MEMBRANE ELECTRICAL BEHAVIOUR IN *NITELLOPSIS OBTUSA*

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Summary

The electrical properties of the two membranes, plasmalemma and tonoplast, bounding the cytoplasm of the brackish water characean, *Nitellopsis obtusa*, were studied. In a solution approximating the natural pond water, the electrical potential difference between cytoplasm and outside was −141 mV, and between vacuole and cytoplasm +19 mV. The resistance of the plasmalemma was 80,000 Ω cm^2, and of the tonoplast, 10,000 Ω cm^2.

During an action potential, the peak values of the p.d.'s across plasmalemma and tonoplast were −25 and +57 mV respectively. The action potential was accompanied by a transient decrease in the resistance of the plasmalemma to 5700 Ω cm^2, and of the tonoplast to 2500 Ω cm^2.

The effects of changes in external potassium and calcium concentrations on the p.d.'s and resistances of the membranes were examined.

During an action potential there was an increased chloride efflux from the vacuole to the outside of the cell. It is concluded that as in other characean species, a transient increase in the permeability of both plasmalemma and tonoplast to chloride ions is responsible for the action potential.

I. INTRODUCTION

In a number of papers (Hope and Walker 1961; Findlay 1964; Findlay and Hope 1964*a*, 1964*b*; Hope and Findlay 1964) the electrical properties of the two membranes, plasmalemma and tonoplast, bounding the cytoplasm in the large internodal cells of the freshwater characean, *Chara corallina*, have been described. It was shown that most of the potential difference and electrical resistance between the vacuole of the cell and the external solution is located at the outer cytoplasmic membrane, the plasmalemma, whose electrical properties are dependent on the concentrations of potassium, sodium, and calcium ions in the external solution.

The action potential in *Chara corallina* consists of a transient change in both p.d. and resistance of the two membranes—a fast, positive-going "spike" across the plasmalemma, and a slower change across the tonoplast which together give rise to the usually recorded two-component action potential in the vacuole. Similar types of vacuolar action potentials have been measured in *Chara globularis* (Gaffey and Mullins 1958), *Nitella* sp. (Findlay 1959), *Nitella flexilis* and *Chara braunii* (Oda 1960), and *Nitella translucens* (Williams and Bradley 1968).

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Measurements of ionic fluxes through the membranes of *Chara globularis* (Gaffey and Mullins 1958), *Nitella clavata* (Mullins 1962), *Chara corallina* (Hope and Findlay 1964), and *Nitellopsis obtusa* (Haapanen and Skoglund 1967) have shown that during the action potential there is a marked increase in efflux of potassium and chloride ions from the cell, from which it is inferred that the action potential results from a transient increase in the permeability of the plasmalemma to chloride ions.

The characean species described above grow in fresh water, where the concentrations of the main ions K^+, Na^+, Cl^-, and Ca^{2+} are less than 4 mM. There are, however, some species which grow in brackish water, although there is not much published data about their electrical properties. Kishimoto and Tazawa (1965) have described some electrical properties of *Lamprothamnium succinctum*, an ecorticate species which grows in ponds where the average salt concentration is about two-thirds that of seawater. In its normal environment this species is not excitable, but becomes so in a solution where most of the ions are replaced by an osmotic equivalent of mannitol. Findlay (unpublished data 1964) has found that an ecorticate species of *Chara* from salt lakes north of Sydney (salt concentration about one-half that of seawater) gives the usual type of action potential with components at the plasmalemma and tonoplast.

This paper describes experiments with *Nitellopsis obtusa*, a species that in the Norfolk Broads, England, grows in approximately one-twentieth seawater. The p.d. and resistance of the plasmalemma and tonoplast and the efflux of chloride ions in the resting state of the cell and during the action potential have been measured. It is shown that the electrical behaviour of *Nitellopsis obtusa* is similar to that of *Chara corallina*, *Nitella translucens*, and the freshwater *Nitellopsis obtusa* used by Haapanen and Skoglund (1967), but certain aspects appear to be in conflict with conclusions reached by MacRobbie and Dainty (1958) from ionic flux studies with the brackish water *Nitellopsis obtusa*.

II. Materials and Methods

Cells of *Nitellopsis obtusa* were collected from Hickling Broad, Norfolk, England, and either stored in the laboratory in an artificial Hickling water AHW(K1Ca4), or grown in a culture with a base of river mud and AHW(K1Ca4) as solution. AHW(K1Ca4) consisted of NaCl 23 mM, KCl 1·0 mM, $CaCl_2$ 4·0 mM, $MgCl_2$ 3·0 mM, and $NaHCO_3$ 1·5 mM. Whorl cells were used for practically all the experiments described in this paper, because they were sufficiently short in relation to the electrical space constant (see below). The cells were usually 1–2·5 cm long and up to 0·6 mm in diameter (internodal cells were usually 10–25 cm long). In *N. obtusa* it is possible to separate all the whorl cells at a single node and thus have up to six cells of approximately the same age and size. The cells, after being cut from the parent culture, were usually soaked in AHW(K1Ca4) for several days before use. Most *N. obtusa* cells, like other characean species, have a crystalline deposit on the outside of the cell wall. In *Chara australis* the deposit is in distinct bands, and in *N. obtusa* it tends to cover the whole cell. Occasionally cells of *N. obtusa* lost most or all of the deposit after soaking in AHW(K1Ca4) for a few days, but this did not occur in any consistent manner. The cells used in experiments described here usually had a deposit covering the entire cell surface, except for gaps between the crystals. Microelectrodes were inserted into the cell through these gaps.

The cell was held in a small Perspex vessel in flowing solution. Two glass microelectrodes, each filled with 3M KCl, were inserted into the vacuole of a cell at the start of an experiment. One measured vacuole potential and the other was used to pass current across the tonoplast

and plasmalemma. The tips of these electrodes had a diameter of about 5 μm, to allow a gush of vacuolar sap into the electrode on its insertion into the cell, and to ensure that the electrode tips were in the vacuole. The recorded vacuolar potential usually reached a steady level about 0·5–1 hr after insertion of the electrode. After this time a third microelectrode, with tip diameter about 2 μm, was inserted into the cytoplasm. Within a few minutes the potential measured with this electrode reached a steady value, almost invariably more negative than the vacuolar potential. The external reference electrode consisted of a thin plastic tube filled with 3M KCl in 3% agar, connected to a calomel half-cell. Similar tubes connected the intracellular microelectrodes to calomel half-cells. A current generator was connected to the appropriate vacuolar microelectrode via a chlorided silver wire. The external current electrode was also a chlorided silver wire.

For R_m, the area specific resistance (Ω cm^2), to be estimated, it is necessary to take into account the cable-like properties of the cell. If it is assumed that an *N. obtusa* cell behaves as a short cable with infinite impedances across the line at the nodes, it can be shown that

$$V_x = \tfrac{1}{2}I\{r_m(r_o+r_i)\}^{\frac{1}{2}}\{\cosh(L-x)/\lambda\}/\{\sinh(L/\lambda)\}, \quad (1)$$

where I is the current flowing through a vacuolar electrode inserted at the midpoint of the cell (length $2L$), V_x is the change in potential inside the cell at distance x from the midpoint, λ is the space constant $[=\{r_m/(r_o+r_i)\}^{\frac{1}{2}}]$, r_m is the resistance for unit length (Ω cm) of membrane $[=R_m/2\pi\rho]$, r_i is the resistance per unit length (Ω cm^{-1}) of internal fluid $[=R_i/\pi\rho^2]$, r_o is the resistance per unit length of external fluid (Ω cm^{-1}), R_m is the resistance for unit area of membrane (Ω cm^2), R_i is the specific resistance of the internal fluid (Ω cm), and ρ is the radius of the cell (cm). In this analysis it is assumed that the cell is bounded by only one "membrane".

The basic quantities that one measures to determine R_m are V_x, I, and A $(=4\pi\rho L)$, where A is the area of the cell (ignoring the ends). It is possible to use $R_m' = V_x.A/I$ as a measure of R_m, providing R_m' is sufficiently large. For a typical *N. obtusa* cell, e.g. $R_i = 80$ Ω cm, $L = 0{\cdot}75$ cm, $x = 0{\cdot}22$ cm, $\rho = 0{\cdot}03$ cm, equation (1) yields the result that, for experimentally determined estimates greater than 5000 Ω cm^2, R_m' differs from R_m by less than 5%.

For measurements of chloride efflux, five cells, all carefully trimmed of adjoining cell material, were first soaked in ^{36}Cl AHW(K1Ca4) for periods ranging from 7 to 21 days, to get the intracellular specific activity of chloride to a sufficiently high level. Each cell was then placed in flowing AHW(K1Ca4) for about an hour, and two vacuolar microelectrodes inserted in the usual way. Two of the cells had previously been soaked in non-radioactive AHW(K1Ca4) for about 24 hr. After the electrodes had been in a cell for about 2 hr, and the vacuolar p.d. had reached a steady level, the flow of AHW(K1Ca4) was stopped for a period of from 15 to 30 min. The stagnant solution, containing ^{36}Cl AHW(K1Ca4) was then removed with a pipette. A second aliquot of inactive AHW(K1Ca4) was placed on the cell, and this too was removed after about 30 sec and added to the first aliquot. Both aliquots were then put on to an aluminium planchet, dried, and counted in a Nuclear–Chicago gas flow counter. Usually two or three samples were taken and counted, with the cell in its resting state. These were followed by a shorter sample time, about 5 min, during which one action potential was initiated in the cell. The whole sequence was repeated several times. At the end of the experiment the electrodes were carefully removed, the cell cut open in a small quantity of distilled water, and one-tenth of the contents dried down on a planchet and counted. The remainder was used to determine the total chloride ion content of the cell, using a standard electrometric titration method.

Throughout this paper, numerical results are given as the mean $\pm$ one standard error of the mean (S.E.M.) with the number of observations given in parenthesis.

III. Results

(a) *Ionic Composition of the Vacuole*

Vacuolar sap samples from each of several whorl cells from a culture about 2 months old were pooled to get a sample of sufficient size to analyse for sodium and potassium concentrations by flame-photometry, and for chloride by electrometric

titration. For five such samples the values obtained were K 95±6 mM; Na 60±4 mM; and Cl 174±4 mM. The cells were ones similar to those used for most of the electrical measurements described in this section. A further analysis of the same culture made about 3 months later showed that both K and Cl had increased by about 20–30 mM.

In the five cells (from another culture) used for measurements of chloride efflux [Section III(*f*)] the mean vacuolar concentration of chloride was 144±18 mM.

(*b*) *Potential Difference, Resistance, and Capacitance of the Plasmalemma and Tonoplast*

The electrical properties of the membranes were investigated with cells in AHW(K1Ca4) and two modifications of this solution, AHW(K0·1Ca1·5), comprising NaCl 23 mM, KCl 0·1 mM, $CaCl_2$ 1·5 mM, and AHW(K1Ca1·5), comprising NaCl 22·1 mM, KCl 1·0 mM, $CaCl_2$ 1·5 mM. Both solutions differ from AHW(K1Ca4) in having less $CaCl_2$, no $MgCl_2$ or $NaHCO_3$, and differ from each other by a factor of 10 in K^+ concentration.

TABLE 1

PLASMALEMMA AND TONOPLAST POTENTIAL DIFFERENCES (mV) RESISTANCES (kΩ cm^2) AND CAPACITANCES (μF cm^{-2}) IN THE RESTING STATE AND AT THE PEAK OF AN ACTION POTENTIAL

Values given are mean values ± standard error of the mean. Number of determinations given in parentheses

Parameter*	AHW(K1Ca4)	AHW(K0·1Ca1·5)	AHW(K1Ca1·5)
ψ_{vo}†	−139 ± 5 (26)	−144 ± 9 (4)	−108 ± 5 (6)
ψ_{co}	−141 ± 6 (18)	−152 ±15 (5)	−129 ± 7 (4)
ψ_{co}^{p}	−25 ± 2 (15)	−38 ± 3 (4)	−45 ±10 (4)
ψ_{vc}	19 ± 3 (18)	21 ± 8 (5)	19 ± 5 (4)
ψ_{vc}^{p}	57 ± 3 (15)	56 ±10 (4)	53 ±11 (4)
R_{vo}	99 ±21 (17)	113·4±12·2(3)	56 ±38 (5)
R_{co}	80 ±12 (10)	88·3±24 (5)	58 ±15 (4)
R_{co}^{p}	5·7 ± 2·9 (5)	3·7± 2·5(3)	3·3± 1·2(3)
R_{vc}	10·0 ± 1 (10)	10·5± 3·9(4)	10·8± 3·3(4)
R_{vc}^{p}	2·5 ± 0·8 (5)	2·2± 1·0(3)	3·7(2)‡
C_{co}	1·23± 0·08 (5)	—	—
C_{vc}	2·82± 0·26 (4)	—	—

* The superscript *p* denotes measurement at the peak of the action potential.

† Values of the vacuolar p.d. within the first 30 min after insertion of the microelectrodes.

‡ The mean of the two values 2·5 and 4·9 is given.

The p.d.'s, ψ_{co} and ψ_{vc}, across plasmalemma and tonoplast respectively, or their sum, ψ_{vo}, were measured at intervals throughout the course of an experiment usually lasting 2–3 hr. Mean values of ψ_{co} and ψ_{vc} were calculated for each cell and then a mean taken of values from a number of cells. The value of ψ_{vo}, about 30 min after the insertion of the electrode into the vacuole, was often more negative than the average value for the whole experiment. This is because ψ_{vo} and ψ_{co} often depended, among other things, on the number of action potentials that had been initiated and their frequency, prior to the measurements of p.d. After each action potential ψ_{co} and ψ_{vo} were 20–30 mV less negative than before, and took several minutes

to return to their original level. The resting value of ψ_{vc} was not affected in this way. If the action potentials were of sufficient frequency, ψ_{co} did not return to its original level, but remained at a depolarized level. In some cells, ψ_{co} continued to become smaller in magnitude after each action potential, and eventually the plasmalemma became almost completely depolarized. Cells behaving in this way usually died within an hour or so, and results from them were disregarded. The results of measurements of ψ_{vo}, ψ_{co}, and ψ_{vc} are shown in Table 1.

The resistance of the plasmalemma and that of the tonoplast were measured by taking a mean value of the change in p.d. across each membrane which occurred when short (about 1 sec) pulses of inward and outward current flowed between vacuole and the outside. The applied current was 0·1–0·5 μA cm^{-2} of cell surface. R'_m was usually 50–100 kΩ cm^2, and consequently was taken as the value for R_m. Resistance measurements were made during the course of an experiment and a mean value determined. The results for a number of cells are shown in Table 1.

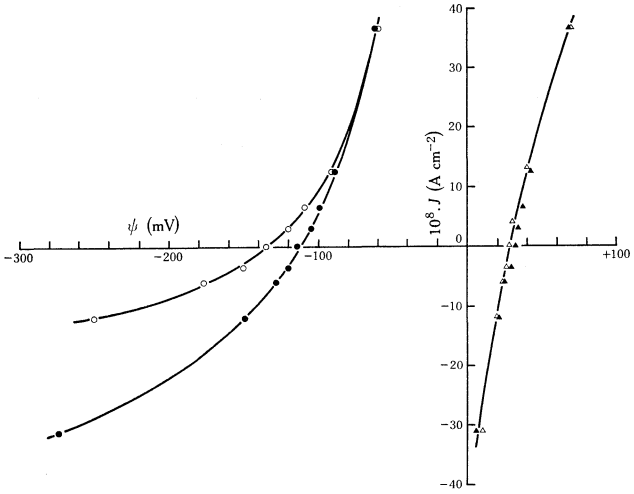

Fig. 1.—Potential difference across the plasmalemma, ψ_{co} (●,○), and the p.d. across the tonoplast, ψ_{vc} (▲,△), both as a function of J, the current density. ○,△ Cells in AHW(K0·1Ca1·5). ●,▲ Cells in AHW(K1Ca1·5).

The capacitance of each membrane was obtained by measuring the time constant for the change in membrane p.d. when a rectangular pulse of current was applied. It was assumed that both membranes behave as a parallel resistance–capacitance circuit, with time constant, τ_m, given by R_mC_m, where R_m is the area specific resistance and C_m the capacitance per unit area. Values of C_m are shown in Table 1. These values were calculated on the basis that τ_m is the time taken for the change in p.d. to reach 63% of its final value. Strictly speaking, τ_m will lie somewhere between 63%, for a simple resistance–capacitance circuit, and 84%, the value for an infinitely long cable.

(*c*) ψ–J *Characteristics of Plasmalemma and Tonoplast*

Figure 1 shows ψ_{co} and ψ_{vc} as functions of J, the current density, with the cell in AHW(K0·1Ca1·5) and AHW(K1Ca1·5). Replacement of the chloride ions in AHW(K0·1Ca1·5) with sulphate ions had very little effect on the curves.

(*d*) *Transient Changes in Potential Difference and Resistance of Plasmalemma and Tonoplast during the Action Potential*

The action potential at the plasmalemma had two phases, an initial positive-going "spike", reached about 100–500 msec after the stimulus, followed by a second peak (which may be more of a plateau) of smaller magnitude at about 3 sec. After

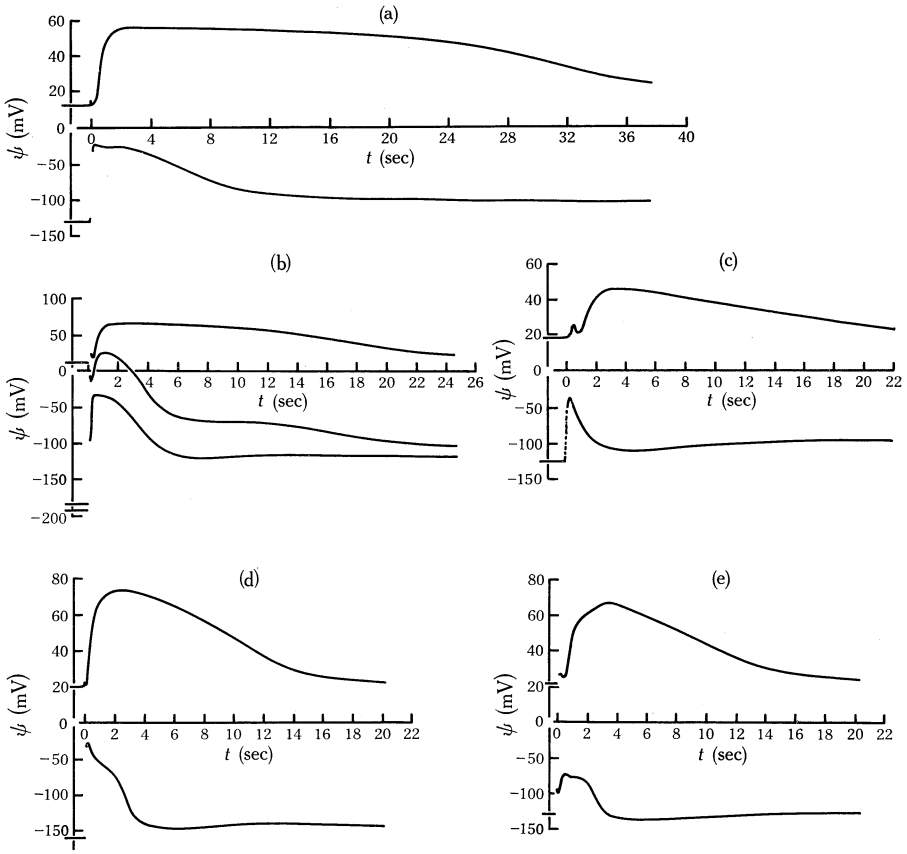

Fig. 2.—Various types of action potential. The upper curves are for the tonoplast, and the lower curves for the plasmalemma. In (*b*) the middle curve is the action potential between the vacuole and the outside. Note that, except in (*b*), the scale for the p.d. across the tonoplast is not the same as that for the p.d. across the plasmalemma. (*a*) A typical action potential in a cell in AHW (K1Ca4). (*b*) As for (*a*), but in a different cell, showing the course of ψ_{vo} as well. (*c*) An action potential in a cell in AHW(K1Ca4), where only the first component of the action potential at the plasmalemma occurred, and where the tonoplast action potential did not show a plateau. (*d*) The action potential in a cell in AHW(K0·1Ca1·5). The second peak in the plasmalemma component is diminished, and there is no plateau in the tonoplast component. (*e*) Cell in AHW (K1·0Ca1·5). Remarks similar to those for (*d*).

the two action potential peaks the p.d. was generally less negative and the resistance lower than the original resting levels and recovery towards the resting levels took several minutes. Occasionally, the second peak was absent, and the initial peak was followed by a quick recovery of the p.d. towards the original level, or even below it

with an eventual return to the resting level. This behaviour occurred more often in cells with a reduced calcium concentration in the external medium.

The action potential at the tonoplast was a positive-going change in p.d. of magnitude 40–60 mV, reaching a peak in about 5 sec. The time course of this action potential was usually determined by the type of action potential at the plasmalemma. If the action potential at the plasmalemma had the usual two peaks, the action potential at the tonoplast showed a plateau which lasted up to 20 sec. In cells where the second peak of the plasmalemma action potential was absent, or diminished, the

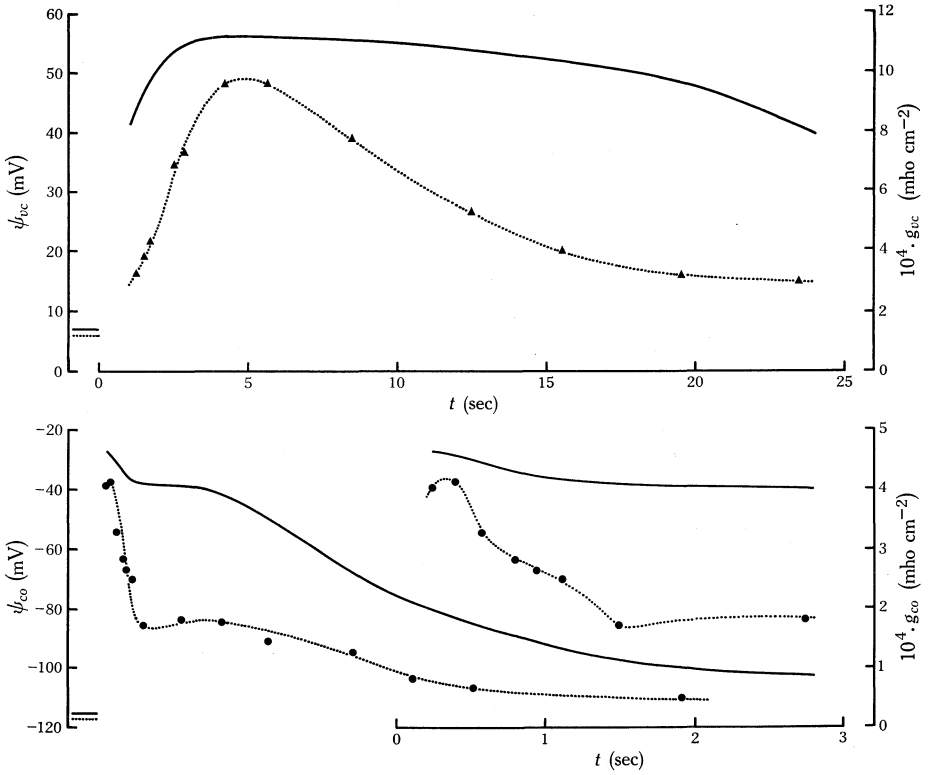

Fig. 3.—Time course of plasmalemma and tonoplast p.d. and conductance during an action potential. The continuous curves are p.d. and the dotted curves are drawn visually through the calculated values of conductance. The values of conductance and p.d. prior to the start of the action potential are shown by the lines preceding time zero. The curves in the bottom right of the diagram are on the expanded time scale shown.

tonoplast action potential did not have a plateau. The action potential at the tonoplast was also closely linked to that at the plasmalemma in the sense that an action potential could not be initiated at the tonoplast alone, but only appeared after the start of an action potential at the plasmalemma. The various types of action potential are shown in Figure 2.

The resistance of the two membranes during the action potential was measured by following the initiating pulse with a further series of current pulses. For the first second or so, these pulses had a magnitude of about 70% of the initiating pulse, and

after this were about 20%. The larger pulses were arranged to coincide approximately with the time during which the resistance of the plasmalemma was low, and hence larger current pulses were needed to ensure that changes in p.d. at the plasmalemma were of sufficient magnitude to be measured with reasonable accuracy. Changes in conductance of the plasmalemma and tonoplast during the action potential are illustrated in Figures 3 and 4. No corrections have been made as the resistance of plasmalemma plus tonoplast does not go below 5000 Ω cm^2, and errors due to the cable-like properties of the cell in estimating the resistance should be less than 5%.

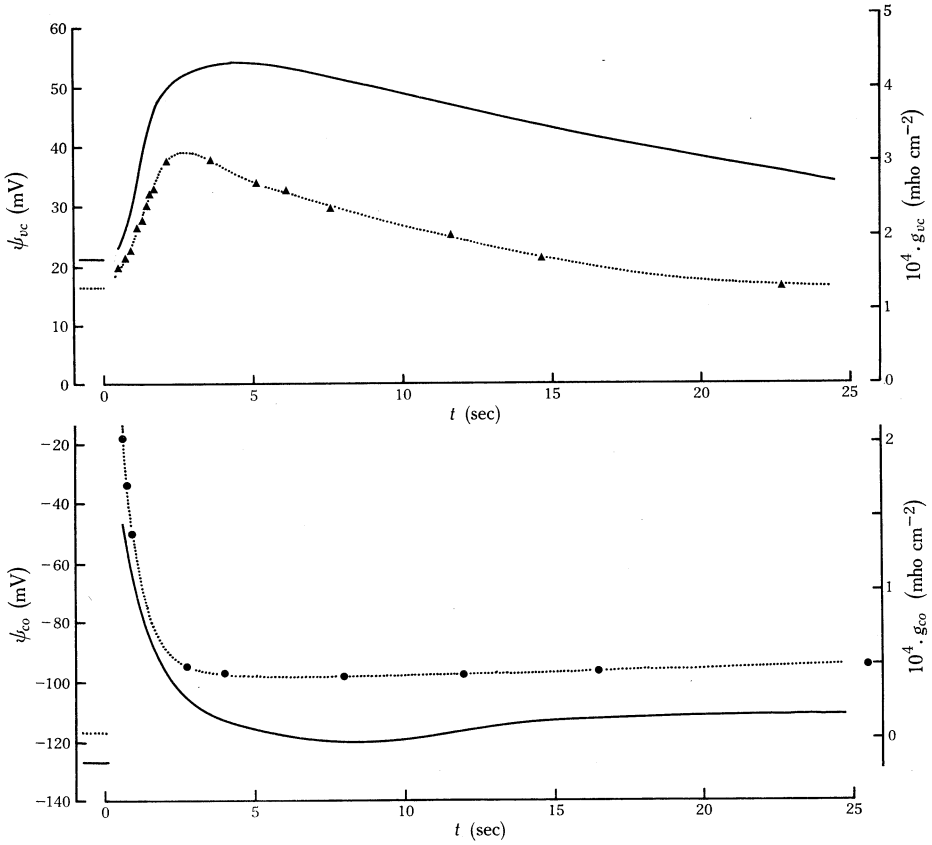

Fig. 4.—As for Figure 3, but for the case where the second component of the action potential at the plasmalemma was absent. Note that the peak value of g_{vc} occurs earlier than in Figure 3.

In quite a number of cells, after one or two action potentials the resting p.d. of the plasmalemma became less negative, and eventually the membrane became inexcitable. After 1 or 2 hr the p.d. was often as low as −30 mV. However, by holding the plasmalemma p.d. at its original level with a continuously applied current it was possible to produce action potentials of normal time course.

(*e*) *Effects of Changes in Concentration of Calcium, Potassium, and Chloride in AHW*

Changes in the concentration of calcium ions in the external medium affected both the resting p.d. and the peak of the action potential at the plasmalemma.

A decrease in [Ca_o] or replacement of the equivalent amount with magnesium from 4 mM to 0·5 mM depolarized the plasmalemma by up to 25 mV and the peak of the action potential became more negative. Complete replacement of calcium by magnesium caused a continuous decline in plasmalemma p.d. to a low level; the cell also became inexcitable. The resistance of the plasmalemma decreased as calcium concentration was decreased. These effects were usually reversible. Changes in [Ca_o] had negligible effects on the tonoplast. Replacement of calcium by strontium at 3 mM or higher, as well as causing the changes attributable to lowered calcium concentration, caused a plateau lasting several seconds to occur in the action potential at the plasmalemma.

A change in external medium from AHW(K0·1Ca1·5) to AHW(K1Ca1·5) caused an average decrease in the plasmalemma p.d. of 21 mV, a decrease in the plasmalemma resistance of 30 kΩ cm^2, and caused the peak of the plasmalemma action potential to become more negative by up to 28 mV—see Table 1. The tonoplast was unaffected. All these effects were reversible.

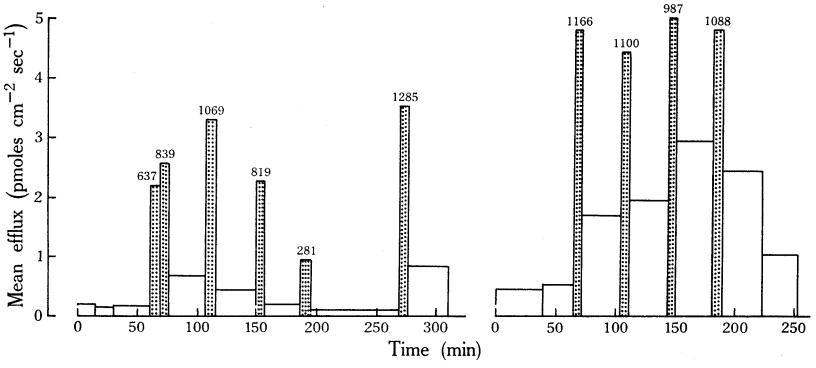

Fig. 5.—Measurements with two cells of mean chloride efflux during the resting state of the cell and during the action potential. The unhatched areas indicate the mean resting efflux, and the hatched areas the mean efflux over the time period that included one action potential. The numbers above each hatched area represent the extra amount of chloride ions (pmoles cm^{-2}) lost during the action potential.

[Cl_o] was increased by adding either tetraethyl ammonium chloride or choline chloride to AHW(K1Ca4) or AHW(K0·1Ca1·5) and decreased by replacement with benzene sulphonate. Decreases in chloride concentration had no appreciable or consistent effect on the peak of the action potential at either plasmalemma or tonoplast, but often caused a shortening of the duration of the action potential at the plasmalemma. Increases in chloride likewise had little effect on the peaks of the action potential, but occasionally produced a prolongation of the action potential at the plasmalemma.

(*f*) *Chloride Fluxes during the Action Potential*

Figure 5 shows the results of measurements on two cells of mean chloride efflux during the resting state of the cell and during the action potential. The unhatched areas in the diagram indicate the mean resting efflux, and the hatched areas the mean efflux over the time period that included one action potential. The extra

chloride efflux caused by the action potential was calculated by subtracting from the total efflux a resting efflux determined by taking the mean of the values for efflux in the periods immediately before and after the action potential. The numbers above each hatched area represent the extra amount of chloride ions (pmoles cm^{-2}) lost during the action potential. In a third cell there was a mean extra chloride efflux of 150 pmoles cm^{-2}. In two other cells the results were not conclusive, but there the action potentials were of shorter duration than normal, and may not have propagated over the whole cell. Nevertheless, it is clear that there is an extra efflux of chloride ions caused by the action potential.

The occurrence of an action potential also caused an overall increase in the resting chloride efflux. This is particularly noticeable in Figure 5.

IV. Discussion

The values obtained for concentrations of K, Na, and Cl are not greatly different from those given by MacRobbie and Dainty (1958) for the same species of *Nitellopsis*, although their cells came from a different source, and their external medium contained less calcium (0·67 mM).

The measurements of vacuolar ionic concentrations at the beginning and end of a 3-month period suggest that there was a net influx of both potassium and chloride ions into the cells, but sodium ions were in a steady state, with zero net flux. The electrochemical equilibrium potentials calculated from the Nernst equation,

$$\psi = (\mathbf{R}T/z\mathbf{F})\ln(C_o/C_i),$$

with the values of concentrations given in Section III(a), are $\psi_K = -115$, $\psi_{Na} = -23$ and $\psi_{Cl} = +40$. The observed p.d. in the vacuole was −139 mV, and thus there was an active transport of sodium ions out of the cells. The net chloride influx, because it was directed against the electrochemical gradient, was an active flux. In fact, almost all the chloride influx would have been active because for passive fluxes of chloride ions with the given electrochemical gradient the ratio of influx to efflux from the Ussing equation would be about 10^{-4}, and this is not the case—see Section III(f). No definite conclusion can be made about potassium because although the net flux is in the direction of the electrochemical gradient, the efflux is not known.

The p.d. across the plasmalemma is sensitive to changes in $[K_o]$ (see Table 1). When AHW(K1·0Ca1·5) was changed to AHW(K0·1Ca1·5), ψ_{co} changed by −23 mV. If a Goldman model is assumed for the plasmalemma with

$$\psi_{co} = 58\log_{10}([K_o]+\alpha[Na_o])/([K_c]+\alpha[Na_c]), \quad (2)$$

where $\alpha = P_{Na}/P_K$ (P_{Na}, P_K are the permeabilities of the membrane to Na and K respectively) and c refers to the cytoplasm, then a change in ψ_{co} of 23 mV for a change in $[K_o]$ from 0·1 to 1·0 mM gives $\alpha = 0{\cdot}02$ and $C_c = 230$ mM with $C_c = [K_c]+\alpha[Na_c]$. For *Nitellopsis obtusa*, MacRobbie and Dainty (1958) have shown that the ratio of potassium concentration to sodium concentration in the cytoplasm is about the same as in the vacuole, hence there is very little discrimination between sodium and potassium at this membrane. They conclude that the sodium and potassium fluxes across the tonoplast are entirely passive. Thus

$\psi_{vc} = 58 \log_{10}([K_c]/[K_v]) = 58 \log_{10}([Na_c]/[Na_v])$. If $([K_v]/[K_c]) = ([Na_v]/[Na_c])$ and $C_c = [K_c] + 0{\cdot}02[Na_c] = 230$ mM, then one gets $[K_c]/[K_v] = 2{\cdot}4$ and $\psi_{vc} = +22$ mV. This value of ψ_{vc} is quite close to the measured values shown in Table 1.

In the three external media described, the resistance of the plasmalemma was five to eight times larger than the tonoplast resistance. The resistance of both membranes, but particularly of the tonoplast is higher than most values reported for other characean cells.

From flux data, MacRobbie and Dainty (1958) have calculated that, in the cells of *Nitellopsis* they used, the tonoplast resistance was about 250,000 Ω cm^2. A calculation for the plasmalemma, based on the constant-field equation of Goldman (1943), and using their flux data gives the resistance of the plasmalemma as 58·2 kΩ cm^2. However, there is considerable evidence (MacRobbie 1962; Walker and Hope 1969; Findlay, Hope, and Williams 1970) to show that for most plant membranes, there is an appreciable discrepancy between the membrane resistance measured electrically, and that calculated from the ion fluxes across the membrane. The discrepancy could be as much as a factor of 10. Assuming this value, MacRobbie and Dainty's estimate of the resistance of the tonoplast is then not too different from that shown in Table 1. Their rather low value of plasmalemma resistance could result from their use of a lower $[Ca_o]$. In Section III(*e*) it is shown that lowering $[Ca_o]$ causes a decrease in the plasmalemma resistance.

The ψ versus J curve for the tonoplast is quite different from that of the plasmalemma, the differential slope resistance $\partial\psi/\partial J$ increasing as ψ becomes more positive; $\partial\psi/\partial J$ for the plasmalemma increases as ψ becomes more negative. There was no indication of "punch-through" (Coster 1965) at either membrane over the range of J examined.

The action potential in *N. obtusa* is similar to the action potential in other characean cells (Gaffey and Mullins 1958; Oda 1960; Mullins 1962; Findlay and Hope 1964*a*), with a fast component at the plasmalemma, followed by a slower component of smaller magnitude at the tonoplast. However, the action potential at the plasmalemma usually has two peaks. The tonoplast component is characterized by a long plateau usually dependent on the occurrence of the second peak in the plasmalemma action potential.

Associated with an action potential is an increased chloride efflux and this evidence is consistent with the idea that during the action potential, the plasmalemma becomes predominantly permeable to chloride, and the p.d. moves towards the electrochemical equilibrium potential for that ion. During this time, there will be a net efflux of chloride ions down the electrochemical gradient. In *Chara globularis* (Gaffey and Mullins 1958), *C. australis* (Hope and Findlay, unpublished data) and freshwater *N. obtusa* (Haapanen and Skoglund 1967) the efflux of chloride is accompanied by an efflux of potassium ions. The values given by Haapanen and Skoglund for the amount of K^+ and Cl^- released by a single action potential, 2–4 $\times 10^4$ pmole cm^{-2} impulse^{-1}, are somewhat larger than those for the brackish water *N. obtusa* reported in this paper.

It is not clear whether one or both peaks in the plasmalemma action potential are due to changes in P_{Cl}. Using the equation,

$$\psi_{co} = 58 \log_{10}([K_o] + \alpha[Na_o] + \delta[Cl_c])/([K_c] + \alpha[Na_c] + \delta[Cl_o]), \qquad (3)$$

assuming α remains unchanged during the action potential, and putting $[Cl_c] = 20$ mM (MacRobbie and Dainty 1958), at the peak of the action potential with $\psi = -26$ mV, one gets $\delta = P_{Cl}/P_K = 4$. The expected change in the peak of the action potential when $[Cl_o]$ is changed from 35 to 60 mM is quite small, about 4 mV. Experimentally, no significant change in peak p.d. was observed, although the addition of tetraethylammonium chloride caused changes in the time course of the action potential.

While it is clear that the action potential at the plasmalemma is caused by changes in membrane permeability, there is less evidence that the action potential at the tonoplast is similarly caused. However, it is unlikely that changes in ionic concentrations in the cytoplasm caused by the action potential at the plasmalemma could produce such large changes in p.d. and resistance at the tonoplast. With each action potential about 800–1000 p-equiv. cm^{-2} of chloride and an equal amount of cation are lost from the cell. If the cytoplasm is 5 μm thick (and assuming all the chloride efflux comes from the cytoplasm) the decrease in concentration of chloride and cation in the cytoplasm is only about 2 mM. This small change would have a negligible effect on the p.d. and resistance of the tonoplast.

If the action potential at the tonoplast does result from a change in permeability to a particular ion, the most likely candidate is again chloride. To account for the change in ψ_{vc}, the electrochemical equilibrium potential for chloride would need to be more positive than the peak of the action p.d.; say +55 mV. From the Nernst equation $[Cl_c]$=20 mM, a reasonable value. If a Goldman model is assumed for the tonoplast, then

$$\psi_{vc} = 58 \log_{10}([K_c]+\alpha[Na_c]+\delta[Cl_v])/([K_v]+\alpha[Na_v]+\delta[Cl_c]). \quad (4)$$

In the resting state δ is small and if it is assumed that α is unchanged, and the peak value of ψ_{vc} is +58 mV and $[Cl_c] = 20$ mM, then $\delta = 15$. If α were to decrease, δ would be less than 15. During an action potential R_{vc} decreases to about one-fifth of its resting value.

One particularly interesting feature of the action potential in *N. obtusa*, and in *C. corallina*, is that the action potential at the tonoplast cannot be made to occur on its own but always occurs with the plasmalemma component. One possible explanation for this behaviour is that the two membranes have the same current threshold for an action potential. Alternatively, the flux of a particular ion into or out of the cytoplasm, resulting from the start of the plasmalemma action potential, causes a change in the tonoplast threshold sufficient to initiate an action potential. In *N. obtusa* there is also the further link between the plasmalemma and tonoplast action potentials in that the tonoplast action potential is prolonged when there is a second peak in the plasmalemma action potential.

The relationship between the peak of the plasmalemma action potential and $[Ca_o]$ is similar to other characean cells (Findlay and Hope 1964*a*) and indicates that the peak value of P_{Cl} is an increasing function of $[Ca_o]$ (see Mullins 1962). At the peak of the action potential at the plasmalemma the term $\delta[Cl_c]$ predominates over $[K_o]$ in equation (3), as shown by the fact that when $[K_o]$ is increased from 0·1 to 1·0 mM with a consequent increase in P_K and decrease in δ, ψ_{co} becomes more negative.

The slow recovery of the p.d. across the plasmalemma following the action potential is most likely a consequence of two factors, a transient local increase in

$[K_o]$ just outside the plasmalemma caused by the diffusional delay in the cell wall and an increased value of P_{Cl} slowly declining to its resting level. This increased P_{Cl} would account for the chloride efflux following an action potential being higher than its resting value. If it is further assumed that above a threshold value of ψ_{co} P_{Cl} slowly increases with time, the behaviour of some cells, where the plasmalemma slowly depolarized further after each action potential, becomes explicable. The application of a hyperpolarizing current would lower ψ_{co} below the threshold value and maintain P_{Cl} at a low level.

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VI. References

Coster, H. G. L. (1965).—A quantitative analysis of the voltage–current relationships of fixed-charge membranes and the associated property of "punch-through". *Biophys. J.* **5**, 569.

Findlay, G. P. (1959).—Studies of action potentials in the vacuole and cytoplasm in *Nitella*. *Aust. J. biol. Sci.* **12**, 412.

Findlay, G. P. (1964).—Ionic relations of cells of *Chara australis*. VIII. Membrane currents during a voltage clamp. *Aust. J. biol. Sci.* **17**, 388.

Findlay, G. P., and Hope, A. B. (1964*a*).—Ionic relations of cells of *Chara australis*. VII. The separate electrical characteristics of the plasmalemma and tonoplast. *Aust. J. biol. Sci.* **17**, 62.

Findlay, G. P., and Hope, A. B. (1964*b*).—Ionic relations of cells of *Chara australis*. IX. Analysis of transient membrane currents. *Aust. J. biol. Sci.* **17**, 400.

Findlay, G. P., Hope, A. B., and Williams, E. J. (1970).—Ionic relations of marine algae. II. *Griffithsia*: Ionic fluxes. *Aust. J. biol. Sci.* **23**, 323.

Gaffey, C. T., and Mullins, L. J. (1958).—Ion fluxes during the action potential in *Chara*. *J. Physiol., Lond.*, **144**, 505.

Goldman, D. E. (1943).—Potential, impedance, and rectification in membranes. *J. gen. Physiol.* **27**, 37.

Haapanen, L., and Skoglund, C. R. (1967).—Recording of the ionic efflux during single action potentials in *Nitellopsis obtusa* by means of high-frequency reflectometry. *Acta physiol. scand.* **69**, 51.

Hope, A. B., and Findlay, G. P. (1964).—The action potential in *Chara*. *Pl. Cell. Physiol., Tokyo* **5**, 377.

Hope, A. B., and Walker, N. A. (1961).—Ionic relations of cells of *Chara australis* R.Br. IV. Membrane potential differences and resistances. *Aust. J. biol. Sci.* **14**, 26.

Kishimoto, U., and Tazawa, M. (1965).—Ionic composition and electric response of *Lamprothamnium succinctum*. *Pl. Cell Physiol., Tokyo* **6**, 529.

MacRobbie, E. A. C. (1962).—Ionic relations of *Nitella translucens*. *J. gen. Physiol.* **45**, 861.

MacRobbie, E. A. C., and Dainty, J. (1958).—Ion transport in *Nitellopsis obtusa*. *J. gen. Physiol.* **42**, 335.

Mullins, L. J. (1962).—Efflux of chloride ions during the action potential of *Nitella*. *Nature, Lond.* **196**, 986.

Oda, K. (1960).—Two components of the action potentials in *Nitella* and *Chara*. *Sci. Rep. Tohoku Univ.* (Ser. IV, Biol.) **26**, 205.

Walker, N. A., and Hope, A. B. (1969).—Membrane fluxes and electric conductance in Characean cells. *Aust. J. biol. Sci.* **22**, 1179.

Williams, E. J., and Bradley, J. (1968).—Voltage-clamp and current-clamp studies on the action potential in *Nitella translucens*. *Biochim. biophys. Acta* **150**, 626.