IONIC RELATIONS OF CELLS OF *CHARA AUSTRALIS*

IX. ANALYSIS OF TRANSIENT MEMBRANE CURRENTS

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[*Manuscript received December* 2, 1963]

Summary

The effect of divalent cations and of high chloride ion concentration on voltage-clamp currents in the plasmalemma of *C. australis* was investigated. Either calcium or strontium ions were necessary in the medium for a transient current to appear during a voltage clamp. The transient current with strontium was about 20% of that with calcium present. Barium, cadmium, magnesium, manganous, or nickelous ions were unable to replace calcium in this function.

In media containing a chloride concentration of 30 mN, the transient current reversed in sign if the membrane was clamped at potentials more positive than about −50 mV.

An analysis of the peak transient current as a function of membrane potential was made with the assumptions that a negative transient current corresponded to (*a*) an inward current of calcium ions, or (*b*) an outward current of chloride ions. The observations were fairly well fitted by the theory involving chloride ion current if it was assumed that the peak permeability to chloride was of the order of 10^{-4} cm sec^{-1} and that the chloride activity in the cytoplasm was 1–10 mN.

It was concluded that calcium ions mediate an increase in the permeability of the plasmalemma not to chloride specifically but to anions such as Cl^-, Br^-, and NO_3^-, following a suitable stimulus. The same peak anion permeabilities were reached and the time courses of the permeability changes were similar during action potentials and during voltage clamps.

I. INTRODUCTION

In Part VII of this series (Findlay and Hope 1964) the action potential in *Chara australis* was described in terms of potential changes occurring across the plasmalemma and tonoplast. A quantitative description was sought in terms of transient changes in membrane permeability towards calcium or chloride ions. Both potential difference and resistance changes in each membrane associated with the action potential were almost equally well fitted by equations employing large peak permeabilities to calcium or chloride. It was thus not possible on this evidence alone to decide between the two possibilities.

Findlay (1962) showed by means of a voltage-clamp technique that large transient currents may flow across the plasmalemma when it is depolarized above a threshold level, in a *Nitella* sp. By improvements in technique (Findlay 1964) the relations between transient current and time at a clamped potential, and between peak current and potential, have been determined for the plasmalemma alone, whereas earlier results related to the two cytoplasmic membranes in series.

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The present paper is concerned with the effects on the clamp currents of replacing calcium with other divalent ions and of large increases in external chloride concentration.

A theoretical analysis analogous to that made of the action potential (Findlay and Hope 1964) is attempted of the clamp currents across the plasmalemma.

II. Materials and Methods

These have been described previously (Findlay 1964; Findlay and Hope 1964).

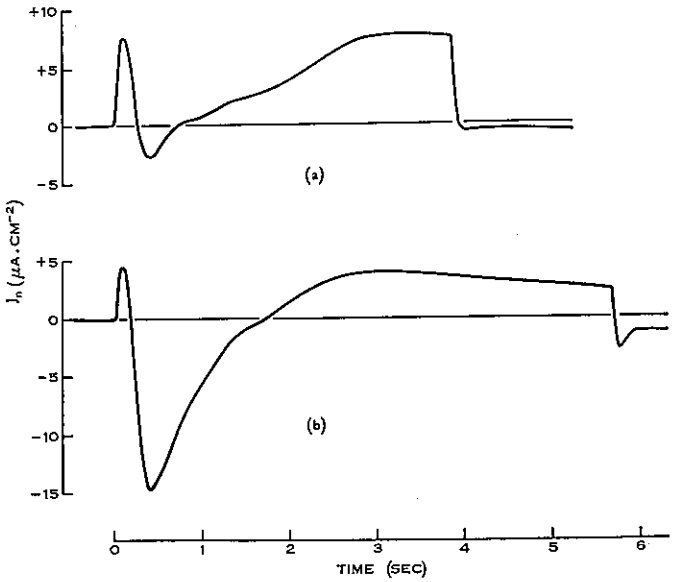

Fig. 1.—Comparison, for voltage clamps of the cytoplasmic potential at −50 mV, between the membrane current (*a*) with the cell in a solution containing 1·0 mN $SrCl_2$, 0·1 mN KCl, and 1·0 mN NaCl; and (*b*) with 1·0 mN $CaCl_2$ replacing 1·0 mN $SrCl_2$.

III. Results

(*a*) *Clamp Currents—Cells in Strontium Solutions*

The current flow during a voltage clamp of the plasmalemma potential difference at −50 mV and with a solution 1 mN $SrCl_2$, 1 mN NaCl, and 0·1mN KCl was compared with that in the standard artificial pond water, containing 1 mN $CaCl_2$ instead of $SrCl_2$. Calcium was exchanged away from the outside of the protoplast with the aid of $MgCl_2$. Figures 1(*a*) and 1(*b*) show a comparison of this sort. The peak net transient current, J'_n, was always less with strontium than calcium. Figure 2 shows J'_n (solid circles) and $J_{n(5)}$ (open circles) plotted against E_{co}. The points were obtained from quick "scans" of the membrane potential as described previously (Findlay 1964). The continuous lines are theoretical relations derived as explained in Section IV.

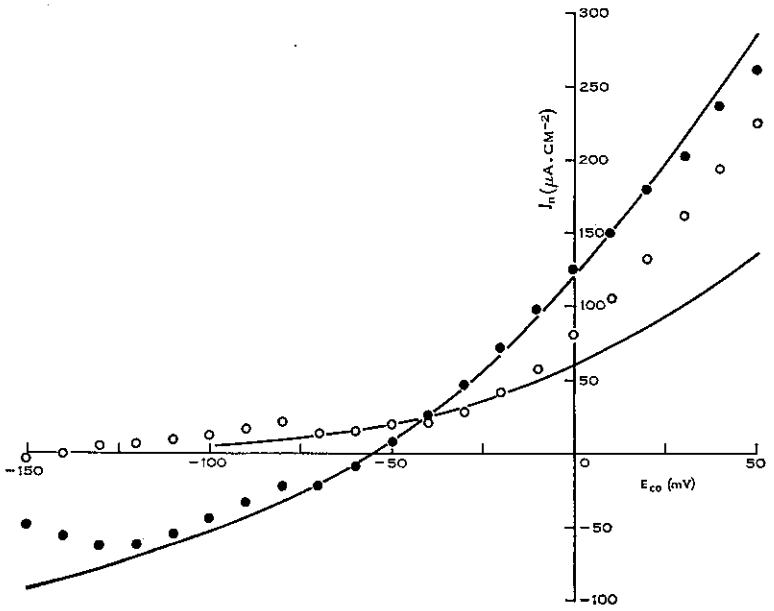

Fig. 2.—Net peak transient current, J'_n (●), and the membrane current 5 sec after the start of the clamp, $J_{n(5)}$ (○), from a cell with external medium 1·0 mN $SrCl_2$, 0·1 mN KCl, and 1·0 mN NaCl. The points were obtained from scans of the cytoplasmic potential. The curves are theoretical relationships obtained by procedures described in the text.

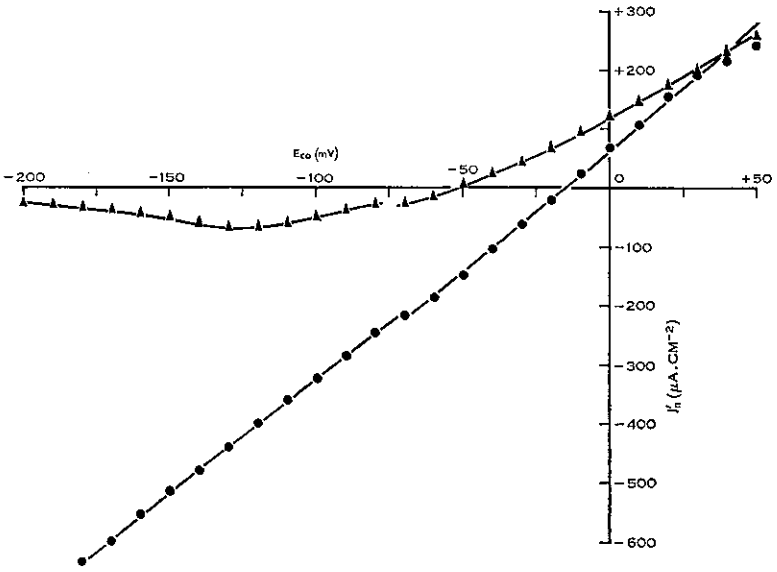

Fig. 3.—Net peak transient current, J'_n, as a function of E_{co} with external solution containing 1·0 mN $SrCl_2$, 1·0 mN NaCl, and 0·1 mN KCl (▲); and 1·0 mN $CaCl_2$ replacing 1·0 mN $SrCl_2$ (●).

Figure 3 shows a comparison of J_n' for the same cell first in $SrCl_2$ then in $CaCl_2$, each 1 mN. The scanning method was used to obtain the points; the discontinuity at about −85 mV is due to the fact that separate scans were made at 10-min intervals, firstly from −70 to +50 mV, then from −80 to −200 mV. Perfect overlap is obtained only if the scans were made at equal times after clamping. The continuous lines have no theoretical significance in Figure 3.

The transient current disappeared in the absence of strontium and varied with the external concentration of strontium (cf. calcium: Figure 10 in Findlay 1964).

A change in the sign of the slope in the transient current curve (such as at about −120 mV in Figure 3) was generally noted when strontium replaced calcium.

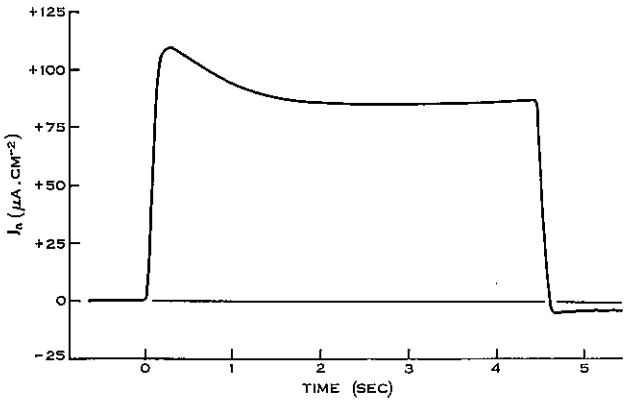

Fig. 4.—Membrane current during a voltage clamp of the cytoplasmic potential at −49 mV, with the cell in a solution containing no divalent ions.

(b) *Clamp Currents in Solutions of other Divalent Ions*

After treating cells with solutions of 3 mN $MgCl_2$ for about 15 min the transient current (designated J_t') disappeared, at whatever potential the membrane was clamped, if media containing barium, cadmium, magnesium, manganous, or nickelous ions were used.

Figure 4 shows the current during a clamp at −49 mV, in the absence of divalent cations.

(c) *Clamp Currents—Cells in Solutions of High and Low Chloride Concentration*

Keeping calcium present at a concentration of 1 mN, the relation between clamp current and potential was examined in solutions containing between 2·1 and 30·1 mN Cl^-. The chloride concentration was increased by an addition of tetraethylammonium chloride, 2-amino-2-(hydroxymethyl)-1,3-propanediol chloride (Tris chloride), or choline chloride.

Figure 5 is a composite of tracings made of the clamp currents across the plasmalemma when it was clamped at the sequence of levels shown, in a medium containing 30·1 mN Cl^-. The transient current clearly reversed at a potential of approximately −50 mV.

Figure 6 shows the clamp currents when three different concentrations of chloride were employed. The solid and dotted lines are again theoretical expectations based on a certain peak transient permeability to chloride, as explained below. In a further single experiment the clamp currents were found to be almost identical when media containing 18 mN NO_3^- and 2·1 mN Cl^- or 20·1 mN Cl^- and zero NO_3^-, were used.

The absence of calcium ions reduced the transient current to a low value, even when the external chloride concentration was high (20–30 mN).

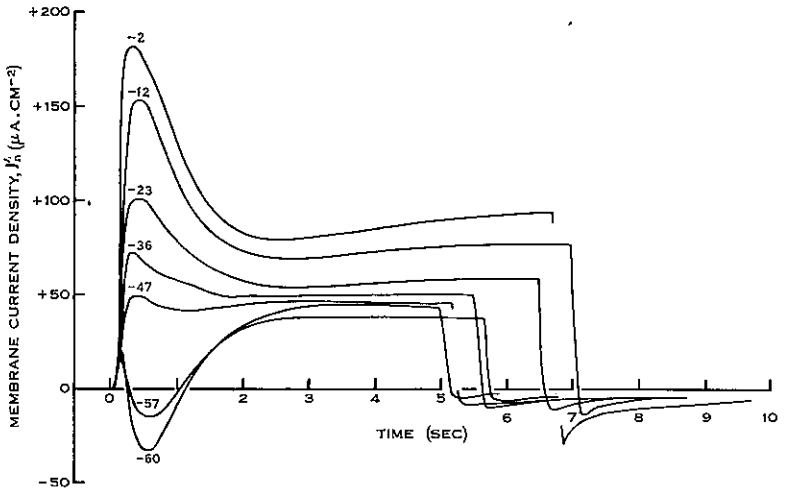

Fig. 5.—Superposition of a number of tracings of the membrane current during voltage clamps of the cytoplasmic potential at several levels. The cell was bathed in a medium containing 1·0 mN Ca^{2+}, 1·0 mN Na^+, 0·1 mN K^+, 28 mN tetraethylammonium chloride, and 30·1 mN Cl^-. The transient current J_n' shows a reversal at a cytoplasmic potential of about −50 mV.

(*d*) *Clamp Currents before and after the Transient Current*

Figure 4 [and Fig. 5(*a*) in Findlay 1964] shows that when the transient current is not appreciable the initial net current, $J_{n(0)}$, decreases to a steady value, $J_{n(5)}$, at, say, 5 sec after the potential has been clamped at the new value. This is obscured in Figure 1 by the initiation of a large transient current about 0·1 sec after the clamp has started. The relation between $J_{n(0)}$ and $J_{n(5)}$ was examined in a number of experiments by means of "scans" from the resting potential in a depolarizing direction. In the experiment of Figure 7 a scan from −150 to +30 mV was made in a short time before the transient current could be initiated. Then, 5 sec later, a further scan was made. The continuous lines are theoretical relations between J_n and E_{co}.

IV. Discussion

(*a*) *Clamp Currents before and after the Transient Current*

It has been shown previously (Hope and Walker 1961; Findlay and Hope 1964) that from measurements of the resting potential and resting resistance of the plasma-

lemma several parameters can be calculated, viz. P_K, P_{Na}, and $K_c+(P_{Na}/P_K)Na_c$, where K_c and Na_c are the potassium and sodium activities in the cytoplasm. Hence

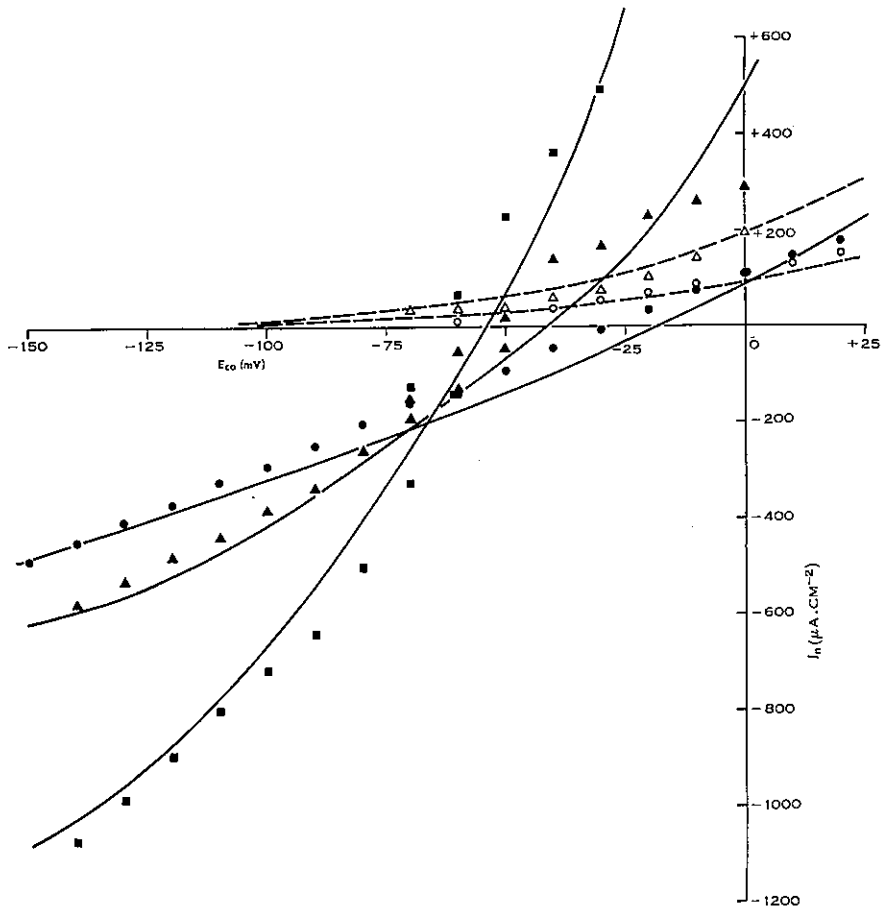

Fig. 6.—Clamp current when three different concentrations of external chloride were employed. J'_n : 2·1 mN Cl^- (●), 8·1 mN Cl^- (▲), 30·1 mN Cl^- (■); $J_{n(5)}$: 2·1 mN Cl^- (○), 8·1 mN Cl^- (△). The chloride concentration was increased by the addition of 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) chloride. The solid and dotted lines are theoretical curves and are explained in the text.

it should be possible to predict the net current before or after the transient from the equation:

$$J_n = J_K+J_{Na}$$

$$= \frac{E_{co}F^2}{RT} \cdot \frac{P_K K_o+P_{Na}Na_o-(P_K K_c+P_{Na}Na_c)\exp(E_{co}F/RT)}{1-\exp(E_{co}F/RT)}, \qquad (1)$$

(a positive current being equivalent to an outward cation current) provided the permeabilities stay constant when the membrane potential is far from the resting value.

Equation (1) is derived from the Goldman assumption of a linear potential gradient in the membrane (Briggs, Hope, and Robertson 1961). For example, in cells C93 and C95 from which some values of $J_{n(0)}$ and $J_{n(5)}$ are plotted in Figure 7, $P_K = 9 \times 10^{-6}$, $P_{Na} = 2{\cdot}6 \times 10^{-6}$ cm sec^{-1}, and $K_c + (P_{Na}/P_K)Na_c = 140$ mN, at the resting potential of -150 mV. Equation (1) yields the continuous line A in Figure 7 as the expected current needed to depolarize the membrane to the potentials shown. The curve B is the relation between J_n and E_{co} if P_K is reduced to 4×10^{-6} and the other parameters remain constant.

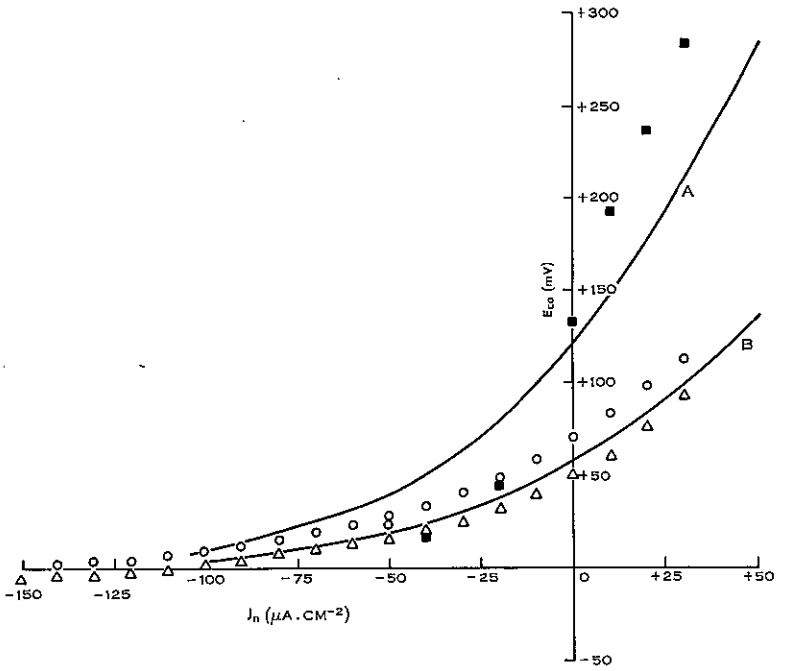

Fig. 7.—Initial net current, $J_{n(0)}$ (■), from cell C95 and the net current 5 sec after the start of the clamp, $J_{n(5)}$ (○, △), as functions of E_{co}. The latter are from two cells, C93 and C95, respectively. The continuous lines are theoretical curves as explained in the text.

It is seen that the points for $J_{n(5)}$ from the two experiments plotted in Figure 7 fit the latter relationship very well. The initial current $J_{n(0)}$ is a closer fit to the curve using resting values of P_K than that using a reduced P_K. It would appear likely that after a clamp to a much depolarized level or after the passage of the transient current, P_K decreases while P_{Na} stays constant. In the extreme case of P_K becoming negligible compared with P_{Na} all the current would be carried by sodium ions and therefore the net current would be zero at the equilibrium potential for sodium ions:

$$E_{(J_n = 0)} = 58 \log_{10}(Na_o/Na_c). \qquad (2)$$

If Na_c is about 30 mN (Findlay and Hope 1964) the potential according to equation (2) should be -86 mV. This is the least negative value observed. In Figure 7 it is about

-105 and -140 mV for the two experiments and -135 mV for the lower theoretical curve.

Which permeability to potassium and sodium is appropriate to the time when large transient currents are flowing is a question more difficult to answer. The indications [e.g. in Figure 5(*a*) of Findlay 1964] are that P_K rapidly decreases to a steady value, and hence the reduced value is used below to calculate the peak net transient current in terms of a transient permeability to calcium or chloride.

(*b*) *Transient Current according to the Calcium Hypothesis*

At present there seem to be two possibilities to describe the transient current—either as an inward current of calcium ions or as an outward current of chloride ions; in both cases a net negative current would be observed provided the transient current exceeded the postulated outward (positive) current of potassium and sodium.

The current disappears in the absence of calcium (cf. Fig. 4) and varies with, but is not directly proportional to, the external calcium concentration (cf. Fig. 10, Findlay 1964). Strontium appeared to be the only bivalent ion able to replace calcium and at an equal concentration J'_n was usually about one-fifth of that in calcium solutions (cf. Fig. 3).

Firstly it will be supposed, as during an action potential, that during a voltage clamp if the plasmalemma potential is raised above a threshold the permeability to calcium (or strontium) quickly increases to a peak value $P_{Ca}(P_{Sr})$ and declines. Then:

$$J'_t = \frac{2F^2E_{co}P_{Ca}}{RT} \cdot \frac{Ca_o - Ca_c[\exp(2E_{co}F/RT)]}{1 - \exp(2E_{co}F/RT)}, \tag{3}$$

Ca_c, the calcium activity in the cytoplasm, is unknown but J'_t does not depend markedly on Ca_c in the range 0·01–1 mN except when E_{co} is zero or positive.

In Figure 8 theoretical curves for J'_n and $J_{n(5)}$ are fitted to experimental results from cell C95 obtained by scanning E_{co}. The theoretical curve $J_{n(5)}$ was calculated from equation (1), using the reduced value 4×10^{-6} cm sec^{-1} for P_K, and J'_t was calculated from equation (3). J'_n was then determined from the equation $J'_n = J'_t + J_{n(5)}$. It can be seen in Figure 8 that the experimental points lie close to the theoretical curves over a large range of potential. Also shown in Figure 8 for comparison is a theoretical curve for $J_{n(5)}$ assuming $P_K = 9\times10^{-6}$ cm sec^{-1}. This curve of $J_{n(5)}$ is the same as that in Figure 7.

While the observed net peak transient current, J'_n, could generally be fitted by a "Goldman curve", with P_{Ca} of the order of 10^{-3} cm sec^{-1}, P_{Ca} was not constant with calcium concentration.

Equation (3) would indicate peak inward calcium currents almost proportional to Ca_o when E is in the range -150 to 0 mV (the other quantities being kept constant), but this is not observed. For example, in Figure 10 of the previous paper (Findlay 1964), the inward current was not more than trebled as Ca_o was increased from 0·3 to 3 mN. With this model it is possible to get an estimate of Ca_c from the potential at which J'_n crosses $J_{n(5)}$, for this should be the equilibrium potential for calcium ions: where no net current flows in either direction. In Figure 8 the equilibrium potential

was +25 to +30 mV, whence Ca_c was 0·05 mN; from Figure 9 of Findlay (1964) the estimate is similar. It was with this result in mind that Findlay and Hope (1964) used a value of the order of 0·01 mN for Ca_c in calculating action potential peaks.

The clamp currents when strontium replaced calcium in the external solution are also predicted approximately by an analogous model if P_{Sr} is $3–10 \times 10^{-5}$ cm sec^{-1}.

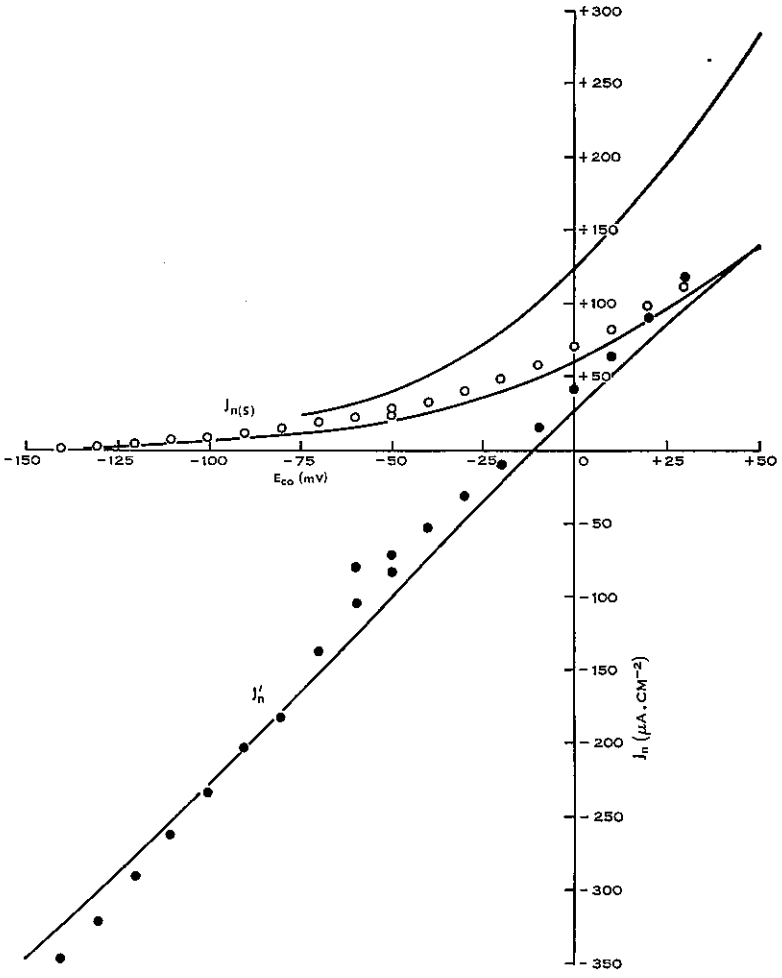

Fig. 8.—J'_n and $J_{n(5)}$ as functions of E_{co} from cell C95. The continuous lines are theoretical curves as explained in the text.

In Figure 2 the line through most of the solid circles is the calculated J'_n, this time with J'_t calculated from an equation analogous to (3), $P_{Sr} = 8 \times 10^{-5}$, $Sr_c = 0·01$ mN, but with J_{K+Na} calculated on the basis of resting permeabilities ($P_K = 9 \times 10^{-6}$, $P_{Na} = 2·7 \times 10^{-6}$ cm sec^{-1}). It is seen that in this experiment $J_{n(5)}$ (open circles) is less than the current expected from the resting permeabilities but greater than the current using half resting P_K (the solid line).

(c) *Transient Current according to the Chloride Hypothesis*

If the transient current is carried by chloride ions then the current should be the following function of membrane potential:

$$J'_t = J_{Cl} = \frac{F^2 E_{co} P_{Cl}}{RT} \cdot \frac{Cl_o - Cl_c[\exp(-E_{co}F/RT)]}{1 - \exp(-E_{co}F/RT)}. \quad (4)$$

P_{Cl} and Cl_c are unknown. It should be possible to estimate Cl_c from the value of E_{co} at which the transient current reverses sign, this being the equilibrium potential for chloride ions [cf. equation (2)]. However, there is usually doubt about the value of E_{co} at which this takes place since the appropriate value of potassium and sodium current is unknown [see Section IV(a)].

If $J_{n(5)}$ is the same as the outward current flowing at the time of the peak chloride current the equilibrium potential for chloride is the potential at which the curve of J'_n crosses that of $J_{n(5)}$. Such a reversal was not observed in dilute chloride solutions unless the membrane was clamped at a positive potential; whether (e.g. as in Fig. 8) it is then interpreted as a true reversal or not depends on the choice of J_{K+Na} at the time of the transient current. When the reversal occurred at -50 to -60 mV the transient current quickly became very much greater than any probable J_{K+Na} (Fig. 6).

In Figure 6 are three curves relating J'_n and E_{co}, calculated by means of equations (1) and (4). By trial and error it was found that the best fit was obtained by assuming that Cl_c was 2·1, 2·5, and 4 mN when the cell was in external solutions of 2·1, 8·1, and 30·1 mN Cl^-. Such an increase in cytoplasmic chloride is not unreasonable as a result of active transport across the plasmalemma. The values of P_{Cl} used to calculate the three curves were 4, 4, and 5×10^{-4} cm sec^{-1} respectively. The theory describes the observed currents fairly well when the (chloride) current is outwards, but not so well when the chloride current is inwards. It is difficult to see how the "reversed" current, which becomes obvious when Cl_o is made 20–30 mN, could be accounted for otherwise than as a net inward current of external chloride ions. The transient permeability mechanism is, however, not specific for chloride since when nitrate ions were substituted for 90% of the external chloride the "reversed" current was the same. In earlier experiments (Fig. 4 of Findlay and Hope 1964) substitution of bromide or nitrate for chloride caused little change in the peak of the action potential; this agrees with the idea that the plasmalemma, in the excited state, does not distinguish between these anions.

(d) *Permeability Changes*

Similar values of peak permeability to chloride predicted both the peak reached during an action potential (Fig. 4 of Findlay and Hope 1964) and the peak transient current during a voltage clamp (Fig. 6 above). The time courses of the permeability changes are probably also similar [cf. Fig. 1(b) in this paper and Fig. 1(b) of Findlay and Hope (1964)]. The same conclusion regarding peak permeability may be reached from a comparison of the conductance at the peak of the action potential [Fig. 7(a), Findlay and Hope 1964] with the slope of the curve of J'_n against E_{co} (Fig. 8 above).

This slope, $\partial J'_n/\partial E_{co}$, is the "slope conductance", and, at a membrane potential corresponding to the peak of the action potential, has an average value of 3700 μmho. cm^{-2} (from Fig. 11, Findlay 1964). In Figure 7(*a*) of Findlay and Hope (1964), the value is 4000 μmho. cm^{-2}, the calcium (1 mN) and chloride (2·1 mN) concentrations being the same.

V. Conclusions

The above analysis was made with a view to deciding between two possibilities proposed as the main factor in transient membrane behaviour in the plasmalemma of *C. australis*. Gaffey and Mullins (1958) and Mullins (1962) have claimed, mostly on the basis of tracer fluxes, that a transient increase in permeability to chloride causes the action potential and, by inference, carries the transient current observed during a voltage clamp. Findlay (1961, 1962) and Hope (1961) believed that the electrical evidence favoured a transient calcium permeability.

An analysis of the peak potentials and conductances reached during action potentials (Findlay and Hope 1964) was inconclusive. By suitable choice of parameters, the true values of which are unknown at present, the data could be fitted by either of the two hypotheses, the chloride mechanism being somewhat the more probable.

In the present paper similar difficulties were encountered in interpreting the currents flowing during voltage clamps. Except in experiments involving a high external concentration of chloride ions, the model predicted equally well a transient inward current of calcium or outward current of chloride ions.

The analysis suffers from at least two difficulties. The "constant field" model involves the usual assumption of a linear potential gradient in the plasmalemma, with little justification. Also there were unknowns for which likely values had to be guessed, namely, the calcium or chloride activity in the cytoplasm. Nevertheless, the following conclusions are favoured:

(1) On depolarization the permeability of the plasmalemma to chloride increases to a peak value, usually 10^{-4} cm sec^{-1}, and returns to the resting level. The cytoplasmic chloride activity is concluded to be 1–10 mN. With moderate depolarizations ($E_{co} = -150$ to 0 mV) the transient current is carried out of the cytoplasm by chloride ions. This outward current does not vary greatly in the range 0·1–1 mN Cl_o (Findlay 1961), presumably because Cl_i does not vary over this range. However, when Cl_o is raised to 30 mN, the transient chloride current is inward and very large at potentials more positive than about −50 mV (Fig. 6).

(2) The plasmalemma in the excited state does not distinguish between Cl^-, Br^-, and NO_3^- in the external medium.

(3) The transient anion permeability depends on the concentration of calcium in the medium. If the calcium is dilute enough the phenomena of action potential and transient clamp current disappear. Strontium is a less effective replacement for calcium.

The effect of divalent ions on transient anion permeability may be related to the hydrated size of the divalent ions since Ca^{2+} and Sr^{2+} have

similar equivalent conductivity, 59·50 and 59·45 mho. cm^2. equiv^{-1} respectively, at 25°C. The crystallographic radii are 0·99 and 1·13 Å. If these ions actually enter pores in the membrane, as suggested by Mullins (1959), and thus affect transient anion permeability, it would seem that they do so in the hydrated state. Otherwise, Cd^{2+}, with the same crystal radius, should be electrically an analogue to Ca^{2+}, which it is not. However, admittedly there is no evidence that the effect of Ca^{2+} on transient anion permeability depends on Ca^{2+} entering pores in the membrane.

(4) The permeability to potassium decreases following a voltage clamp to a much depolarized level, as shown by the decrease in outward current $J_{n(5)}$ after the transient, compared with $J_{n(0)}$ before it.

(5) The permeability changes during the action potential and during a voltage clamp are similar in magnitude. Consequently chloride fluxes of similar size to those observed in clamping, i.e. 100–1000 μA.cm^{-2} may be expected to flow during an action potential although the net charge transfer is zero when potassium and sodium fluxes are taken into account. Thus it is not surprising that Mullins (1962) observed a chloride loss during activity of about 1000 times that needed to change the potential by discharging the membrane capacitance.

VI. References

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