THE ROLE OF PH IN THE PUNCH-THROUGH EFFECT IN THE ELECTRICAL CHARACTERISTICS OF CHARA AUSTRALIS

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Summary

The role of pH in determining the electrical characteristics of the membranes of *C. australis* was investigated. Voltage-current characteristics were obtained with external solutions of pH $4 \cdot 5$ -9 $\cdot 0$. The chord resistance to hyperpolarizing currents decreased as the external pH was increased. For large hyperpolarizations ($\Delta E < -170 \text{ mV}$) this effect was much greater than for small hyperpolarizations ($\Delta E \simeq -25 \text{ mV}$).

The membranes of these cells display the punch-through effect, that is a rapid decrease in the slope resistance at large hyperpolarizations.

The punch-through potential became more negative as the pH was increased (from $\sim -250 \text{ mV}$ at pH 4.5 to $\sim -450 \text{ mV}$ at pH 9.0).

An analysis is made of the effect of concentration of fixed charge on the electrical characteristics of double-lattice, fixed-charge membranes. It is shown that the punch-through potential is a function of the fixed-charge concentration so that if the latter arises from free ionized amine or acid groups on proteins, then the punch-through potential becomes more negative as the pH is increased.

I. INTRODUCTION

The voltage-current (V-I) characteristics of the membranes of *Chara australis* are very similar to those of a membrane consisting of two regions of fixed charge, of opposite sign, in contact.

For moderate changes in potential both systems display rectification. At large hyperpolarization the differential resistance decreases rapidly and finally approaches zero at a particular membrane potential difference. This rapid decrease in the membrane slope resistance, which has been termed "punch-through", occurs at about -170 mV hyperpolarization, that is for a plasmalemma potential difference (p.d.) of $\sim -300 \text{ mV}$, in cells of *C. australis* (Coster 1965). Recently Williams and Bradley (1968) have reported a similar effect in cells of *Nitella translucens*.

The presence of fixed or indiffusible ions in electrolytic systems have long been recognized as playing a major role in the electrical characteristics of such a system (e.g. Donnan 1911). The electrical properties of single fixed-charge membranes have been dealt with by Meyers and Sievers (1936) and by Teorell (1953).

A detailed analysis of the electrical characteristics of a double fixed-charge membrane has been given by Coster (1965). Recently George and Simons (1966) showed that for membranes whose thickness is ≤ 50 Å, some of the assumptions made in the former analysis are not justified. However, more recent computer studies (Coster, George, and Simons 1969) show that for membranes with a thickness >

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70 Å the analysis given by Coster (1965) is valid to a good approximation. Isotope tracer experiments have been made to determine the identity of the ionic conductance at punch-through in cells of C. *australis*, and these results have been published separately (Coster and Hope 1968).

The present paper is concerned with the role of pH in the electrical characteristics of the membranes of C. australis cells. The effect of changes in the concentration of fixed charges on the electrical characteristics of a double fixed-charge membrane is also considered, and these results are compared with those obtained for the C. australis cells.

II. Methods

The general electrophysiological techniques used have been described in detail by Walker (1955). Current was passed through the membranes via a microelectrode inserted into the vacuole and a Ag/AgCl grid in the external solution. The current could be scanned using a linearly increasing (or decreasing) ramp from a sweep generator. A resistance of 10⁶ ohm in series with the sweep generator ensured that the current passed was controlled by the output voltage of the sweep generator and was not affected by changes in membrane resistance. The potential differences across the plasmalemma (E_{oc}) and tonoplast (E_{cv}) were measured between microelectrodes inserted into the vacuole and cytoplasm, and an electrode in the external medium.

The rate at which the current was scanned could be varied between 2 and 50 μ A/sec (0.5–10 sec for a complete scan). In most of the experiments the current was scanned at a rate of ~ 8 μ A/sec. The effect of current scanning rate is discussed below.

Some experiments were also done using a voltage clamp technique similar to that described by Findlay (1964).

In the experiments, the pH of the artificial pond water* used as the external solution was adjusted with HCl or NaOH. V-I curves were obtained with pH values between 4.5 and 9.0.

III. EXPERIMENTAL RESULTS

(a) Voltage-Current Characteristics of the Plasmalemma and Tonoplast in Artificial Pond Water

Figure 1 shows the typical responses of the tonoplast and plasmalemma potential differences to a linearly increasing current which hyperpolarized the plasmalemma.

It can be seen that starting from the resting potential the resistance of the plasmalemma, defined as $R_{pl} = \Delta E/J$, where ΔE is the change in p.d. and J is the current density, first increased slowly and then decreased rapidly until at $E_{oc} \simeq -370 \text{ mV}$ the V-I curve folded over. That is, the differential resistance beyond this point had decreased drastically, since an increase in the current had a negligible effect on the potential difference.

At $E_{oc} = -380$ mV the differential resistance (though not the chord resistance) was zero. The fold in the V-I characteristics is referred to as punch-through.

Although the resistance of the tonoplast was very much smaller than that of the plasmalemma the general form of the relationship between its p.d. and the current density was similar to that of the plasmalemma. Punch-through also occurred in the tonoplast. At punch-through E_{cv} was negative (i.e. vacuole negative with respect to the cytoplasm; the resting value of E_{cv} is $\sim +10$ mV). The V-I characteristics of the plasmalemma, cytoplasm, and tonoplast in series were similar to those of the

* 0.1 mn KCl, 1.0 mn NaCl, 0.2 mn CaCl₂; pH 6.8

plasmalemma alone as would be expected from the results shown in Figure 1. Thus, the V-I relationships obtained with the electrodes in the vacuole and bathing medium can be taken as closely approximating those for the plasmalemma. For the investigation of the effect of pH on the V-I characteristics, the measurements were hence confined to the two membranes in series (i.e. to vacuolar recordings).



Fig. 1.—The separate responses of the potential differences across the plasmalemma and the tonoplast of a cell of C. australis to a linearly increasing hyperpolarizing current. The external solution was artificial pond water. RP, resting potential differences.

Fig. 2.--Results of a voltage clamp experiment, showing the current required to clamp the membrane at a particular potential as a function of the potential. The current electrode was a platinum-in-glass electrode inserted longitudinally into the cell, and the external solution was artificial pond water.

(b) Time Variations in the Voltage–Current Characteristics

Experiments were done to find the effect of current scanning rate on the V-Icharacteristics. For current scanning rates between 2 and 50 μ A/sec there was little effect on the V-I relationship.

At low current scanning rates the shape of the V-I curves, particularly near punch-through, was dependent on the scanning rate. In general the slope resistance near punch-through increased as the rate of current scanning was decreased. The punch-through potential, taken as the potential at which the membrane resistance first reached its minimum positive level in the hyperpolarizing scan, appeared to be largely independent of the current scanning rate.

V-I curves were also obtained with a voltage clamp technique. A typical result is shown in Figure 2. In general the overall appearance of such a V-I curve is similar to that obtained with the current scanning technique. Punch-through, i.e. a rapid decrease in the membrane resistance, was still observed, although usually the limiting value of this slope resistance was much higher than those obtained by the former method. With the current scanning technique the V-I curves often showed a region, beyond punch-through, which had a negative membrane resistance.

This effect was enhanced by faster scanning rates. No such effect was observed in the voltage clamp experiments.

(c) Effect of pH

(i) Voltage–Current Characteristics

Figure 3 shows the results of an experiment with pH of the external medium $(pH_o) = 5 \cdot 9$, 7 · 2, and 9 · 0. It can be seen that pH_o has a large effect on the V-I curves. From Figure 3 the chord resistance (i.e. $\Delta E/J$) was calculated for $\Delta E = -25$,





-100, and -170 mV. This is shown in Figure 4. It can be seen from this figure, which was typical of the results obtained, that increasing pH_o decreased the membrane resistance, and more so as ΔE became more negative.

Figure 5 shows the membrane conductance (i.e. $\partial J/\partial E$) as a function of ΔE for $\mathrm{pH}_o = 5.9, 7.2$, and 9.0. The conductances were calculated from the curves of E against J already shown in Figure 3. It can be seen that the conductances for moderate hyperpolarizations (i.e. $\Delta E \sim -140 \text{ mV}$) in high pH_o were greater than those in low pH_o . However, the rapid increase in conductance associated with punch-through swamped this effect beyond $\Delta E = -150 \text{ mV}$.

(ii) Punch-through

The effect of pH_o on punch-through was very marked. From Figure 3 it can be seen that the punch-through potential became more negative as pH_o was increased. In going from $pH_o = 4.5$ to 9.0 the punch-through potential shifted from -250 to



Fig. 4.—Membrane resistance at various values of ΔE as a function of pH_o. The resistance was calculated from voltage-current curves similar to those shown in Figure 3 and for the same cell.

Fig. 5.—Membrane conductance $(\partial J/\partial E)$ as a function of the change in membrane potential (ΔE) for various pH_o values. The conductance was calculated from the voltage-current curves shown in Figure 3. Asymptotes of each curve are indicated by vertical lines.



Fig. 6.—Punch-through potential as a function of pH_o for cells of *C. australis*. Different symbols represent experiments on different cells.

-425 mV despite the fact that the resting potential had shifted some 25 mV in the other direction over this pH range.

Figure 6 shows the effect of pH_o on the punch-through potential for three cells.

IV. THEORY

(a) The Double Fixed-charge Membrane Model

The membrane model to be considered here consists of a double lattice in which one side has fixed positive charges and the other fixed negative charges. The distributions of fixed charges in each half of the membrane in this model are assumed to be homogeneous and effects due to the lattice network which fixes the charges are neglected.

The profiles of potential and ion distribution for such a membrane have been given by Mauro (1962) and an analysis of the electrical characteristics has been given by Coster (1965).

For completeness, the basic features of this analysis are reviewed here as a basis for the extension of this treatment to be discussed in the next section.

The junction of a fixed positive-charge region with a fixed negative-charge region gives rise to a transition region which is almost completely depleted of mobile ions and where subsequently the space charge density is high. This transition region is also referred to as the depletion layer. The width of the depletion layer in the N^+ and N^- lattices is denoted by λ_N + and λ_N - respectively (cf. figs. 2 and 3, Coster 1965). A large junction potential exists across this depletion layer and it can be shown that, as a consequence of the ion depletion, any applied bias will appear almost completely across this region. When a potential V is applied across the membrane the current density is given (Coster 1965) by

$$J = q[(D_p P_m / W_N +) + (D_n N_m / W_N -)] \exp[(q V / \mathbf{k}T) - 1],$$
(1)

where D_p is the diffusion constant for positive mobile ions, q is the absolute value of the electronic charge, **k** is the Boltzmann constant, T is the absolute temperature, and P_m is the minority positive ion (i.e. co-ion) concentration in the N^+ fixed-charge lattice near the solution-lattice boundary. At zero applied bias the minority or co-ion concentration is constant and equal to P_m everywhere in the fixed-charge lattice outside the depletion layer. W_{N^+} is the width of the N^+ fixed-charge lattice excluding the width of the depletion layer, i.e. $W_{N^+} = W_o - \lambda_{N^+}$, where W_o is the total width of the fixed-charge lattice. Similar definitions hold for D_n , N_m , and W_{N^-} for negative ions. W_{N^+} and W_{N^-} are a function of the applied bias. This is a consequence of the fact that the applied bias appears across the depletion layer. Any changes in the potential (and hence field) must be balanced by changes in the space charge regions in this layer. The actual expressions can be obtained by solving Poisson's equation,

$$d^2\Psi/dx^2 = -\rho(x)/\epsilon, \qquad (2)$$

where Ψ is the electrostatic potential, ρ is the net or space charge density (in C/m³), and ϵ is the dielectric constant. Then, following the previous reference, in the depletion layer,

$$W_{N^{+}} = W_{o} - \{(2\epsilon/qN^{+})[(\mathbf{k}T/q)\ln(N^{+}/N_{o}) - K(E_{m}+V)]^{\frac{1}{2}},$$

$$W_{N^{-}} = W_{o} - \{(2\epsilon/qN^{-})[(\mathbf{k}T/q)\ln(N^{-}/P_{i}) - (1-K)(E_{m}+V)]\}^{\frac{1}{2}},$$
(3)

and

where
$$E_m$$
 is the resting potential (inside with respect to outside), $N_o(=P_o)$ is the concentration of anions in the outside solution, $P_i(=N_i)$ is the concentration of cations in the internal solution, and K is the fraction of the applied bias (and the resting and junction potential) that appears across the N^+ half of the depletion layer (for a completely symmetrical situation $K = 0.5$).

Inspection of equation (3) reveals that W_N + and W_N - decrease as V becomes more negative (i.e. the hyperpolarizing direction). Eventually W_{N+} or W_{N-} or both approach zero. At this stage the depletion layer width has approached that of the whole lattice. From equation (1) it can be seen that at this stage the current increases extremely rapidly with potential. That is, the differential resistance approaches zero. This is the punch-through effect. The overall shape of the voltage-current characteristics predicted by this model is very similar to that obtained for the membranes of cells of C. australis (Fig. 2; see also Coster 1965, fig. 5).

It should be pointed out that the analysis breaks down as soon as λ_N + becomes comparable with the width of the fixed-charge lattice since it is assumed that the profiles are able to attain steady values within the lattice (outside the depletion layer). This is obviously impossible when $W_{N^{\pm}} \rightarrow 0$. Thus, the actual punch-through potential calculated from equation (3) by putting $W_{N+} = 0$ represents a limiting case for which the analysis and hence equation (3) is not valid.

(b) Effect of Changes in Fixed-charge Concentration

In principle the membrane need not be symmetrical in the sense that (1) the concentration of fixed charges in the two lattices need not be equal, and (2) the widths of the N^+ and N^- regions need not be equal.

Considering the depletion layer as a whole and remembering that the majority of uncompensated fixed charge occurs in this region, overall electric neutrality must be obeyed, and hence it follows that

$$\rho^+ \lambda_N + = \rho^- \lambda_N -, \tag{4}$$

where ρ^+ and ρ^- are the space charge densities in the N^+ and N^- half of the depletion layer respectively. The fixed charges are almost completely uncompensated by counter-ions in this region, i.e. $\rho^+ \approx N^+$ and $\rho^- \approx N^-$, and thus

$$N^+ \lambda_N^+ \simeq N^- \lambda_N^-. \tag{5}$$

Consider the special case when $N^+ > N^-$; then to balance the overall charge $\lambda_N \rightarrow \lambda_N$. That is, the depletion layer (i.e. space charge region) will extend further

into the N^- region than into the N^+ region. This effect is shown qualitatively in Figure 7.

Further, if $\lambda_N - > \lambda_N +$, then the fraction of the membrane resting potential and any applied bias that appears across the N^- half of the depletion layer (1-K)will be larger than the fraction K across the N^+ half. It then follows from equation (3) that W_N^- will decrease more rapidly than W_N^+ with increasing reverse (i.e. hyperpolarizing) applied bias. That is, the depletion layer will grow more into the N^-



Fig. 7.—Profiles of the electrostatic potential Ψ and the depletion layer thickness in the N^+ and N^- lattices when $N^+ \simeq N^-$, $N^+ > N^-$, and $N^+ < N^-$. The depletion layer in each case is shaded. The relative magnitude of the lattice junction potential Ψ_j is also shown qualitatively. Note that $V_i = -(1-K)E_m$ and $V_o = -KE_m$, where E_m is the resting potential.

lattice than into the N^+ lattice as the reverse bias is increased. From equation (1) it follows that the current carried by negative ions (i.e. chloride ions) for this special case will increase as W_{N^-} decreases. Punch-through will then occur when $W_{N^-} \to 0$ and J_{anions} becomes very large. An analogous argument of course will apply if $N^+ < N^-$ in which case punch-through would be associated with a large increase in cation conductance.

For the special case when $N^+ \neq N^-$ and punch-through is associated with a large increase in either anion or cation conductance, the punch-through potential can be deduced from equation (3) by letting either W_{N^-} or $W_{N^+} \rightarrow 0$. Thus for $W_{N^-} \rightarrow 0$,

$$(E_m + V_{pt}) = [(\mathbf{k}T/q)\ln(N^-/P_i) - (qN^-W_o^2/2\epsilon)]/(1-K).$$
(6)

Note that $(E_m + V_{pt})$ is a negative quantity. The fraction K of the depletion layer junction potential, resting potential, and applied bias that appears across the N^+ half of the depletion layer can be related to the width of the depletion layer by solving Poisson's equation in the depletion layer. Two integrations of equation (2) and substitution of (5) reveals that the potential across the N^+ half, $\Psi_{\lambda N^+}$ is given by

$$\Psi_{\lambda_N^+} = K \Psi_j \propto N^+ (\lambda_N^+)^2,$$

$$\Psi_{\lambda_N^-} = (1 - K) \Psi_j \propto N^- (\lambda_N^-)^2.$$
(7)

and similarly

Hence, by making use of equation (5), $\left[\frac{1}{(1-K)}\right]$ in equation (6) is given by

$$1/(1-K) = 1 + (N^{-}/N^{+}).$$
 (8)

Substitution of equation (8) into (6) shows that the punch-through potential is a function of the ratio N^{-}/N^{+} .

Figure 8 shows a plot of the variation of the punch-through potential with the concentration of fixed positive charges, N^+ , in the double membrane. For this plot it was assumed that when $N^- = N^+ = 6 \times 10^{25}$ charges/m³ (i.e. 0.1N) the punchthrough potential $(E_m + V_{pt}) = -300 \text{ mV}.$



Fig. 8.—Punch-through potential as a function of the concentration of fixed positive charges in the doublelattice membrane. N^+ is in units of $0 \cdot \ln$. The punchthrough potential was calculated from equations (6) and (8), assuming that when $N^- = N^+ = 6 \times 10^{25}$ charges/m³ (0.1N) the punch-through potential $(E_m + V_{pt}) =$ -300 mV.

V. DISCUSSION

The order of magnitude of the resting resistance of these cells was found to be consistent with those measured by Walker (1960), Hope and Walker (1961), and Findlay and Hope (1964).

The V-I characteristics near the resting potential can be fitted reasonably well by the Goldman equation (Hope and Walker 1961). For large hyperpolarizations, however, this is not so. The steep increase in conductance of the membranes which eventually leads to ionic punch-through cannot be described by any simple, constantfield model, such as that of Goldman (1943).

Chloride fluxes, measured with tracers, show that the chloride conductance rises very steeply at large hyperpolarizations and accounts for most of the total conductance near punch-through (figs. 2 and 3 in Coster and Hope 1968).

For the fixed-charge membranes this result suggests that W_N - is decreasing more rapidly than W_{N+} and that punch-through occurs when $W_{N-} \rightarrow 0$. Following the treatment in Section IV this is possible when $N^+ > N^-$.

The contribution of hydrogen ion fluxes to the total conductance is likely to be very small (Coster and Hope, unpublished results). This is also consistent with the fact that as pH_o was increased the membrane resistance to hyperpolarizing current decreased (see Fig. 4). It appears more likely that the effect of pH is an effect on the membrane properties.

It should be pointed out here that the pH in contact with the membrane is not necessarily the same as the pH of the external solution due to the buffering effect of the cell wall, which is known to be a Donnan phase (Dainty and Hope 1959; Spanswick and Williams 1965).

The effect of pH_o on the membrane properties can be visualized quite readily in the case of fixed-charge membranes if the fixed charges arise from ionized groups

such as COO⁻ and NH_3^+ on proteins. The density of these charges will then depend on the pH of the solution in contact with the membrane and the titration characteristics of the amine: acid groups on the protein. An increase in the pH should produce a decrease in the acidic form of free amine: acid groups, resulting in an increase in N^- and a decrease in N^+ . The punch-through potential is a function of the ratio N^-/N^+ in the double fixed-charge membrane as shown in Figure 8. On this scheme punch-through occurs when $W_{N^-} \rightarrow 0$, and this would be accompanied by a large increase in anion conductance (i.e. chloride).

The analogy between the electrical behaviour of the fixed-charge membranes and that of C. australis is striking and it is indeed tempting to suggest that the membranes of C. australis contain a double fixed-charge system.

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