ mM respectively. The potassium and sodium concentrations are unrealistic but the values for Cl^- are feasible, although an explanation is needed for the increase in $[Cl_c]$ with increasing $[K_o]$. To complete the argument, it must be assumed that when the applied current causing the punch-through is switched off the permeability of the tonoplast to chloride rapidly decreases again.

When the depolarizing effects on the p.d. across the plasmalemma of the alkali cations Rb^+, Cs^+, Na^+, and Li^+ were compared with that of K^+, it was found that either $P_K > P_{Rb} > P_{Cs} > P_{Na} > P_{Li}$, or $P_{Rb} > P_K > P_{Cs} > P_{Na} > P_{Li}$. Eisenman (1962) has developed a theory of ion specificity involving the free energy changes between hydrated ions in solution and electrostatically bonded ions in ion-exchange systems, particularly in the case of glasses, which can be made to act as specific cation electrodes. This theory can readily be extended to membrane specificity. Of the possible cation specificity series our results correspond to Eisenman's series III or IV; when $P_K = P_{Rb}$ the borderline between these series is indicated. The field strength of the anions to which the permeating (or adsorbing) cations bond, determines the particular series or permeability order. The spread or span of permeabilities (e.g. between K^+ and Li^+) depends on the state of hydration of the ion exchanger; presumably in the present experiments a permeability span greater than or equal to 500 : 1 (cell 3, Table 3) corresponds to a highly hydrated system. Eisenman (1963) has gathered much data on the effect of the alkali cations on bio-electric potentials and other phenomena. The series IV is also found to be the order of depolarizing ability of

cations on the squid axon resting potential, the p.d. at the inside of frog skin, and the resting potential in several muscle tissues.

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