EFFECTS OF TEMPERATURE ON MEMBRANE PERMEABILITY TO IONS

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Summary

The effects of varied temperature on membrane potential difference (p.d.) and resistance were measured in single cells of the green, fresh water alga *Chara corallina* and the red, marine alga *Griffithsia pulvinata*.

Lowering the temperature depolarized the plasmalemma in both genera and increased the resistance. The influx of potassium into *G. pulvinata* was markedly decreased by lowered temperature, that of sodium less so.

Calculations were made of the permeability ratio α ($= P_{Na}/P_K$) from the data on change of p.d. with temperature, assuming that the p.d. is determined by K^+ and Na^+ permeability only, which is strongly indicated for *Chara* and *Griffithsia*.

In *C. corallina*, α was constant from room temperature down to 13°C (short-term temperature changes) or 7°C (long-term), then α increased between 13 or 7°C (respectively) and 2°C. Taken with the changes of resistance, this indicated that P_{Na} and P_K decreased equally down to a critical temperature region, after which P_{Na} decreased less than P_K, with decreasing temperature. In *G. pulvinata*, α was found to increase continuously with decreasing temperature, consistent with P_K being affected more strongly than P_{Na}.

Individual permeabilities were calculated as functions of temperature from the resistance data and confirmed the conclusions from the behaviour of α. Also, calculations of permeability change with temperature from the influxes in *G. pulvinata* further showed that P_K was more strongly dependent on temperature than P_{Na}.

The slopes of $\log_{10}P_{K,Na}$ versus $1/T$ (Arrhenius plots) were interpreted as enthalpies of activation for the permeation of Na^+ and K^+ through the plasmalemma. ΔH^*_K was 10–46 kcal mole^{-1}, ΔH^*_{Na} 3–16 kcal mole^{-1}. The enthalpies were not generally constant with temperature, indicating either a change of membrane structure ("phase change") with cooling, or else different processes becoming limiting in the permeation, with decreasing temperature.

I. Introduction

The use of imposed temperature changes as a means to study the activation of various physiological processes has a long history. The effect of temperature change on membrane resting potential was studied in *Valonia* by Blinks (1942) and Thorhaug and Drost-Hansen (1966). In the present studies we were particularly interested in using effects on membrane resistance to give a means of estimating activation energies for membrane ion permeation. After this work was commenced, Hogg, Williams, and Johnstone (1968) published results of similar experiments made with *Nitella translucens*, interpreting them with the aid of the theory of rate processes (Zwolinski, Eyring, and Reese 1949).

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Hogg, Williams, and Johnstone (1968) made an ingenious analysis of the data on potential difference (p.d.) and resistance in *Nitella*, leading to estimates for potassium and sodium permeability, P_K and P_{Na}, as functions of temperature. However, since their recordings of p.d. were made with electrodes in the vacuoles of the cells, there is doubt as to whether the permeabilities deduced are those of the plasmalemma, unless it can be shown that the tonoplast p.d. and resistance (and changes therein) are negligible. Also, the theoretical analysis made use of a relation between membrane resistance and P_K, P_{Na} (Hope and Walker 1961) that depends on the Goldman (1943) assumption of a linear potential gradient. This equation for resistance has only occasional correspondence with observation; in particular it fails to describe observed changes in flux of potassium and sodium when the membrane p.d. is altered by a voltage or current clamp (Walker and Hope 1969). Williams and Hogg (1970) find that constant permeabilities are not appropriate for describing membrane resistance in *Nitella* when external concentrations are changed.

Finally, confirmation of permeability changes with temperature, calculated from electrical measurements, need to be obtained from measurements of flux with temperature, as Hogg, Williams, and Johnstone (1968) stated.

Some of these objections have been attended to in the present experiments. In *Chara corallina*, however, other difficulties have prevented a clear-cut picture being obtained of the enthalpies and entropies of activation for ion diffusion through the plasmalemma. Using cells of *Griffithsia pulvinata*, of which the electrical properties have recently been examined (Findlay, Hope, and Williams 1969), and in which the plasmalemma has substantially passive influxes of potassium and sodium, it has been possible to estimate P_K and P_{Na} as functions of temperature from both electrical and flux measurements.

II. Materials and Methods

Plants of *C. corallina* were cultured in a medium containing (mM) 0·5 $NaHCO_3$, 1·0 NaCl, 0·2 $NaNO_3$, 0·017 KH_2PO_4, 0·05 K_2SO_4, 0·1 $MgSO_4$, 0·1 $CaCl_2$ and (μM) 3·6 $FeSO_4$, 0·91 $MnSO_4$, 0·76 $ZnSO_4$, 0·014 $(NH_4)_6Mo_7O_{24}$, modified from Barr and Broyer (1964). Cells cut from these plants were first soaked for 20–24 hr in 10 mM NaCl, to exchange some of the cell wall divalent cations for monovalent cations. Cells were then transferred to the experimental medium, FPW, comprising NaCl, 2; KCl, 0·2; and $CaCl_2$, 0·05 mM.

G. pulvinata was collected and stored, and used in an artificial sea water, ASW, comprising NaCl, 490; KCl, 10; $CaCl_2$, 11·5; $MgCl_2$, 25; $MgSO_4$, 25; $NaHCO_3$, 2·5; NaBr, 1·0, all mM. The methods used to obtain electrophysiological measurements have been described previously (*Chara*—Hope and Walker 1961; *Griffithsia*—Findlay, Hope, and Williams 1969), as have procedures for measuring fluxes of sodium and potassium across the plasmalemma of *Griffithsia* cells (Findlay, Hope, and Williams 1970). Briefly, the influx of potassium and sodium at the plasmalemma was estimated from the uptake of the appropriate tracer into batches of single cells placed in labelled ASW for 20 min, at various constant temperatures. The cells were rinsed vigorously with ASW for 3 min before counting to remove extracellular radioactivity. Cells were pretreated at the experimental temperature for 30 min after 24 hr in ASW at room temperature.

During the electrical measurements, a thermistor (STC type F23D) was used to measure the temperature close to the cell when cooled solution flowed past it. The thermistor was made part of a bridge circuit from which an output of 0–50 mV corresponded linearly to the temperature range 0–25°C. A current generator was switched on periodically with the aid of a synchronous motor, for 1–2 sec every 1–2 min. This enabled the cell resistance to be measured frequently, through the changes in transmembrane p.d. superimposed on the resting p.d. Both this and temperature were continuously recorded. In *Chara* the length constant was 2–3 cm at room

temperature, while the actual length of the cells used was less than 2·5 cm, so no corrections were made for cable attenuation of the injected current. The consequent errors involved in measuring resistance were less than 5% (Walker and Hope 1969) at room temperature, and progressively less the lower the temperature.

III. Results

(*a*) Chara corallina

Two series of experiments were performed, in which temperature changes were for brief periods or extended periods. The second series corresponds to the conditions used by Hogg, Williams, and Johnstone (1968), where measurements were made in a constant-temperature room. Brief (2–5 min) changes in temperature seemed more desirable in that other parameters such as cytoplasm and wall concentrations would not be expected to change. However, the electrophysiological experiments involving long-term temperature changes correspond more closely to the experiments seeking to measure changes in ionic flux with temperature.

(i) *Changes in Electrical Properties Following a Temperature Change*

C. corallina cells treated as described in Section II, and used in the FPW medium had a mean resting potential ψ_{vo} of $-148 \pm 1 \cdot 8$ mV (mean $\pm$ standard error of the mean of about nine cells) at a mean room temperature of $22 \cdot 6 \pm 0 \cdot 2$°C. The resting resistance averaged $8 \cdot 6 \pm 0 \cdot 7$ kΩ cm². Cells were usually potassium-responsive and depolarized reversibly when the medium was changed to 0·6 K, 1·6 Na, 0·05 Ca (mM).

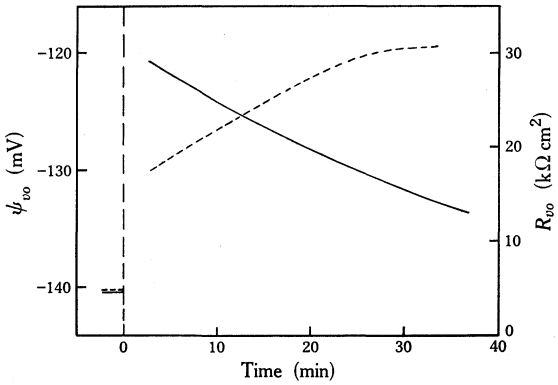

Fig. 1.—Changes in vacuolar p.d. (ψ_{vo}, ———) and resistance (R_{vo}, – – – – –), as a function of time, in *C. corallina* following a temperature change from 22 to 8°C at time zero.

Vacuolar recordings were made because cytoplasmic recordings can only be obtained for some minutes before sealing of the tip of the cytoplasmic electrode intervenes. However, it was found that the p.d. across the tonoplast was on the average 10 mV (vacuole positive to cytoplasm), while the tonoplast resistance was about 300 Ω cm². The p.d. was similar to that observed by Findlay and Hope (1964) but the resistance was lower. Neither the p.d. nor resistance of the tonoplast changed very much with temperature. A 10% change could probably have been detected but accuracy at these levels was not high.

Changes in p.d. and resistance were usually reversible if the temperature change was brief. After a reduction in temperature, it was observed that p.d. and resistance

immediately took new values but thereafter drifted with time at the new, constant temperature. The initial change in p.d. was a depolarization, followed by a drift back towards a more negative value, while the resistance increased suddenly, then continued to increase. Figure 1 shows the p.d. and resistance (means of two runs with the same cell) after a change from 22 to 8°C, as functions of time. In the short-term experiments the values of p.d. and resistance 2–5 min after the temperature change were used for calculation of permeability changes.

The collected results from about nine cells are shown in Figures 2(*a*) and 2(*b*) in which p.d. and resistance, suitably grouped in ranges of temperature, are plotted.

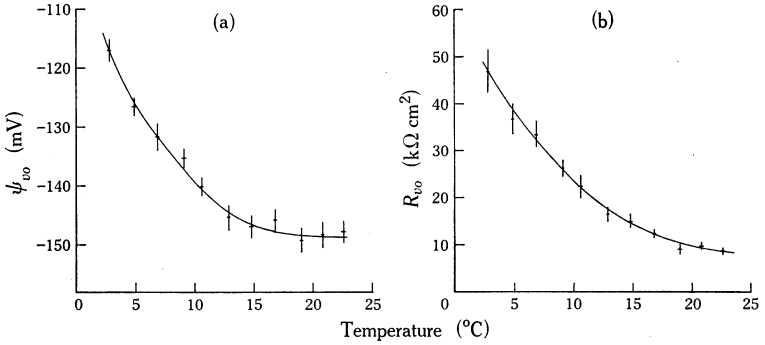

Fig. 2.—(*a*) Mean vacuolar p.d. (ψ_{vo}) in *C. corallina* plotted against temperature. The p.d. is that 2–5 min after a temperature change. Results from nine cells were grouped in ranges of temperature such as 2–3·9°C, 4–5·9°C, etc. The line joining the means was fitted visually. Standard error of the mean also shown. (*b*) Mean resistance (R_{vo}) plotted against temperature. The conditions and temperatures correspond to those in Figure 2(*a*).

(ii) *Effects of Prolonged Temperature Changes on Potential Difference and Resistance*

The series of experiments involving long-term changes in temperature is not strictly comparable with the series already described; the cells were from a different culture tank and the experiments were done about 6 months after the first set. Usually two temperatures below room temperature were investigated with each cell in a sequence over about 8 hr, e.g. 22–4–22–12–22°C. The resting p.d. at room temperature in the second set was about −160 mV (22°C) and p.d.'s at all temperatures were more variable among cells. The mean resting resistance was about 12 kΩ cm² at 22°C. However, as expected from the trends in Figure 1, the cells were less depolarized, and the resistance was higher, at comparable temperatures below about 10°C, compared with the short-term experiments. As well as the greater variability among cells, after a temperature drop followed by a restoration to room temperature, the p.d. showed a tendency to oscillate slowly; in any case there was usually hysteresis. The generally constant values of p.d. and resistance after 2 hr were noted.

The thermostat in the controlled-temperature enclosure allowed variations of 1–2 degC about a mean near the cell, with a 5–6 min cycle after a steady mean temperature was reached. Because p.d. was recorded continuously, and resistance about every 1·5 min, it was possible to calculate the temperature coefficient of the p.d. and resistance, at a few mean temperatures. This has helped to establish the trend of the graphs plotted in Figures 3(*a*) and 3(*b*), since their slopes are known at several temperatures. In the theoretical treatment of these results, it was necessary to use values from the smoothed-out curves, and hence the conclusions are provisional upon the smooth curves representing the actual dependence of ψ and R on temperature.

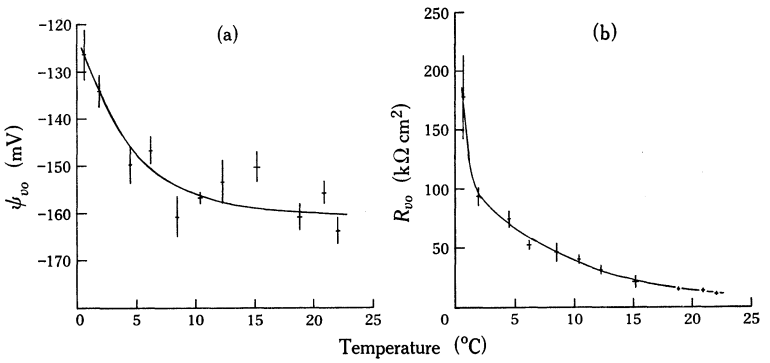

Fig. 3.—(*a*) Vacuolar p.d. (ψ_{vo}) in *C. corallina*, as a function of temperature, 1–2 hr after the change in temperature. A different set of cells from those of Figure 2. The mean and standard error of the mean was obtained by similar grouping into ranges of temperature. (*b*) Vacuolar resistance (R_{vo}) 1–2 hr after temperature change, plotted against temperature.

(*b*) Griffithsia pulvinata

(i) *Electrophysiological Measurements*

It was possible to make prolonged cytoplasmic and vacuolar recordings while setting the temperature between about 19 and 3°C. The p.d. of the cytoplasm (ψ_{co}) reached a steady value a few minutes after insertion but it was necessary to wait 30–60 min for the plasmalemma resistance to become constant at room temperature. The tabulation below gives the mean resting values of p.d. and resistance at plasmalemma and tonoplast in seven cells, at the mean room temperature of 19°C:

ψ_{co} −82·0±0·6 mV	R_{co} 186±16 Ω cm²
ψ_{vc} +36·4±1·4 mV	R_{vc} 10·8±1·0 kΩ cm²

These values agree generally with those found by Findlay, Hope, and Williams (1969) for *G. pulvinata* and other species, except that the tonoplast resistance R_{vc} is here twice as large as observed previously. This is possibly an effect of longer storage times. There was less drift in the p.d. and resistance following a change in temperature, and the effects of temperature were generally reversible, compared with the observations using *C. corallina*. The values of p.d. and resistance 5–30 min after the

new temperature of the bathing solution had become constant were used in plotting Figures 4(*a*) and 4(*b*), which show mean results for the plasmalemma. Results for the

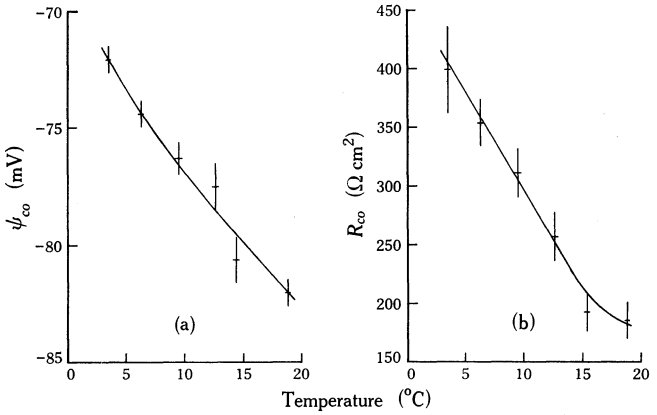

Fig. 4.—(*a*) Plasmalemma p.d. (ψ_{co}) in *G. pulvinata* plotted against temperature. The mean and stardard error of the mean of seven cells are given. Results grouped in ranges of temperature 2·0–4·9°C, 5·0–7·9°C, etc. (*b*) Resistance of the plasmalemma (R_{co}) plotted against temperature for the same cells and ranges of temperature as in Figure 4(*a*).

tonoplast have not been presented because it is not known what ions determine the p.d. and resistance. The effect of lowered temperature was to depolarize the p.d. and raise the resistance, as observed with the plasmalemma.

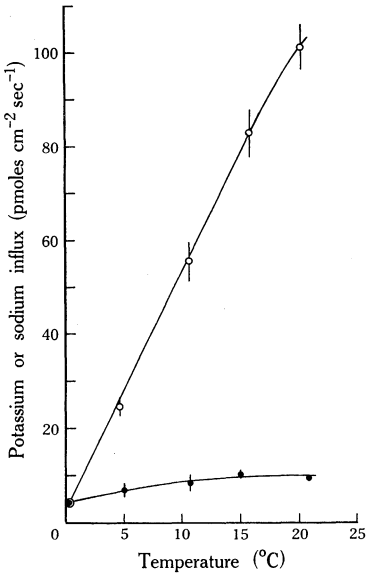

Fig. 5.—Potassium (o) and sodium (●) influx at the plasmalemma, in *G. pulvinata*, plotted against temperature. The means and standard error of the means are given for 10 cells.

(ii) *Influx of K^+ and Na^+*

Figure 5 shows the mean influxes in batches of 10 cells, between 20°C and just above 0°C. The inherent variation (between cells) in the sodium influx was found to be

greater than that in the potassium influx; this has been observed also in another marine alga, *Valoniopsis* (Findlay *et al.*, unpublished data).

IV. Discussion

(*a*) *Changes of Potential Difference with Temperature*

(i) *Interpretation of the Potential Difference*

The general effect of lowering temperature was to depolarize the cells, particularly in the range 2–15°C. There are good reasons for supposing that passive permeability to potassium and sodium determine the potential difference across the plasmalemma, in both *Chara* and *Griffithsia* spp. The contribution of chloride permeability to the p.d. in *C. corallina* can be shown to be small through a consideration of chloride efflux (Hope, Simpson, and Walker 1966; Findlay *et al.* 1969). A few experiments were done in the present context to confirm that the same level of efflux was appropriate for these particular cells. The total efflux was about 0·4 pmole cm^{-2} sec^{-1} at 21°C, reducing to 0·1 pmole cm^{-2} sec^{-1} at 6°C. However, most of this efflux is probably exchange diffusion (Findlay *et al.* 1969) and it is estimated that P_{Cl}/P_K for the plasmalemma of *C. corallina* is less than 0·002 through use of the Goldman equation for flux in terms of permeability, etc.:

$$\phi_j = -\frac{P_j z_j \mathbf{F}\psi_{co}}{\mathbf{R}T} \cdot \frac{c_j^o - c_j^c \exp(z_j \mathbf{F}\psi_{co}/\mathbf{R}T)}{1 - \exp(z_j \mathbf{F}\psi_{co}/\mathbf{R}T)}. \quad (1)$$

where $c_j^{o,c}$ are the concentrations* of the ions j in the external and cytoplasmic phases, **R** the gas constant, T temperature in °K, **F** the Faraday, and z_j the valency.

In *G. pulvinata*, substitution of non-permeant anions for half the chloride in ASW had no observable effect on ψ_{co} and it was assumed that there, too, P_{Cl}/P_K was very small (Findlay, Hope, and Williams 1969).

The only other ion species seriously suggested as playing a part in determining ψ_{co} is H^+ (or H_3O^+) (Kitasato 1968). Reasons are given by Walker and Hope (1969) for supposing that the evidence for implicating H^+ directly is inconclusive.

In view of the above it is proposed to use the usual simple equation for p.d. based on potassium and sodium permeation, viz:

$$\psi_{co} = \frac{\mathbf{R}T}{\mathbf{F}} \ln \frac{P_K c_K^o + P_{Na} c_{Na}^o}{P_K c_K^c + P_{Na} c_{Na}^c}. \quad (2)$$

(ii) *Effects of Temperature*

Effects of temperature on ψ_{co} can be interpreted as effects on P_K and P_{Na} (as well as the obvious effect through the term $\mathbf{R}T/\mathbf{F}$). During short-term experiments the concentrations should remain constant. It is not possible to interpret the data on the changes in tonoplast electrical properties with temperature in *G. pulvinata* without more knowledge about cytoplasm concentrations, particularly of chloride, since the tonoplast is suspected to be preferentially chloride-permeable (Findlay, Hope, and Williams 1969).

* Activities should be used in this and following equations but this refinement is not justified while activity coefficients are unknown for cellular compartments.

Using equation (2) it is possible to calculate values of α ($= P_{Na}/P_K$) for each temperature from the observed p.d. For *C. corallina* it is sufficient for the present analysis to let $\psi_{co} = \psi_{vo} - 10$, the tonoplast p.d. having been observed to stay constant at about 10 mV. In *G. pulvinata*, ψ_{co} was directly measured. Equation (2) is equivalent to

$$\psi_{co} = \frac{RT}{F} \ln \frac{c_K^o + \alpha c_{Na}^o}{c_K^c + \alpha c_{Na}^c} \qquad (2a)$$

The extent of depolarization when c_K^o was increased (from 0·2 to 0·6 mM for *C. corallina*, from 10 to 20 mM for *G. pulvinata*) enables α to be calculated for room temperature, as well as $c_K^c + \alpha c_{Na}^c$. The mean values of these parameters at room temperature are tabulated below:

	α	$c_K^c + \alpha c_{Na}^c$	T
C. corallina	0·082	178	22°C
G. pulvinata	0·0070	355	19°C

The estimates for *G. pulvinata* agree well with those from the previous study already referred to, as does the value of α for *C. corallina*, but $c_K^c + \alpha c_{Na}^c$ for *C. corallina* is larger than expected from the measurements of Vorobiev (1967) made with potassium-selective electrodes, where a_K^c was about 100 mM. The present observations would be

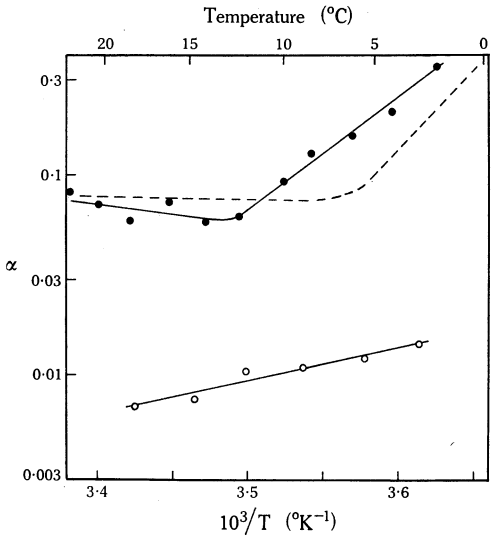

Fig. 6.—Permeability ratio α for the plasmalemma calculated from data of Figure 2(*a*) (●) and Figure 3(*a*) (– – – –) for *C. corallina*, and from Figure 4(*a*) (○) for *G. pulvinata*. α is on a log scale, and is plotted against the reciprocal of absolute temperature.

compatible with Vorobiev's estimate provided the effective c_K^o were somewhat less than 0·2 mM. It has been proposed that depletion of K^+ near the plasmalemma could be caused, for example, by active transport during the growing life of the cells (Barry and Hope 1969). It is difficult to bring the cell wall in *C. corallina* into equilibrium with a new external medium if the wall has previously been in a medium containing divalent cations such as Ca^{2+} and Mg^{2+}. The trends of permeability changes with temperature do not depend greatly on the cytoplasm activities assumed (as can be

confirmed by using different values in the calculations) though the absolute values of permeability subsequently calculated from the membrane resistance do depend on these assumptions. The significance of these permeabilities is, however, dubious because of use of the Goldman assumption for obtaining a relation between membrane resistance and P_K, P_{Na}. This is discussed further in Section IV(*b*), below.

Figure 6 shows how α changes with temperature in *C. corallina* and *G. pulvinata*. The dotted line shows the trend, admittedly approximate because of the high variability encountered, of α in the long-term experiments (data from Fig. 3). Unlike the results from *Nitella*, α in *C. corallina* was constant or slightly decreasing in the range 22·6–13°C (short-term experiments) or 22–8°C (long-term experiments) after which it increased as the temperature dropped. An approximately straight-line relation, on a log scale, between α and $1/T$ is apparent between 13 and 2·6°C, and 7 and 1°C respectively.

(*b*) *Changes of Resistance with Temperature*

(i) *Relation between Resistance and Permeability*

Hope and Walker (1961) showed that, for the model in which the plasmalemma is exclusively potassium- and sodium-permeable,

$$R_m = \frac{\mathbf{R}T[1/C^o - 1/C^c]}{\mathbf{F}^2 \ln(C^c/C^o)}, \tag{3}$$

where R_m is the membrane resistance, $C = P_K c_K + P_{Na} c_{Na}$ for cytoplasm or medium. The permeabilities are those defined in the Goldman equation (1). It is seen that use of equation (3) enables P_K and P_{Na} to be calculated for the various temperatures, since R_m has been measured,* and α estimated from equation (2a). The other parameters used in equation (3) are those already used for equation (2a). This procedure was followed by Hogg, Williams, and Johnstone (1968) in discussing their results with *N. translucens*, and the same will be done here for comparison, though equation (3) has doubtful validity, as pointed out in Section I. In *G. pulvinata*, where the ionic strength is high and about equal on each side of the plasmalemma, constant permeability coefficients are more likely, according to Sandblom and Eisenman (1967).

(ii) P_K, P_{Na} *as Functions of Temperature*

Figures 7(*a*) and 7(*b*) show the calculated permeabilities plotted on a log scale against $1/T$. The slope of such a graph (an Arrhenius plot) is related to an enthalpy of activation, with a small correction term (Johnson, Eyring, and Polissar 1954), as is the slope of the function $\ln(hP_j/kT)$ used by Hogg, Williams, and Johnstone (1968) derived from the theory of rate processes. In the usual model for diffusion in a membrane, the activation enthalpy corresponds to the kinetic energy necessary to surmount the largest potential energy barrier; this is often shown as being at the interface between membrane and aqueous medium (Davson and Danielli 1952).

* Recording in the vacuole of *C. corallina* means that the resistance measured is that of the plasmalemma and tonoplast in series. However, the resistance of the tonoplast was only a small percentage of the total and no correction has been made.

In contrast to *Nitella*, *C. corallina* [Fig. 7(*a*)] does not have a constant enthalpy of activation either for K^+ or Na^+. For K^+ there is a fairly sudden change from about 10 kcal mole^{-1} to 30 kcal mole^{-1} at 13°C, the same temperature as the discontinuity in log α (Fig. 6). The shape of the relation between log P_K and $1/T$ depends little on what values are assumed for the concentrations $c_j^{o,c}$ [see Section IV(*a*)(ii) above] but the shape of log P_{Na} depends somewhat on whether a "depleted" value is used for c_K^o or not. Nevertheless it appears that the enthalpy of activation for Na^+ diffusion in the plasmalemma of *C. corallina* is about 14 kcal mole^{-1} above about 13°C and much less (4–5) below 13°C. These values refer to the short-term temperature changes. The trends for P_K and P_{Na} are shown dotted in Figure 7 (*a*) for the long-term experiments,

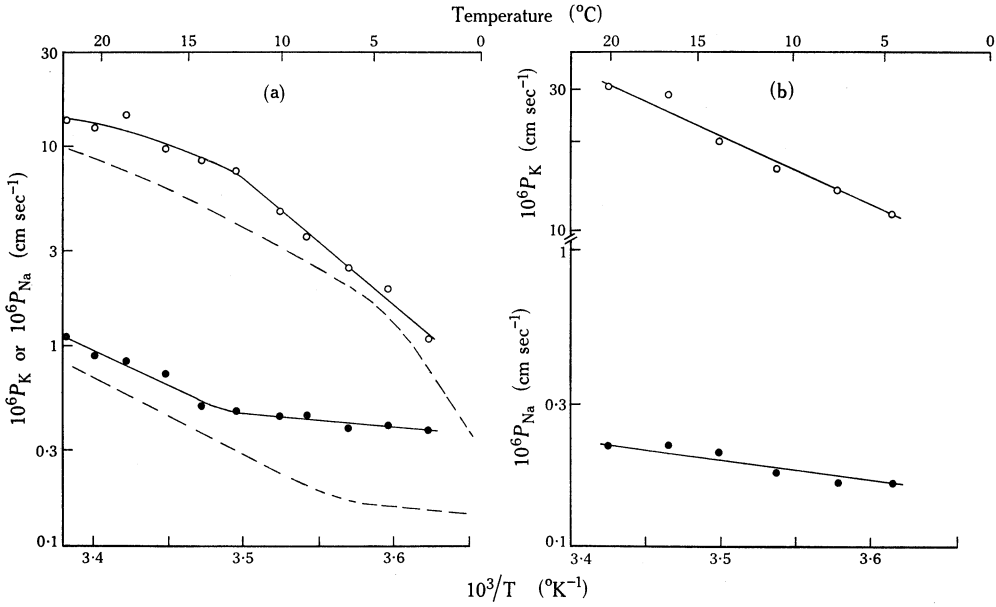

Fig. 7.—(*a*) Permeabilities (P_K, ○; P_{Na}, ●) at the plasmalemma calculated from short-term (———) and long-term (– – – –) temperature changes for *C. corallina*, and plotted on a log scale against the reciprocal of absolute temperature. (*b*) P_K (○) and P_{Na} (●) for the plasmalemma of *G. pulvinata*, calculated from electrical data [Figs. 4(*a*) and 4(*b*)], plotted on a log scale against the reciprocal of the absolute temperature.

from which it can be seen that similar changes in enthalpy of activation seem to occur at a temperature in the region of 6°C, more particularly for K^+. In *G. pulvinata*, P_K is seen in Figure 7(*b*) to decrease monotonically, on a log scale, when plotted against $1/T$, implying a constant enthalpy of activation for diffusion in the temperature range 19–3°C. ΔH_K^* was 10·4 kcal mole^{-1}. P_{Na} decreased much less with temperature, with ΔH_{Na}^* being about 3 kcal mole^{-1}.

(*c*) *Permeabilities Calculated from Influxes*

The results with *G. pulvinata* in which influx was measured as a function of temperature for both sodium and potassium (Fig. 6) may be used to get independent

estimates of P_K, P_{Na} from the following relation between influx and p.d., permeability, and external concentration (see, for example, Dainty 1962):

$$\vec{\phi}_j = \frac{P_j \mathbf{F}\psi_{co}}{\mathbf{R}T} \cdot \frac{c_j^o}{1-\exp(\mathbf{F}\psi_{co}/\mathbf{R}T)}, \tag{4}$$

where $\vec{\phi}_j$ is the influx of ions j (K^+ or Na^+). $1-\exp(\mathbf{F}\psi_{co}/\mathbf{R}T)$ is nearly unity for p.d.'s $\geqslant -75$ mV so we have:

$$P_j = \vec{\phi}_j/[(\mathbf{F}\psi_{co}/\mathbf{R}T)c_j^o]. \tag{5}$$

The result of plotting log $P_{K,Na}$ versus $1/T$, calculated from the data on influxes, is shown in Figure 8. Once again a greater slope is observed for K^+ than Na^+. The slope for K^+ is greater at any temperatures than that of the graph of log P_K (electrical), and a very steep slope appears at temperatures between 0 and 4°C, a region not reached in the electrical measurements.

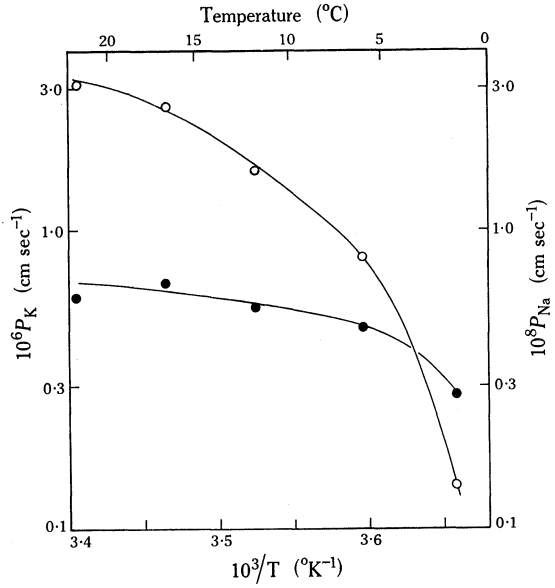

Fig. 8.—P_K (○) and P_{Na} (●) for the plasmalemma of *G. pulvinata* calculated from influxes (Fig. 5), plotted on a log scale against $1/T$.

It will also be noticed that the permeabilities calculated from fluxes are for potassium, one-tenth, and for sodium, about one-quarter of those from electrical data. This sort of discrepancy is frequently found in giant algal cells (MacRobbie 1962; Hope 1963; Williams, Johnstone, and Dainty 1964). In particular, the discrepancy for K^+ is in the same direction as that noted between conductance calculated from the resting fluxes and the plasmalemma electrical resting conductance, and of a similar magnitude (Findlay, Hope, and Williams 1970). Also, α calculated from fluxes is less by a factor of 3–4 than α (electrical) but it is to be noted that estimations of α are very sensitive to small errors in the p.d. observations. For example, in one cell, a depolarization from $-81{\cdot}0$ (K10 ASW) to $-67{\cdot}6$ mV (K20 ASW) yields $\alpha = 0{\cdot}0083$, $C^c = 352$, while in a second cell, the corresponding values are $-81{\cdot}8$, $-66{\cdot}3$; $\alpha = 0{\cdot}0035$, $C^c = 301$.

(*d*) *Interpretation of the Permeability Changes*

The following tabulation summarizes the calculated mean enthalpies of activation for K^+ and Na^+ in the relevant ranges of temperature:

Species	ΔH^*_K (kcal mole^{-1})	ΔH^*_{Na} (kcal mole^{-1})	Temp. Range (°C)	
C. corallina	28	3·5	3–13	Short-term
	10·1	14	13–25	
	46	4	2–7	Long-term
	16·8	16·5	7–22	
G. pulvinata				
Electrical	10·4	3·3	3–20	
Fluxes	14	3·4	5–20	

These data suggest the following explanation of membrane behaviour:

(1) *C. corallina*: The potential energy barriers for permeation of the plasmalemma by K^+ and Na^+ are approximately equal until a critical temperature range is reached, which may correspond to a phase change or partial solidification of the membrane. If the temperature is lowered and kept constant, a drift occurs, over 30–60 min, in which P_{Na} decreases still further and P_K only a little, over the values for 2–3 min after the change in temperature. This corresponds to the shifting of the critical point or range to a lower temperature. Both the changes in slope of log P versus $1/T$, and the change in critical temperature with time might be interpreted as structural changes in the membrane. Wartiovaara (1949) interpreted data on the permeability of *Nitella* to non-electrolytes as indicating that a gradual change in membrane state took place over the temperature range 20–0°C, rather than any sharp phase change. At temperatures below the "critical one", it is noteworthy that the more permeant ion species, K^+, has a much greater potential energy barrier than Na^+. This was observed also by Hogg, Williams, and Johnstone (1968) over the temperature range 2–18°C in *N. translucens* where $\Delta H^*_K = 23$, $\Delta H^*_{Na} = 11$ kcal mole^{-1}.

(2) *G. pulvinata*: The potential energy barriers are fairly constant over a wider temperature range than in *C. corallina*, for both K^+ and Na^+, with the possibility of a phase change at a temperature near zero. Again $\Delta H^*_K > \Delta H^*_{Na}$ although $P_K > P_{Na}$.

It is possible to eliminate two extreme methods of permeation. Firstly, ions do not penetrate through very large pores or slits in which the only friction encountered is with water molecules as in a dilute solution. In this case ΔH^* would be expected to be about $\frac{3}{2}$ $\mathbf{R}T$ per mole or about 0·9 kcal mole^{-1}. Secondly, neither K^+ nor Na^+ are unhydrated ions within the membrane, unless the water of hydration is replaced by another type of solvation at the edge of the membrane with little activation energy for the exchange. Otherwise ΔH^* would be expected to be much higher, the free energy of hydration for K^+ being about 90 kcal mole^{-1}.

For a fuller description of permeation, it is necessary to know the entropies of activation for K^+ and Na^+. Though these were calculated by Hogg, Williams, and

Johnstone (1968) for permeation of K^+ and Na^+ through the plasmalemma of *N. translucens*, their values depend on the absolute values for P_K and P_{Na}, and on assumed values for two other parameters of the membrane.

Theories of ion specificity in cation-selective glasses, which are in some ways similar to cell membranes (Doremus 1967; Eisenman 1967) stipulate that relative permeability depends on several factors, the partition coefficient for each ion between the membrane and solution, and the relative mobilities of the ions within the membrane. It can be shown that $P_j = \beta_j u_j \mathbf{R}T/\delta$, where β_j for ions j is the partition coefficient a_j^m/a_j^o (activity just inside the membrane, over that in the bulk solution), u_j is mobility, and δ the membrane thickness. Separate estimations for the quantities β and u do not exist for biological membranes, nor for ion-exchange resin membranes, so far as we are aware, but extensive data are available for glass membranes (Eisenman 1967). Some measurements have also been made of the effect of temperature on the mobility of Na^+ and Ag^+ in glasses, but not on the partition factors that determine the relative numbers of K^+ and Na^+ on adsorption sites. Hence, at present, we are unable to suggest in which factor the effect of temperature is greater, mobility or partition. Preliminary experiments with a cation-exchange resin membrane have revealed no large difference in the enthalpies of activation between the diffusion coefficients of K^+ and Na^+, ΔH^* being about 3–6 kcal mole^{-1}. The meagre data on ion migration in glass (quoted by Doremus 1967) suggests that the faster ion has the higher temperature coefficient of mobility. In the cell membranes under consideration, it is not necessary that $u_K > u_{Na}$, since the partition factor may outweigh a mobility ratio $u_K/u_{Na} < 1$ as it does in certain glasses (Eisenman 1967) and still yield $P_K > P_{Na}$.

Finally, heterogeneity in the membrane permeation paths should not be ruled out. The above considerations apply to a homogeneous set of common pathways for K^+ and Na^+; much of the data would also be consistent with a model containing many highly specific pathways for K^+ containing moderate potential energy barriers, with the addition of a few relatively open pores for Na^+, in which the potential energy barriers were smaller. These pores would be in the nature of a "leak" for Na^+, and, if variable in number with age, etc. might account for the large variability in sodium permeability, not observed for potassium.

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