IONIC RELATIONS OF CELLS OF *CHARA AUSTRALIS*

VII. THE SEPARATE ELECTRICAL CHARACTERISTICS OF THE PLASMALEMMA AND TONOPLAST

By G. P. FINDLAY* and A. B. HOPE*

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Summary

A study of the electrical properties of the two membranes bounding the cytoplasm in *Chara* cells was made. Three electrodes were employed: a metal probe to pass current, inserted longitudinally into the vacuole, and two glass microelectrodes, to measure potential differences, inserted into the vacuole and cytoplasm.

Across the plasmalemma (outer membrane) the resting potential was −140 to −170 mV relative to solutions containing 0·1 mN potassium. An action potential was accompanied by a fall in the resistance of the plasmalemma to about 300 Ω cm². Increase of external calcium ion concentration caused the peak of the action potential to change in a positive direction. Increase of external chloride concentration by addition of sodium or choline chloride caused the peaks to become more negative. Substitution of chloride by bromide or nitrate had little effect on the peaks.

The tonoplast (inner membrane) had a mean resistance of approximately 1000 Ω cm². The vacuole was at a potential of about +10 mV with respect to the cytoplasm. Ionic activities of potassium and sodium in the cytoplasm probably exceeded those in the vacuole by a factor of 1·5. A stimulus large enough to cause an action potential in the plasmalemma always caused a slow action potential across the tonoplast as well. The tonoplast potential difference usually changed from +10 to +40 or +50 mV, and returned to the resting level in about 20 sec while its resistance fell to a minimum of about 300 Ω cm².

These aspects are discussed in terms of a model involving a linear potential gradient and passive permeabilities to the ions of potassium, sodium, calcium, and chloride. With this model, the transient behaviour of both membranes is more easily described by a transient increase in permeability to chloride ions rather than to calcium ions.

I. INTRODUCTION

Previous papers in this series have described the electrical characteristics of single cells of *Chara australis* in terms of the permeability of the "membrane" to various ions. Equations were developed which related permeabilities and ionic activities to the "membrane potential" and "membrane resistance" (Hope and Walker 1961). These were measured between the vacuole and external medium. Earlier evidence had suggested that most of the potential difference (p.d.) and resistance was attributable to the plasmalemma (Walker 1955, 1960). The action potential was also recorded between the vacuole and medium, and on two lines of evidence was ascribed to a transient increase in permeability of the "membrane" to calcium (Hope 1961; Findlay 1962).

* Plant Physiology Unit, Division of Food Preservation, CSIRO, and School of Biological Sciences, University of Sydney.

Refinements of technique described in this paper have enabled simultaneous measurements to be made of both the plasmalemma and tonoplast transmembrane p.d.'s and resistances, and of their separate behaviour following stimulation producing an action potential. This paper deals also with the possible role of calcium and other bivalent cations, and of chloride, in the action potential. A theoretical analysis is made of the electrical behaviour of both membranes in terms of Goldman's (1943) model. This model assumes a linear potential gradient across the membrane.

II. Material and Methods

The material used was *Chara australis* R.Br. var. *nobilis* A.Br.; it was collected from a field pond and stored in its native pond water in the laboratory for several days. Cells for experiment were separated from a filament of cells and mounted on a Perspex slide, and irrigated with a flowing solution of one of several artificial pond waters (the composition of these is given where necessary in the text).

Cells used were generally 1·1–1·3 mm in diameter and 1·1–1·8 cm in length. Up to three electrodes were inserted into the cell. One, a metallic electrode (50 μ in diameter and 0·6–1·0 cm long) was inserted through the end wall of the cell along the longitudinal axis. A second electrode (a glass microelectrode with a tip diameter of *c.* 10 μ and filled with 1N KCl) was inserted for the duration of the experiment through the longitudinal wall into the vacuole to a depth of 100–200 μ. One or two criteria enabled its location to be known with certainty as the vacuole. A rush of cell contents usually occurred into the glass electrode for a fraction of a second, being stopped by an accretion of crystals and other particles from the vacuole (calcium oxalate?, cf. Walker 1955). This indicated that the tonoplast had been ruptured. Furthermore, with very transparent cells the contact angle of the cytoplasm with the probe could be seen to be about 90° when such a gush had occurred. A temporary stoppage of cytoplasmic streaming accompanied the gush and at the same time an action potential could be recorded between the probe and medium.

Shallow insertions into the flowing cytoplasm were made from time to time with a finer glass microcapillary filled with 3N KCl, the tip diameter being probably less than 2 μ. A favourable insertion was one in which no gush occurred and in which streaming did not temporarily cease. Preliminary experiments were carried out with very young and transparent cells in which, in addition, the end of this probe could be seen to be in the flowing cytoplasm.

Surrounding the cell was a coil of Ag/AgCl wire having a coil diameter of 3 mm; it extended the full length of the cell, but was divided into two sections. The longitudinal metal electrode and the surrounding Ag/AgCl coil constituted the current electrodes, and ensured a uniform radial current flow across cell membranes for a length of about 7 mm. The potential of the vacuole or the cytoplasm was measured with respect to an externally placed probe filled with 3N KCl in 3% agar and of tip diameter 20–30 μ. This probe was situated about 50 μ from the cell surface, adjacent to the inserted microelectrodes.

The Metal Electrode: An alloy wire of composition 75% Pt–25% Ir and of initial diameter 50 μ was electropolished in a saturated solution of NaCN and NaOH (Worbarsht, MacNichol, and Wagner 1960) to a tip diameter of about 5 μ.

The electrode was then insulated with glass, leaving the final 7 mm exposed, in the following way. Glass tubing was drawn out as for ordinary glass microelectrodes. The metal probe was then placed inside the glass tubing, of which the external diameter was about 100 μ, and the glass further drawn out to the diameter of the metal. The softened glass fused to the metal for a length of 2–4 mm. The end of the glass insulation was then fused smoothly to the Pt/Ir electrode with the aid of the microforge filament. Finally the probe was coated with platinum black to prevent electrical polarization.

TABLE 1

POTENTIAL DIFFERENCES ACROSS THE PLASMALEMMA (E_{co}) AND TONOPLAST (E_{vc}) IN THE MEDIA SHOWN

Expt. No.	E_{co} (mV)	E_{vc} (mV)	Concentration of Medium (mN):			
			KCl	NaCl	$CaCl_2$	$SrCl_2$
C81–C87	—	4·4±1·8	0·1	1·0	0·5	
C107	−177	+12	0·1	1·0	0·5	
	−135	+10	1·0	0·1	0·5	
C109	−131	+14	0·1	1·0	0·5	
	−112	+10	1·0	0·1	0·5	
C110	−148	+16	0·1	1·0	0·5	
	−121	+19	1·0	0·1	0·5	
C111	−148	+7	0·1	1·0	0	
	−101	0	1·0	0·1	0	
	−136	+2	0·1	1·0	0	
C113	−152	+17	0·1	1·0	1·0	0
	−155	+15	0·1	1·0	0	1·0
	−156	+20	0·1	1·0	0	3·0

During insertion of the electrodes the cell was held in stocks to prevent its movement. If the metal electrode were well aligned with the long axis of the cell, the cytoplasm continued to stream during the insertion. Occasional small losses of sap were not sufficient to cause lasting damage to the cell since, after a good insertion, the vacuole potential reached its normal value after 20–30 min.

III. RESULTS

(*a*) *Resting Potential*

Simultaneous measurements were made of the potential between the cytoplasm and the external medium, E_{co}, and between the vacuole and cytoplasm, E_{vc}, with the cells in a variety of solutions. Some results are given in Table 1. The mean

p.d. across the tonoplast in a series of experiments with small transparent cells was $+4{\cdot}4 \pm 1{\cdot}8$ (7) mV. (The standard error of the mean is given throughout, with the number of values in parentheses.) In a second series, using cells of the average dimensions given in Section II, E_{vc} was $+11 \pm 1{\cdot}7$ (8) mV; that is, the vacuole was positive with respect to the cytoplasm. This p.d. did not change in any obvious way with changes in the external medium. If it did change, the change was undetectable because of variation in E_{vc} due to other causes (see Section IV). Changes in K_o, the external potassium concentration, led to changes in E_{co}, both in the presence or absence of calcium.

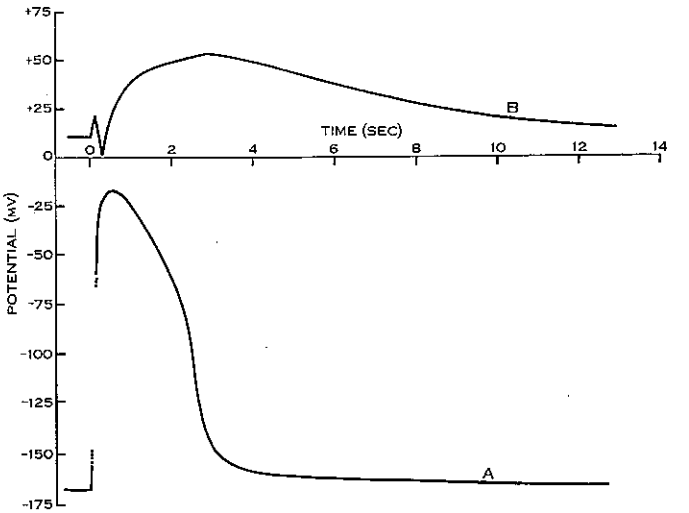

Fig. 1.—Tracings of action potentials across the plasmalemma (*A*) and the tonoplast (*B*), occurring in the same cell following a current pulse between the vacuole and external medium applied at time zero. Part of the initial rise in potential in *A* during the application of the current pulse has been omitted.

(*b*) *Resting Resistance*

The resting resistance of the plasmalemma was $12{\cdot}1 \pm 2{\cdot}5$ kΩ cm^2 in five cells, which is similar to the overall resistance found by Hope and Walker (1961).

The mean resistance of the tonoplast in the small transparent cells mentioned in Section III(*a*) was 960 ± 260 (6) Ω cm^2 and in four older cells was 1100 ± 100 Ω cm^2.

(*c*) *Membrane Time Constant*

The response of the plasmalemma p.d. to a pulse of constant current about 100 msec long was recorded by photographing the trace of a cathode ray tube connected to the electrometer output. The time constant, or time for the p.d. to reach 63% of its final value was, in two cells, 15 and 23 msec. The capacitance of the plasmalemma was calculated from this as described in Section IV.

The response of the tonoplast was much quicker than that of the plasmalemma and the time constant has not been estimated.

(*d*) *The Action Potential*

(i) *With Calcium Present*

When a cell was stimulated by a pulse of current tending to drive positive ions out of the cell and when a threshold was reached, an action potential occurred

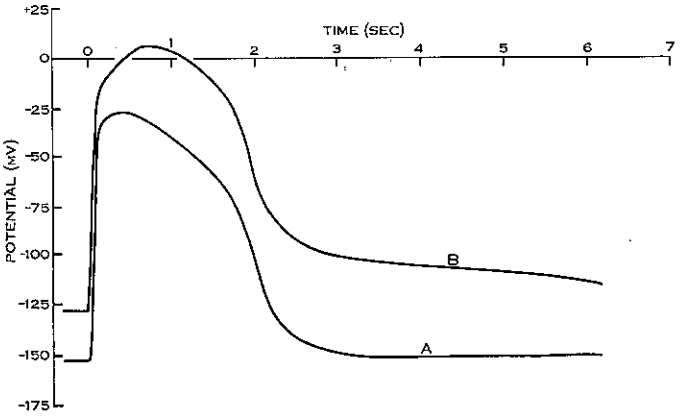

Fig. 2.—Tracings of action potentials across the plasmalemma (*A*) and the plasmalemma and tonoplast together (*B*). The latter measurement was made with an electrode in the vacuole.

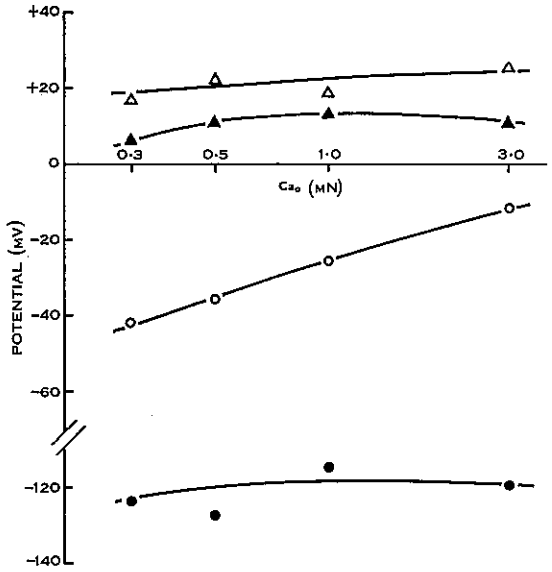

Fig. 3.—Resting potentials across the plasmalemma (●) and tonoplast (▲) and peaks reached during action potentials across the plasmalemma (○) and tonoplast (△), all as a function of external calcium chloride concentration. In addition the media contained 0·1 mN KCl and 1·0 mN NaCl.

across both the plasmalemma and tonoplast. Simultaneous traces of the time course of E_{co} and E_{vc} during this process are shown in Figure 1. The external

medium contained: KCl, 0·1 mN; NaCl, 1·0 mN; and $CaCl_2$, 1·0 mN. The relation between the two action potentials recorded respectively in the vacuole (as in most previous work) and in the cytoplasm, both relative to the outside of the cell, is illustrated in Figure 2. The vacuolar action potential was similar in shape to earlier published records from cells of *C. australis* (cf. Fig. 1 in Hope 1961). It may be seen that the two separate membrane action potentials correspond to the "fast" and "slow" components mentioned by Findlay (1959).

Changes were made in the concentration of calcium ions in the external solution, and the two action potentials recorded. The use of added $MgCl_2$ to keep the total ionic strength constant (Hope 1961) was dispensed with in the experiment illustrated

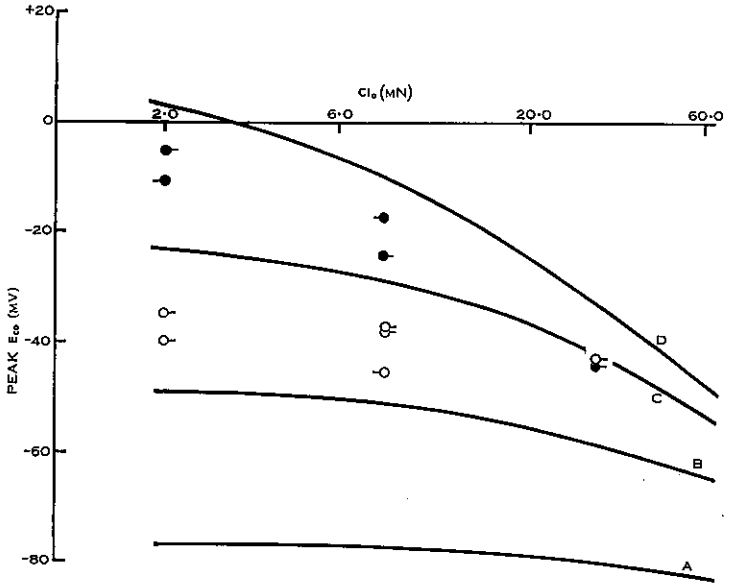

Fig. 4.—Peaks of the action potentials across the plasmalemma as functions of external chloride ion concentration. ● Chloride concentration varied by changing the concentration of tetraethyl ammonium chloride. ○ Chloride concentration changed by altering the proportion of sodium chloride to sodium bromide in a mixture containing a constant concentration of sodium. *A*, *B*, *C*, and *D* are theoretical curves with the parameter $\delta = 0{\cdot}3$, $1{\cdot}0$, $3{\cdot}0$, and $10{\cdot}0$, as explained in Section IV. The bars indicate the direction of increasing or decreasing concentration.

in Figure 3, since it was found with the present batch of cells that magnesium tended to make the membrane refractory when the calcium present was as low as 0·1 or 0·3 mN. In the absence of magnesium, cells were excitable in 0·1 mN calcium.

Changes in external chloride concentration in the presence of a fixed concentration of calcium—usually 1·0 or 3·0 mN—were made in either of two ways. Either choline chloride, sodium chloride, or tetraethyl ammonium chloride was added, or chloride was substituted by bromide or nitrate. Figure 4 shows the effect on the peak potential reached across the plasmalemma in two such experiments. The full lines are theoretical curves explained in Section IV.

(ii) *Solutions without Calcium*

In a medium containing up to 3 mN $MgCl_2$ (+1 mN NaCl, 0·1 mN KCl) it was impossible to initiate an action potential by means of current pulses causing depolarization of up to 130 mV. That is, the cell is refractory in solutions containing magnesium ions, and in the absence of calcium ions. This has been observed previously with these cells (Hope 1961). With *Nitella* (Findlay, unpublished data) action potentials with prolonged periods of depolarization (plateaux) occurred when 1 mN Mg^{2+} replaced external Ca^{2+}.

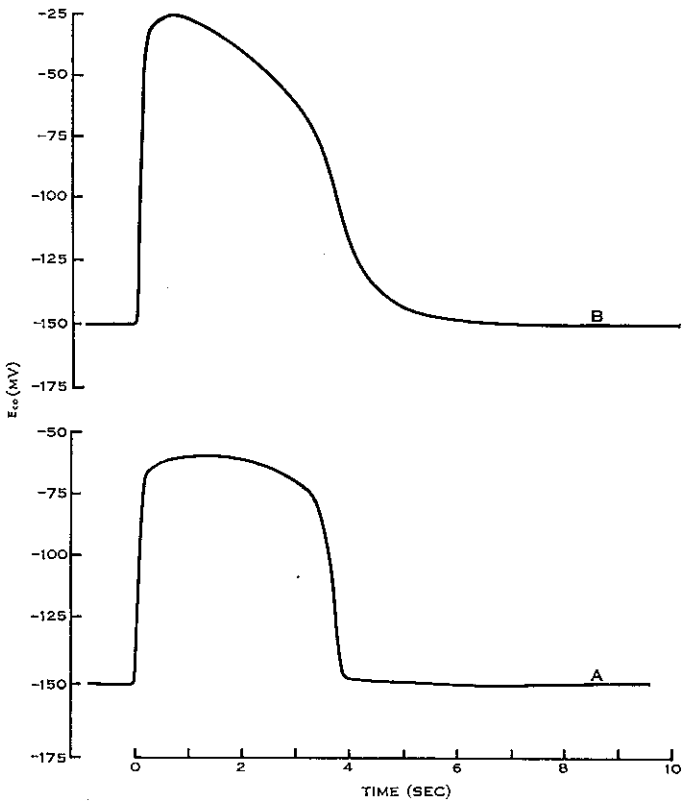

Fig. 5.—Tracings of action potentials across the plasmalemma when strontium chloride (*A*) and calcium chloride (*B*), each at a concentration of 1 mN, was in the external medium.

It was possible to initiate action potentials in *Chara* when strontium chloride replaced calcium chloride. Figure 5, *A* shows such an action potential, which may be compared with one observed in the same cell in the presence of calcium (Fig. 5, *B*). The concentration of strontium or calcium was 1 mN. The action potentials across the tonoplast were similar to that shown in Figure 1, *B* when either strontium or calcium was present.

In these experiments, to ensure that the effective concentration of calcium in the neighbourhood of the plasmalemma was low, the cells were bathed in a flowing solution containing 3 mN $MgCl_2$, in which the membrane was known to be inactive, before treatment with the experimental solution.

The action potential with 3·0 mN NaCl as external solution was similar to that with $SrCl_2$. In some early experiments 1 mN $BaCl_2$ apparently allowed action potentials of the same shape as those in $SrCl_2$ but these did not occur when care

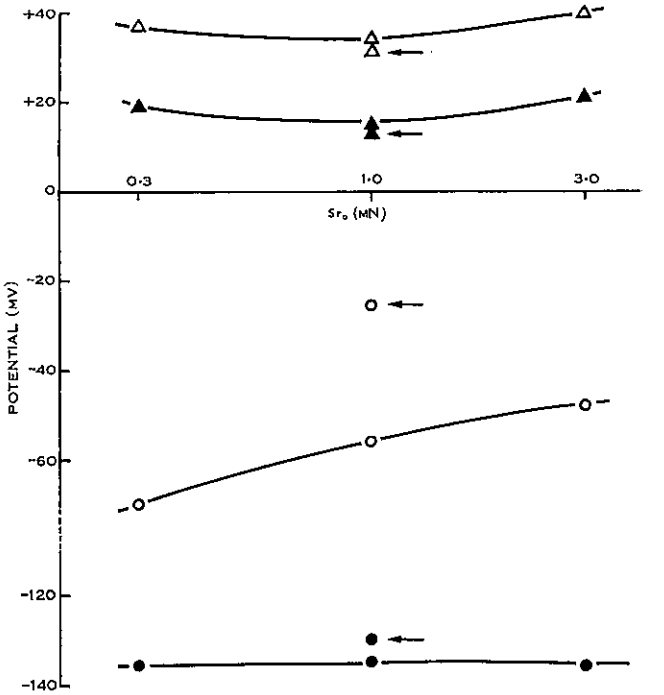

Fig. 6.—Resting potentials across the plasmalemma (●) and tonoplast (▲) and peaks reached during action potentials across the plasmalemma (○) and tonoplast (△), all as a function of external strontium chloride concentration. The arrows indicate corresponding values of the potentials when calcium replaced strontium at 1 mN concentration.

was taken to exchange away cell wall calcium with magnesium. Cells were refractory in solutions of 1 mN Ni^{2+}, Cd^{2+}, and Mn^{2+} ions. Figure 6 shows the peaks of the action potentials as a function of external strontium concentration.

(e) *Changes in Resistance during the Action Potential*

A multivibrator generating square waves of frequency 4–20 pulses/sec was used to pass current through both membranes during the action potential. The changes in potential during this process were estimated from photographs of a cathode ray screen and hence the resistance or conductance calculated. The time

constant of the membranes in the excited state was so short that the response of the potentials to the current was completed during the time of the pulse. In the resting state, however, the plasmalemma capacitance did not charge or discharge completely during a pulse. The resting resistance was therefore measured using long current pulses, manually switched.

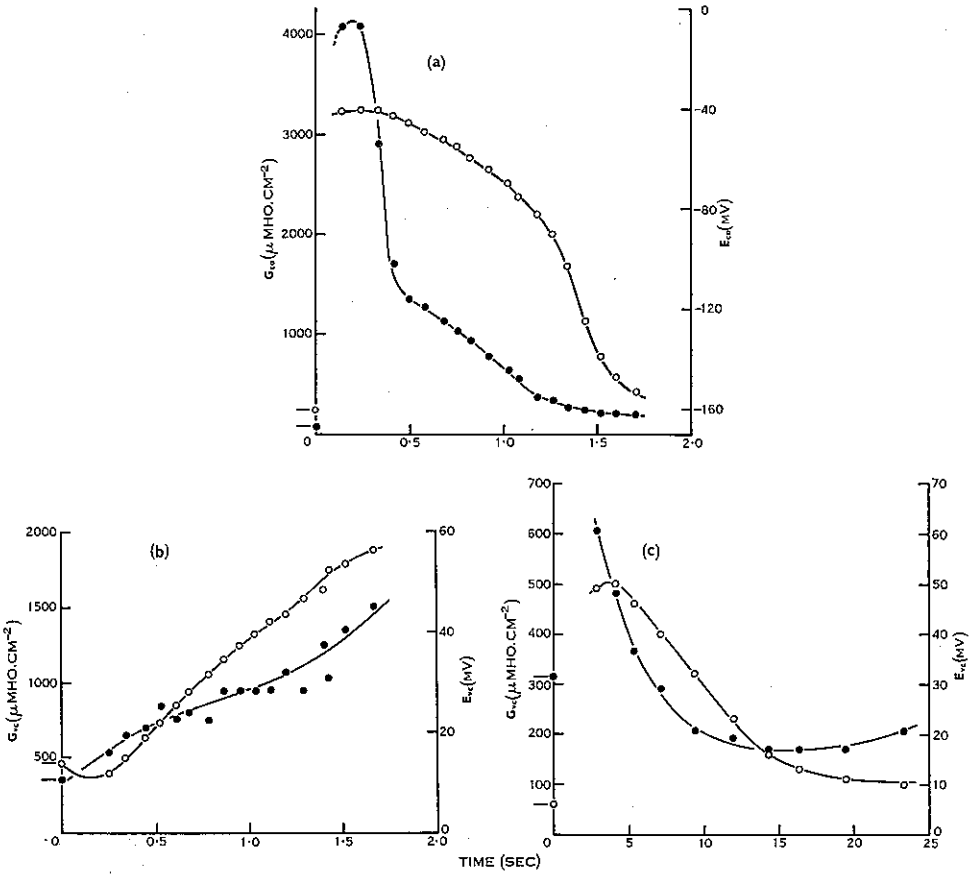

Fig. 7.—Potential difference (○) and conductance (●) plotted against time after stimulation. (*a*) Plasmalemma; (*b*) tonoplast, same cell as (*a*); (*c*) tonoplast in a second cell, over a longer time. The resting levels are indicated by the bars before time zero.

Figure 7 shows the results of two such experiments where the conductance change at each membrane is plotted against time after stimulation, and compared with the corresponding changes in p.d.

IV. Discussion

The separation of overall "cell" potential difference and resistance into components due to the plasmalemma and tonoplast raises questions of interpretation of past results. The new results lead to a clarification of the relation between the phases. The measurements will be considered in order.

(*a*) *Resting Potential*

A small resting p.d. across the tonoplast, in series with that across the plasmalemma but of opposite sign, leads to an underestimate of the magnitude of the plasmalemma p.d. when measurements are made between vacuole and outside. Hope and Walker (1961) used such measurements to estimate two parameters: α (a permeability ratio P_{Na}/P_K), and $C_i = K_i + \alpha . Na_i$.

The effect of an increased E is a negligible alteration in α, but C_i (which should now refer to cytoplasmic ion activities and be termed C_{cyt}) is increased. For example, if the true plasmalemma p.d. is −160 mV, not −150 mV, and $\alpha = 0 \cdot 1$ then C_{cyt} is raised from 74·5 to 110 mN. The value of 74·5 mN should refer to vacuolar ion activity. In fact C_i calculated from overall p.d. measurements was generally found to agree with the measured vacuolar concentrations. Thus the cytoplasmic chemical activities of sodium and potassium exceed those in the vacuole. MacRobbie (1962) observed that the concentration ratio was equal for sodium and potassium in *Nitella translucens* and was about 1·5.

From the p.d. of +10 mV, vacuole positive, observed in the present experiments, an activity ratio can also be estimated from the equation:

$$E_{vc}(\text{mV}) = 58 \cdot 5 \log_{10} K_{cyt}/K_{vac} = 58 \cdot 5 \log_{10} Na_{cyt}/Na_{vac},$$

giving a value for the activity ratio of 1·5. MacRobbie (1962) had in fact predicted this p.d. from the observed activity ratios in *N. translucens*.

In the present experiments the presence of calcium had the effect of increasing α from about 0·1 to 0·2–0·4 in the simple model of passive membrane permeability to sodium and potassium (chloride and calcium permeabilities are neglected), for which, in the resting state:

$$E_{co}(\text{mV}) = 58 \cdot 5 \log_{10}(K_o + \alpha Na_o)/(K_{cyt} + \alpha Na_{cyt}) \qquad (1)^*$$

The value of the potential difference measured with one probe in the cytoplasm and one outside is more indefinite than the p.d. measured between vacuole and medium. This is due to the nature of the cytoplasmic phase, which is not a simple electrolyte. In the vacuole, diffusion potentials between the sap and a glass probe of 5 μ diameter filled with 1N KCl can be assumed negligible. The criteria for satisfactory measurements were a small p.d. between the cytoplasm probe and the reference probe when both were outside the cell, and concordant results with repeated insertions of the probe into the cytoplasm.

(*b*) *Resting Resistance*

The observed resting resistance between the vacuole and cytoplasm of about 1000 Ω cm^2 in series with about 10,000 Ω cm^2 does not lead to great error in interpreting an overall resistance as the plasmalemma resistance.

The ratio of these membrane conductances is roughly equal to the ratio of calculated potassium fluxes (Hope 1963).

* Equation (1) involves no arbitrary assumptions, but to develop later equations a linear potential gradient is assumed, as usual, in the membrane.

(c) *Membrane Time Constants*

The time constant of a circuit containing a parallel resistance R and capacitance C is defined as $R.C$ and refers to the time needed to charge the capacitance to 63% of its final potential with a constant current, or to discharge the capacitor through the parallel resistance to 37% of its initial charge. In the present experiments the mean time constant of the plasmalemma was about 20 msec. That of the tonoplast was much less and could not be measured accurately. Knowing $R.C$, and R from the resting resistance, C is estimated to be 1–2 μF cm^{-2} in this membrane. This is close to often-quoted values of the capacitance of the "membrane" (Cole and Curtis 1938), and recent measurements by Oda (1962) in *Chara braunii* of 2·3 μF cm^{-2}. The effect of the tonoplast on overall measurements of time constants is small, since its time constant is much shorter.

(d) *Action Potential across the Plasmalemma*

The action potential recorded between the vacuole and the external medium is now shown to consist of two components with quite different time courses. Findlay (1959) stated that the shape of the action potential in *Nitella* was the same whether the measuring probe was in the vacuole or cytoplasm. These measurements were made by letting the cytoplasm flow over probes initially inserted into the vacuole. On the cytoplasm covering the probe the tonoplast may have become locally depolarized by KCl from the probe, so that the probe followed the change in potential of the vacuole. In the present experiments electrodes were inserted directly and a short distance into the cytoplasm. With this technique it is consistently found that the action potential across the tonoplast is of simple shape, like that of a nerve cell, and sometimes with the "undershoot" observed in nerve.

With the bivalent ions tested, apart from calcium, action potentials occurred only in solutions of strontium. Sodium ions, in higher concentration than calcium, also initiated an action potential, the threshold being at a higher potential.

The effect of the tonoplast slow component in series with the action potential across the plasmalemma is usually to make the peak of the action potential recorded in the vacuole more positive than the peak of the fast component alone (Fig. 2). This must be taken into account in interpreting the change in peak potential as a function of external ion concentration.

As regards the mechanism of the action potential, Mullins (1962) has advanced further evidence that a transient increase in membrane permeability to chloride causes the action potential, by showing that there is an extra efflux of chloride ions associated with the action potential in *Nitella clavata*, which is an ecorticate species. Mullins (1962) has questioned the suggestion that the transient change in permeability is to calcium ions (Hope 1961; Findlay 1962) and interprets the effect of calcium ions (*a*) in leading to a large transient current during a voltage clamp, and (*b*) in changing the peak potential, as "activation" of the chloride permeability system by calcium ions.

It is now proposed to examine the way in which the peak of the action potential would be expected to change with external ion activities, under the two different assumptions.

(i) *Transient Permeability to Calcium*

Following a stimulus the permeability of the plasmalemma to calcium is supposed to rise from a low value, compared with P_K and P_{Na}, to a peak value $P_{Ca} = \beta . P_K$, the other permeabilities remaining constant. Then it can be shown that, at 21°C,

$$E_{peak}(mV) = 58 \cdot 5 \log_{10} \frac{[(C_{cyt}-C_o)^2+4(C_o+2\beta Ca_o)(C_{cyt}+2\beta Ca_{cyt})]^{\frac{1}{2}}-(C_{cyt}-C_o)}{2(C_{cyt}+2\beta Ca_{cyt})}. \quad (2)$$

Equation (2) reduces to equation (1) if β is made zero. Of the quantities in equation (2), C_{cyt} and C_o may be estimated from the resting potential. It will be shown elsewhere

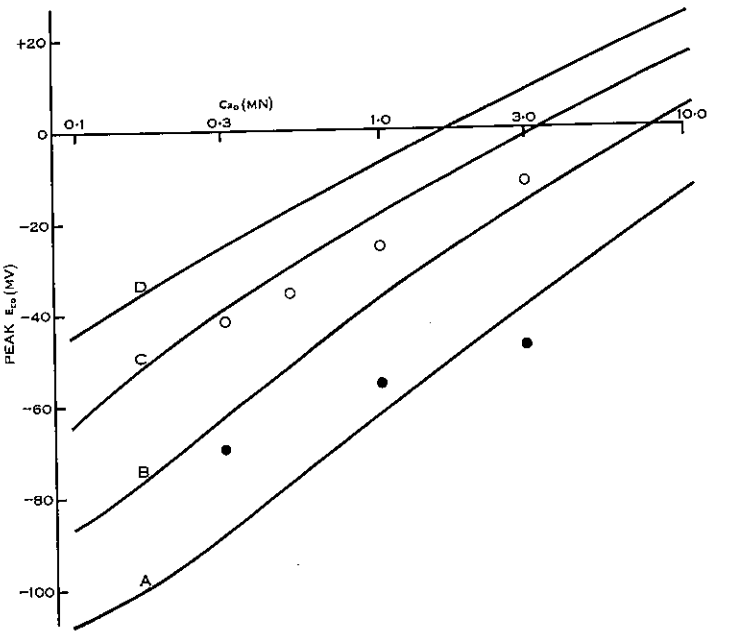

Fig. 8.—Theoretical relationships between the peaks of the action potential across the plasmalemma and external calcium concentration, calculated as described in the text. In *A*, *B*, *C*, and *D* the parameter β ($= P_{Ca}/P_K$) was made 3, 10, 30, and 100, respectively. The points are observed peaks in calcium solutions from the experiment of Figure 3 (○) and in strontium from Figure 6 (●).

that Ca_{cyt}, calcium activity in the cytoplasm, does not exceed about 1 mN, on the assumption that calcium carries the transient current observed during a voltage clamp (Findlay and Hope 1964). Here it will be taken as 1 mN, $\alpha = 0 \cdot 3$, and $C_{cyt} = 70$ mN, for the cell of Figure 3. Then, with β given the values 3, 10, 30, and 100, E_{peak} varies with Ca_o as shown by the full lines in Figure 8. The symbols are the values of peaks taken from Figures 3 and 6 in this paper. It is seen that there is some agreement between predicted and observed peaks in their general level if β is 10–30 but that the increase in peak potential with increase in Ca_o is less

than expected. The peaks are not very dependent on Ca_{cyt} except when β is 30–100. Two aspects of the graphs are worth emphasizing:

Firstly, the slope of E_{peak} *v.* $\log_{10}Ca_o$ approaches +29 mV per tenfold increase in Ca_o at higher values of β and Ca_o, but may, however, be 40–50 mV in dilute solutions of calcium or when β is small. This is observed if the vacuole potential is recorded (Hope 1961; Findlay 1962), but not if the cytoplasm potential is recorded. Across the plasmalemma alone the response to a tenfold increase in Ca_o is 20–30 mV.

Secondly, the peak potential does not reach the electrochemical equilibrium potential for calcium. The membrane potential would change until the inward current of calcium was exactly balanced by an outward current of sodium and potassium. Consequently Ca_{cyt} would not be the value of Ca_o at which the peak potential is zero, as suggested by Hope (1961).

Similar remarks apply to action potentials in strontium where a permeability ratio γ, equal to P_{Sr}/P_K of about 3, is appropriate as seen from Figure 8. Strontium inside the cell is assumed to be very low in activity.

The resting potential is not varied greatly by varying the external calcium (Fig. 3), consistent with the assumption of negligible resting permeability to calcium.

(ii) *Transient Permeability to Chloride*

The equation connecting peak potentials and external chloride activity is:

$$E_{peak}(mV) = 58{\cdot}5 \log_{10}[(C_o+\delta.Cl_{cyt})/(C_{cyt}+\delta.Cl_o)], \quad (3)$$

where $\delta = P_{Cl}/P_K$ in the excited state of the membrane. P_{Cl} is assumed negligible in the resting state. Putting $\delta = 0{\cdot}3$, 1, 3, and 10, in turn, and $Cl_{cyt} = 10$ mN (for reasons which will appear later), the peaks should now reach the values shown in Figure 4 at the external chloride activities shown. The change in potential is now in an opposite direction to that in the theory involving calcium. Only as the term $\delta.Cl_o$ becomes comparable with and greater than C_{cyt} does the peak change greatly with Cl_o. Consequently, as Mullins (1962) has pointed out, experiments such as the one reported by Hope (1961, Fig. 5) are not conclusive, since finding little change in the peaks over a range of Cl_o of 1·6–11·6 mN is not incompatible with the chloride hypothesis.

In the present experiments (Fig. 4) Cl_o was varied up to about 40 mN both by additions of sodium chloride, choline chloride, or tetraethylammonium chloride, and by substitution of bromide or nitrate for chloride, keeping total anion and cation constant in concentration. In all experiments calcium was present at a constant 1–3 mN. When Cl_o is changed by substitution of chloride rather than by addition or subtraction, the peak potential does not change significantly. In the "addition experiment" the peaks changed with Cl_o, following equation (3), with δ between 3 and 10. An alternative explanation is that addition of extra cation reduces P_{Ca} and induces a more negative peak (cf. Fig. 8).

It is clear that these considerations do not allow a choice to be made between the alternative hypotheses. However, calcium is necessary outside the cell to initiate an action potential, strontium being a possible substitute, or sodium at higher concentration.

An interesting aspect of the action potential is the associated sudden stoppage of cytoplasmic streaming which occurs at the time of the peak. It could be supposed that an influx of calcium ions in exchange for potassium leads to the formation of sufficient cross-links between the streaming and stationary components in the cytoplasm to gel it. Subsequently chelation or an active calcium efflux pump could reduce the cytoplasmic activity of calcium (or strontium), and streaming recommence. An explanation in terms of the chloride mechanism is not obvious.

(iii) *Change in Resistance*

The observed minimum resistance or maximum conductance reached during the action potential [Section III(*e*)] can be compared with that expected from the peak permeabilities by means of the following analysis. Using the usual model of a membrane with linear potential gradient it may be shown that the membrane conductance due to ions of species j has the following relation to the potential:

$$G_j = \frac{zF^2P_j}{RT} \cdot \frac{{}_ja_o[1-(1-zEF/RT)\exp zEF/RT]+{}_ja_{cyt}[\exp zEF/RT-(1+zEF/RT)]}{(1-\exp zEF/RT)^2}, \tag{4}$$

where ${}_ja_o$, ${}_ja_{cyt}$ are the ionic activities outside and in the cytoplasm, respectively, and z the algebraic valency. Using values of P_j and E appropriate to the peak of the action potential,

$$G_{\text{total}} = G_{\text{K+Na}}+G_{\text{Ca}}, \text{ or } G_{\text{total}} = G_{\text{K+Na}}+G_{\text{Cl}}$$

may be calculated. P_{K} may be calculated from the observed resting resistance (Hope and Walker 1961):

$$r_o = \frac{RT}{F^2}\left[\frac{(1/C_o)-(1/C_{cyt})}{\ln(C_{cyt}/C_o)}\right]. \tag{5}$$

No further new parameters are involved since $P_{\text{Ca}} = \beta.P_{\text{K}}$ ($P_{\text{Cl}} = \delta.P_{\text{K}}$) and C_o, C_{cyt} have already been estimated. For example, in one experiment (C149), $r_o = 15$ kΩ cm², $E_{co} = -145$ mV, $E_{\text{peak}} = -8$ mV, and the following estimates were obtained using equation (4): $G_{\text{K+Na}} = 1510$ μmho.cm^{-2}, $G_{\text{Ca}} = 8350$ ($\beta = 150$, $\text{Ca}_{cyt} = 1$ mN), and $G_{\text{Cl}} = 1790$ ($\delta = 10$, $\text{Cl}_{cyt} = 10$ mN). Hence, assuming an increase in P_{Ca}

$$r_{\text{min.}} = 1/(G_{\text{K+Na}}+G_{\text{Ca}}) = 100 \ \Omega \text{ cm}^2,$$

and for an increase in P_{Cl},

$$r_{\text{min.}} = 1/(G_{\text{K+Na}}+G_{\text{Cl}}) = 300 \ \Omega \text{ cm}^2.$$

The observed minimum resistance was about 300 Ω cm², but it has not been possible to obtain much accuracy in these experiments.

The effect of the tonoplast resistance, which has usually not fallen to its minimum at the time of the minimum plasmalemma resistance, is to yield a much higher overall minimum resistance if the measurement is made between the vacuole and medium. Thus the value obtained by Fujita (1962) for *Nitella* sp. is probably an overestimate.

(e) *Action Potential across the Tonoplast*

A transient change in potential difference, which by analogy with the plasmalemma may be termed an action potential, occurs across the tonoplast. The condition necessary for this appears to be that an action potential must be initiated across the plasmalemma. The tonoplast action potential is slower and the p.d. change of smaller magnitude than with the plasmalemma (Fig. 1). The resistance of the tonoplast decreases and reaches a minimum near the peak (Fig. 7). This suggests, by analogy with the plasmalemma, that the action potential is due to a transient change in tonoplast permeability to some ion which is not in electrochemical equilibrium in the resting state. Sodium and potassium apparently are in equilibrium (MacRobbie 1962; this paper). On the other hand, calcium in the vacuole is at a much lower electrochemical potential than in the medium, while chloride is much higher (Hope and Walker 1960). The cytoplasmic activities are unknown, but by using the values already employed in Section IV(*d*) it is possible to see whether analogous permeability changes to calcium or chloride could possibly account for the observed peak potentials and conductances.

(i) *Calcium*

There are difficulties in attempting to describe in terms of the calcium hypothesis what happens at the tonoplast. If the cytoplasm contains an activity of about 1 mN calcium then, for the vacuole to go further positive with respect to the cytoplasm following an increase in P_{Ca}, the calcium activity in the vacuole must be very much lower. For example if Ca_{vac} was 0·01 mN the peak of the tonoplast action potential would be about +22 mV (taking β as 150), at which the resistance would have fallen to 680 Ω cm² from 1000. Peaks of +50 mV and minimum resistance of 300 Ω cm² are often observed, which could be attained only if β were extremely large. In any case the measured concentration of calcium in centrifuged (crystal-free) vacuolar sap was 0·3–0·4 mN in these cells.

(ii) *Chloride*

Using an equation analogous to (3) and values for δ, C_{cyt}, Cl_{cyt}, C_{vac}, and Cl_{vac} of 6, 123, 10, 82, and 150, respectively, we have for the tonoplast potential:

$$E_{peak} = 58{\cdot}5 \log_{10} (C_{cyt}+\delta\ Cl_{vac})/(C_{vac}+\delta\ Cl_{cyt})$$
$$= 50{\cdot}0 \text{ mV}.$$

This is close to some of the observed values, which is satisfactory considering we have introduced no new parameters. Using again the same parameters together with potassium permeability, which for a tonoplast resting resistance of 1000 Ω cm² is about 2×10^{-6} cm.sec^{-1} [from equation (5)], it follows that the resistance should fall from 1000 to 330 Ω cm². Once again, this is a plausible value.

(f) *Conclusions*

The revised picture obtained by the present separation of "cell membrane" electrical properties into those attributable to the plasmalemma and the tonoplast is similar to that proposed by Hope and Walker (1961) for the resting plasmalemma.

That is, under the conditions stated the plasmalemma behaves as if passively permeable to sodium and potassium.

The possibility of active chloride or sodium fluxes or of passive chloride fluxes affecting the resting potential and resistance is being re-examined following the remarks by Briggs (1962). While there are conditions in which the plasmalemma p.d. is insensitive to potassium concentration, in the present experiments it was sensitive (Table 1). It was possible to estimate that $P_K \simeq 10^{-5}$, and $P_{Na} \simeq 1\text{–}2 \times 10^{-6}$ cm.sec^{-1}. The cytoplasm activities (mN) were probably K 120, Na 30; and those in the vacuole K 80, Na 20, on the average.

An analysis of the electrical changes in each membrane during excitation (the action potential), together with Mullins' (1962) new evidence has thrown doubt on the role of calcium. A transient increase in chloride permeability to about 10^{-4} cm.sec^{-1} appears to account for most of the facts, together with an average activity of chloride in the cytoplasm equal to 10 mN. A transient calcium permeability of about 10^{-3} cm.sec^{-1} would equally account for some of the phenomena. These doubts will not disappear until there are data on cytoplasmic activities and measurements of chloride and calcium fluxes during activity in these cells.

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