IONIC RELATIONS OF CELLS OF *CHARA AUSTRALIS*

VIII. MEMBRANE CURRENTS DURING A VOLTAGE CLAMP

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Summary

Experiments are described in which "voltage clamps" were applied to the "membrane" of *C. australis* cells comprising tonoplast and plasmalemma and also to the plasmalemma alone. The voltage-clamp system maintained the membrane potential at a predetermined level, and enabled a detailed analysis to be made of the transient electrical phenomena occurring during the action potential. A scanning technique is also described, by means of which the membrane current–potential characteristics could be determined at any particular time during the transient activity of the membrane.

During a voltage clamp of the potential difference across the plasmalemma, a large transient inward current flowed across that membrane. The concentration of calcium ions in the external medium is shown to play an important role in determining the electrical characteristics of the plasmalemma in its transiently active state.

I. INTRODUCTION

In a previous paper (Findlay 1962) the action potential in *Nitella* sp. was described. It was shown that calcium ions appear to be an important factor in the phenomena of potential change and current flow during the action potential. A similar conclusion was reached by Hope (1961) for *Chara australis*.

The preceding paper in this series (Findlay and Hope 1964) has shown that the action potential measured in the vacuole is the sum of potential changes across both tonoplast and plasmalemma, and that the fast component of the action potential occurs across the plasmalemma.

The present paper is mainly concerned with voltage-clamp experiments with *Chara australis*, in which the "clamp" was applied to the potential difference between the cytoplasm and the external medium, across the plasmalemma. Some experiments are also described in which clamps were applied to the potential difference between the vacuole and the outside medium (i.e. across both plasmalemma and tonoplast). The role of calcium ions in determining the electrical characteristics of the plasmalemma during the action potential is examined.

The large cells of *C. australis* allow the insertion of longitudinal metallic electrodes of low electrical resistance, as described by Findlay and Hope (1964). This has resulted in considerable improvement in the feedback control of the membrane potential as compared with that in the earlier experiments with *Nitella*

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(Findlay 1962). In those experiments the use of a high-resistance glass microelectrode filled with potassium chloride limited the amount of current that could flow during the clamp, and often resulted in inadequate control of the membrane potential.

II. Materials and Methods

The material used was *Chara australis* R. Br. var. *nobilis* A.Br., as described by Findlay and Hope (1964).

In the voltage-clamp experiment three electrodes were inserted into the cell, again as described previously. The experimental arrangement for voltage clamping is shown in Figure 1. In this system the membrane potential was controlled by

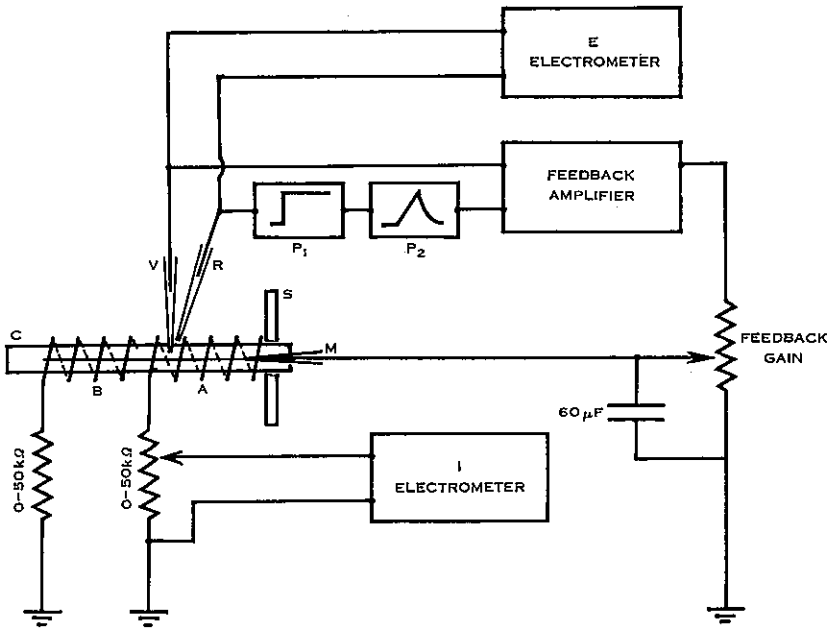

Fig. 1.—Diagram of the experimental arrangement used for voltage-clamp experiments. The following parts are labelled: *C*, *Chara* cell; *V*, electrode in the vacuole or the cytoplasm; *R*, external reference electrode; *S*, stock holding the cell; *M*, intracellular metal electrode; *A*, *B*, separate sections of the external current electrode. The operation of the system is described in the text.

including the membrane in an appropriate feedback circuit. The membrane potential was fed into the feedback amplifier through the control potentiometers. The output of this amplifier was connected to the metal electrode. The operation of the system was basically similar to that described by Findlay (1962). The membrane potential was first clamped at the resting level by backing off the resting potential, and then increasing the gain of the feedback amplifier. To change the membrane potential stepwise, a potential from P_1 was switched in. It was possible to hold the membrane potential to within 1–2 mV of the desired level. The current flowing through a

known area of the cell membrane was measured as the potential difference appearing across the resistance between section A of the external electrode and earth. As the internal platinum electrode extended beyond A and into B, an approximately uniform radial current flowed through the region enclosed by A. The two sections of the external silver electrode were earthed through equal resistances to prevent any appreciable longitudinal current between A and B.

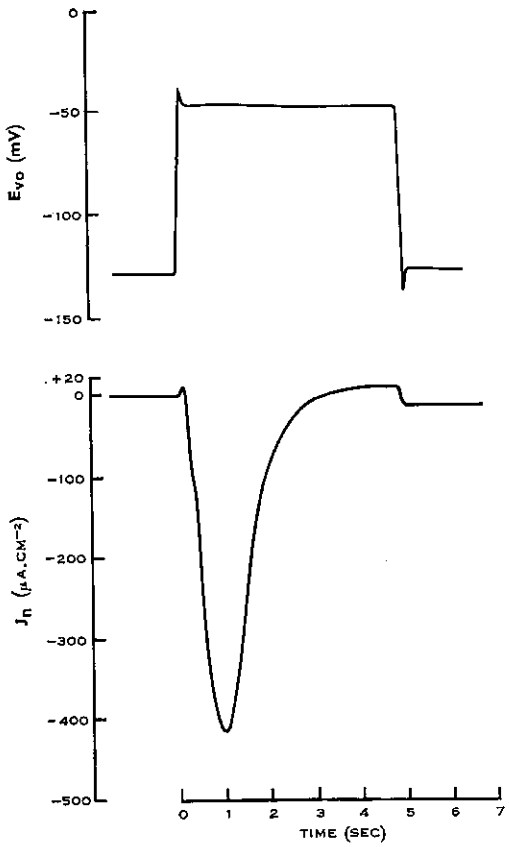

Fig. 2.—Tracings of a voltage clamp of the vacuolar potential and the corresponding membrane current.

Figure 2 shows a typical voltage clamp of the vacuole potential, and the corresponding membrane current. The overshoot in the membrane potential at the beginning and end of the clamp was due to oscillation, or "ringing", in the feedback circuit, as well as overshoot in the pen recorder.

A transistorized single-shot time-base circuit (P_2 in Fig. 1), giving a sweep time of about 0·2 sec, was connected in series with P_1. In some experiments, a step in the membrane potential was first applied from P_1, and then P_2 was switched on manually at the peak of the inward clamp current. The purpose of this was to ascertain the

relationship between membrane potential and current during the short time in which the membrane exhibits its transient permeability change.

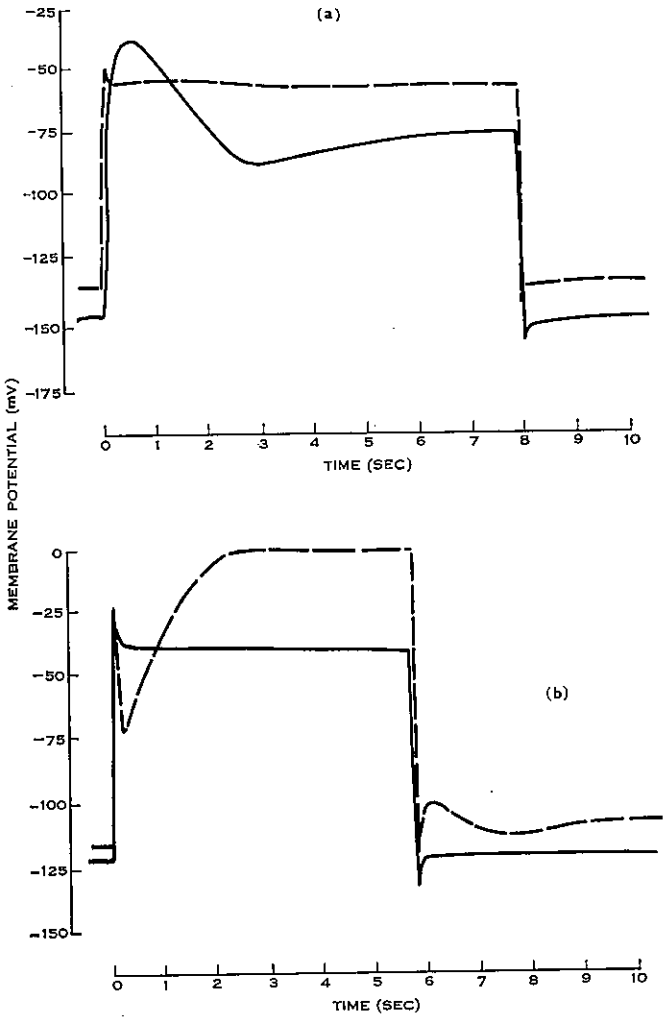

Fig. 3.—(*a*) Tracings of the potential of the vacuole (dotted line) and of the cytoplasm (solid line), during a voltage clamp of the vacuolar potential. (*b*) As for (*a*) but with the voltage clamp applied to the cytoplasmic potential.

III. Results

(*a*) *Voltage Clamps Applied to the Vacuolar and Cytoplasmic Potentials*

Voltage clamps were applied to either vacuolar or cytoplasmic potentials, and the potential of both recorded simultaneously. Figures 3(*a*) and 3(*b*) show the cytoplasmic and vacuolar potentials when the voltage clamp was applied to the vacuolar potential and the cytoplasmic potential respectively.

(b) Membrane Current during a Voltage Clamp

A voltage clamp across either the plasmalemma or the plasmalemma and tonoplast together was maintained by a flow of electric current between the inserted metal electrode and the external silver–silver chloride electrode. This current will be called membrane current because the tonoplast and plasmalemma are electrically in series and any flow of current will be the same through both.

Voltage clamps were applied to the potential of either the vacuole or the cytoplasm for about 5 sec. Figure 4 shows the time course of the membrane current for the vacuole potential clamped at —65 mV, compared with that for the cytoplasm potential clamped at the same level. The form of the clamping current in both cases

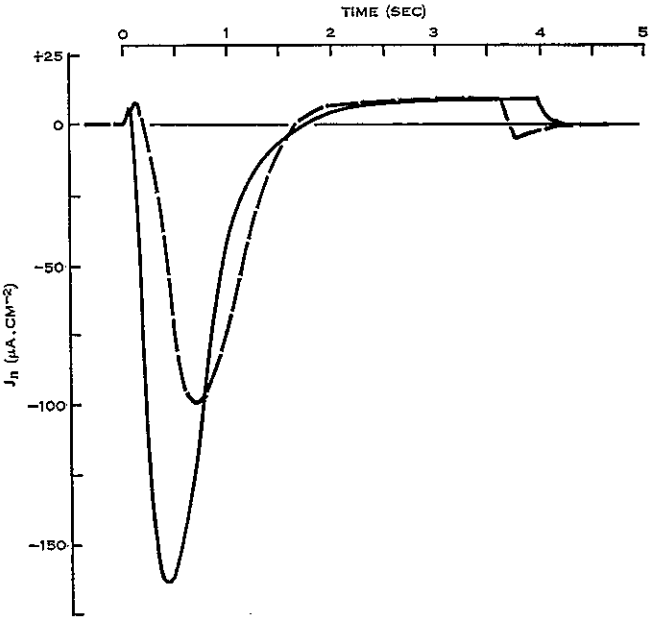

Fig. 4.—Tracings showing the time course of the membrane current when either the vacuolar potential (– – – –) or the cytoplasmic potential (————) was clamped at —65 mV.

is similar. On the application of the voltage clamp an initial outward current (positive in Fig. 4) is followed rapidly (usually within 0·2 sec) by a transient inward current. After this transient inward current has ceased an outward current is apparent until the end of the voltage clamp. The time interval between the start of the voltage clamp and the peak of the transient inward current, 0·2–0·5 sec for clamps of the cytoplasm potential, is consistently smaller than for clamps of the vacuole potential, where the time interval is from 0·5 to 1·0 sec.

Figure 5(*a*) shows the membrane current during a series of voltage clamps at different levels of the cytoplasm potential. Quantities associated with a clamp current, and indicated in Figure 5(*b*), are as follows: (i) the net membrane current flowing at any time, J_n; (ii) the transient inward current, J_t, with maximum value

J'_t; (iii) the net current at the transient peak, J'_n; (iv) the net membrane current flowing at a time, usually 5 sec, after the start of the clamp, $J_{n(5)}$.

In Figure 6, J'_n and $J_{n(5)}$ are shown as functions of the cytoplasmic potential, E_{co}. These graphs are plotted from the curves shown in Figure 5(*a*).

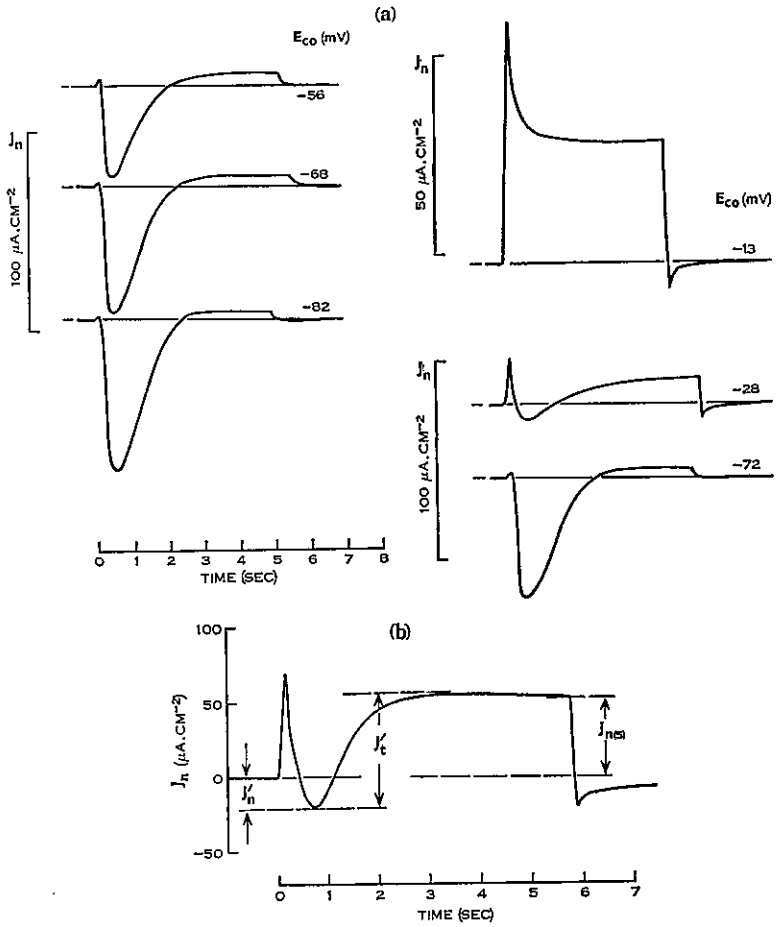

Fig. 5.—(*a*) Series of tracings of the membrane current when the potential of the cytoplasm was clamped at the levels shown to the right of each curve. (*b*) Tracing of the membrane current during a voltage clamp—in this case of the vacuolar potential—indicating the quantities described in the text.

(*c*) *The Instantaneous* E *versus* J'_n *Characteristic*

In the previous section, E versus J'_n was obtained from a series of clamps, each at a different level of potential. If, however, the membrane potential was controlled to follow a depolarizing step of sufficient size to produce electrical activity and subsequently a linear positive or negative function (commencing at or just before the peak of the transient current), it was possible to obtain a continuous

recording or scan of E, and the corresponding J_n'. When scanning either the vacuolar or cytoplasmic potential in a time (0·1–0·2 sec) short compared with the time course of J_n, it is assumed that J_n' as a function of E alone, and not of time, will be obtained.

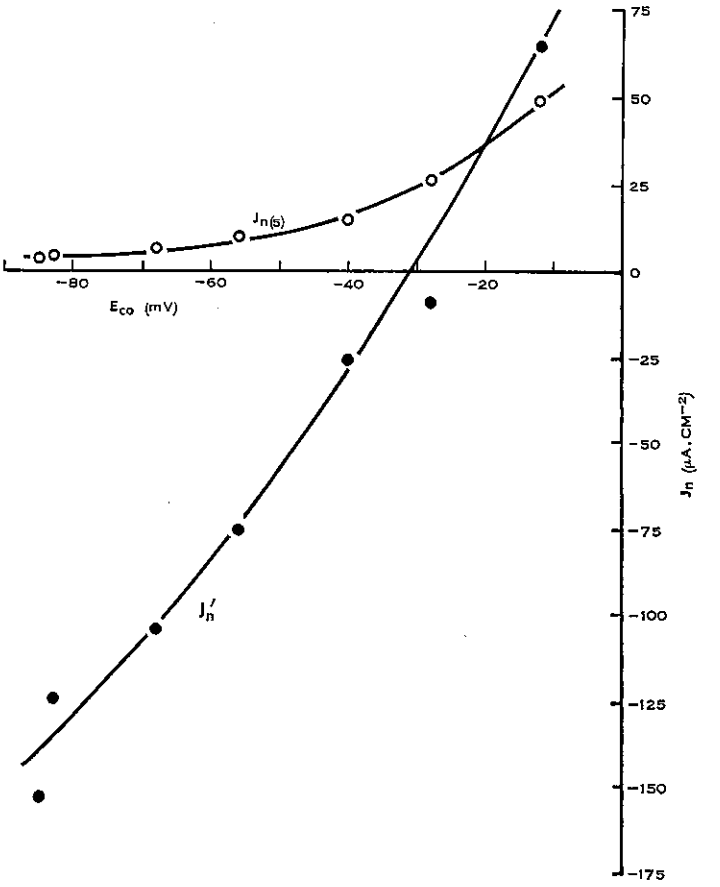

Fig. 6.—The relationship between J_n' and E_{co}, and between $J_{n(5)}$ and E_{co}, obtained from the curves shown in Figure 5(*a*).

After the transient current, J_t, had ceased, $J_{n(5)}$ as a function of E resulted if another scan of E was made 5 sec after the start of the clamp. Figure 7 shows a photograph

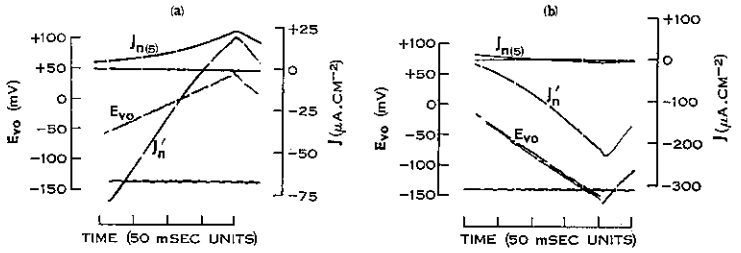

Fig. 7.—Two scans of the vacuolar potential as displayed on a cathode-ray oscilloscope, showing J_n' and $J_{n(5)}$ where E_{vo} was scanned (*a*) in a depolarizing direction and (*b*) in a hyperpolarizing direction.

of two scans of E (in the vacuole), displayed on a cathode-ray oscilloscope, showing J'_n and $J_{n(5)}$, where E was scanned (*a*) in a depolarizing direction and (*b*) in a hyperpolarizing direction. Curves of J'_n versus E (vacuole) obtained from a single scan and from a series of voltage clamps have been compared, and two such curves, with $Ca_o = 1{\cdot}0$ mN, $Na_o = 1{\cdot}0$ mN, and $K_o = 0{\cdot}1$ mN, are shown in Figure 8.

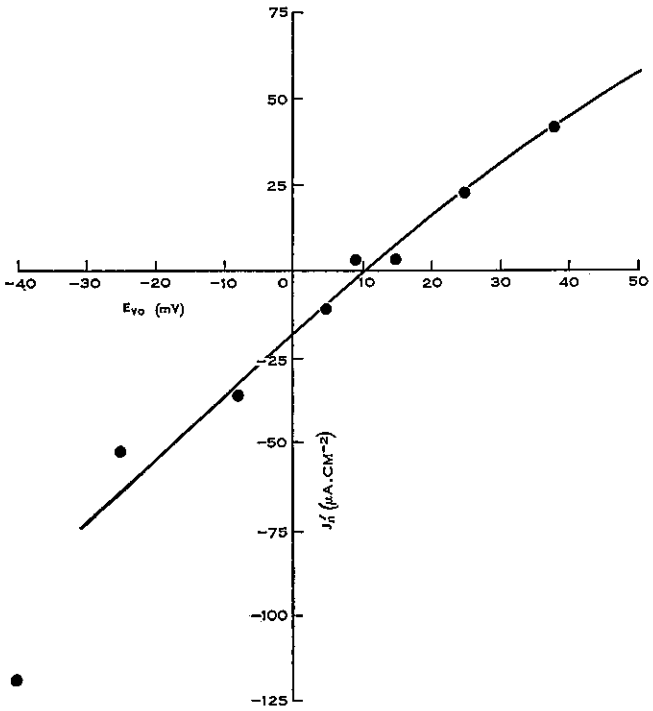

Fig. 8.—Comparison of the relationship between J'_n and E_{vo} obtained with a single scan (continuous line) and from a number of voltage clamps (separate points).

Voltage clamps and scans were applied once every 10 min. By arranging the initial clamped potential level to be less negative for hyperpolarizing scans, it was possible to overlap a hyperpolarizing and a depolarizing scan, and so obtain an extended E versus J'_n characteristic curve. The two curves did not always completely overlap, owing to the difficulty of starting the scan exactly at the peak of the transient current, but the E versus J'_n characteristic determined by a scan at the peak had the maximum slope. In some later experiments the plasmalemma potential was clamped to +10 mV and only one scan, in the hyperpolarizing direction and of magnitude about 150 mV, was applied.

Figure 9 shows the results, from one cell, of an extended scan of the vacuolar potential and one of the cytoplasmic potential. E versus J'_n and E versus $J_{n(5)}$ are shown. The two scans of the cytoplasmic potential do not completely overlap for the reason given above. The peak values of the action potential in cytoplasm and vacuole are indicated by the arrows.

(d) The E *versus* J_n' *Characteristic as a Function of External Calcium Concentration*

All the previous measurements described in this paper were made with cells bathed in an artificial pond water containing 1·0 mN Ca^{2+}, 1·0 mN Na^+, 0·1 mN K^+, and 2·1 mN Cl^-. The E versus J_n' characteristic was also investigated as a function of the external calcium concentration, Ca_o. The following external solution was used: 3·0 mN $Mg^{2+}+Ca^{2+}$, 0·1 mN K^+, 1·0 Na^+, and 4·1 mN Cl^-. Changes were made in the concentration of the external calcium ions, with concomitant changes in Mg_o to keep the total ionic concentration of the external solution constant.

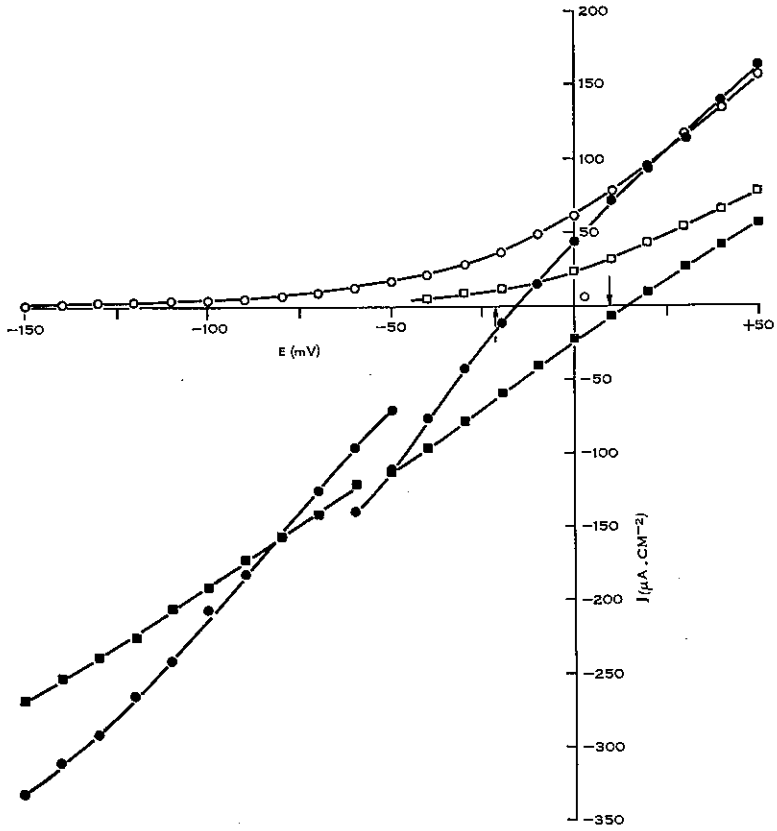

Fig. 9.—Extended scans, in one cell, of the cytoplasmic potential showing J_n' (●) and $J_{n(5)}$ (○), and of the vacuolar potential showing J_n (■) and $J_{n(5)}$ (□).

Figure 10 shows J_n' versus E with different Ca_o, for clamps of cytoplasm potentials. These results were obtained from a number of "scans". Also indicated are the values of E reached at the peaks of the action potentials in the appropriate calcium concentrations.

The quantity $\partial E/\partial J_n'$ may be calculated from the curves of E versus J_n', and is the slope resistance, R, with units of ohm. cm². This resistance has been calculated

from scans of the cytoplasm potential, for different Ca_o, with an arbitrarily chosen $E = -100$ mV, and also for values of E at which $J_n' = 0$. Figure 11 shows the results from 14 cells.

IV. Discussion

The results of experiments on *Chara* cells described in this paper show that it is now possible to examine the electrical properties of the plasmalemma alone, using voltage-clamping techniques. Previous voltage-clamping experiments in *Nitella* (Findlay 1962) only allowed a study of the composite "membrane", consisting of plasmalemma and tonoplast in series.

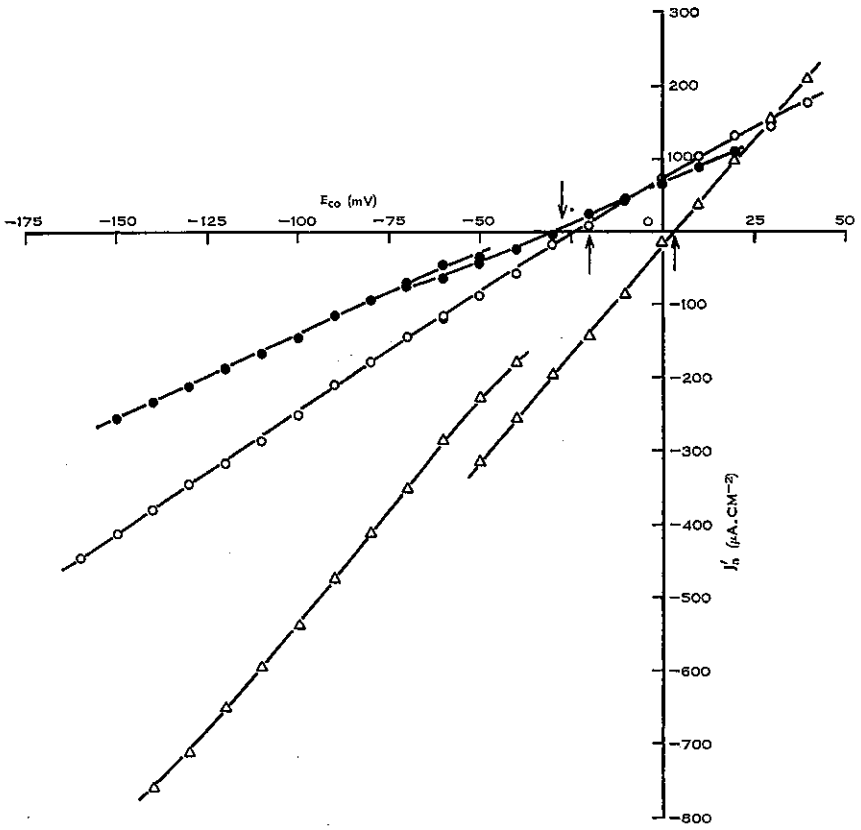

Fig. 10.—Results, from one cell, of an extended scan of E_{co}, showing J_n' as a function of Ca_o: 0·3 mN Ca_o (●); 1·0 mN Ca_o (○); 3·0 mN Ca_o (△).

The membrane currents flowing during voltage clamps applied to either the potential of the vacuole or to the potential of the cytoplasm show a close similarity in form, and for comparison voltage clamps of both potentials have been considered in this paper. A more detailed study, however, has been made of voltage clamps of the potential of the cytoplasm, i.e. of the potential difference across the single

membrane, the plasmalemma. As yet, it has not been possible to apply a voltage clamp to the potential difference across the tonoplast.

The relationship between the net peak inward current, J'_n, and the membrane potential during the voltage clamp indicated a clear dependence of the transient electrical characteristics of the plasmalemma on the concentration of calcium ions in the external solution. Figure 11 shows that the net plasmalemma resistance, at the peak of the transient current, $\partial E_{co}/\partial J'_n$, decreases as the external calcium concentration increases.

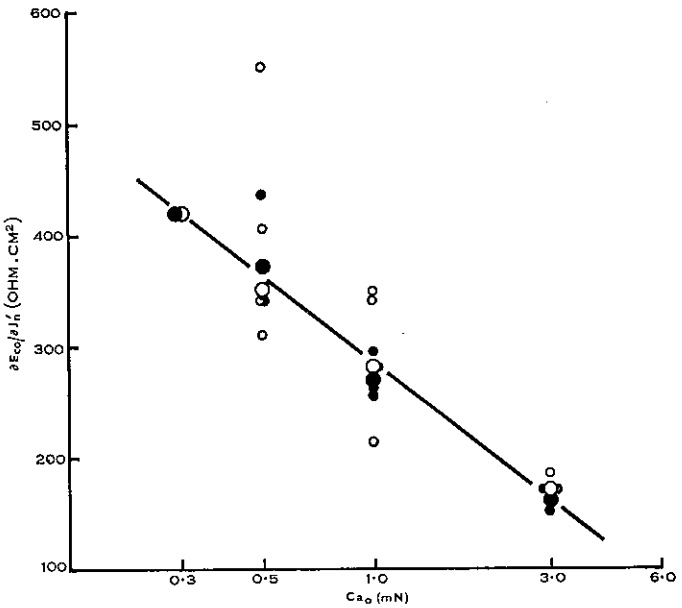

Fig. 11.—Relationship between $\partial E_{co}/\partial J'_n$ and Ca_o. Results are from 14 cells. ● Values of $\partial E_{co}/\partial J'_n$ for $E_{co} = -100$ mV. ○ Values of $\partial E_{co}/\partial J'_n$ for $J'_n = 0$. The larger circles are the appropriate mean values.

The technique of scanning the membrane potential during the voltage clamp has enabled the E versus J'_n characteristics over a wide range of E to be obtained at the one time. Previously, E versus J'_n had been determined from a series of voltage clamps at different levels. Because of fluctuations in the membrane characteristics with time, accurate determinations of E versus J'_n were not always possible to obtain. During such scans, however, the change in membrane potential will also produce a capacitative current given by

$$J = -C\partial E/\partial t,$$

where C is the capacitance of the membrane, and is about 1–2 μF.cm^{-2} (Findlay and Hope 1964). Scans changed the membrane potential at rates up to 1·0 V/sec. Hence $\partial E/\partial t$ had a maximum value of 1–2 μA.cm^{-2}. Thus the curve of E versus J'_n obtained from a scan will only be slightly displaced from its true value. Corrections of this small error were usually not made.

The net peak current, J'_n, is assumed to be the sum of an inward current, J_t, and an outward current. Thus when $J'_n = 0$ the inward current will be balanced by an outward current of the same magnitude. If the results from voltage-clamp experiments in which the membrane potential is controlled are applicable to a study of the action potential itself, then the value of E at which $J'_n = 0$ is of special significance. At the peak of the action potential $\partial E/\partial t$ is zero, and consequently the net membrane current would be zero also. It has been found in a large number of experiments that the value of E (either for the vacuole or the cytoplasm) at which $J'_n = 0$ does in fact coincide with the peak of the action potential.

V. Acknowledgment

The author thanks Dr. A. B. Hope for much helpful advice and criticism.

VI. References

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