IONIC RELATIONS OF CELLS OF *CHARA AUSTRALIS* R.BR.

IV. MEMBRANE POTENTIAL DIFFERENCES AND RESISTANCES

By A. B. HOPE* and N. A. WALKER†

[*Manuscript received September* 2, 1960]

Summary

Experiments are described in which the electric potential difference and resistance between the cytoplasm and the external medium were measured in cells of *Chara australis*. The method was designed to eliminate the effect of the negatively charged Donnan system of the cell wall. Both the potential difference and the resistance are attributed to the outer cytoplasmic membrane. It is shown that they may be quantitatively explained by the passive diffusion of potassium and sodium ions across the membrane with permeabilities of the order of 10^{-5} and 10^{-6} cm sec^{-1} respectively. The resistance–voltage characteristic of the membrane is accurately predicted by the constant field equation of Goldman (1943). The significance of these findings is discussed.

I. INTRODUCTION

The resting potential of single cells of the Characeae has been the subject of much investigation. The resting potential is the electric potential difference (p.d.) between the vacuolar sap of the cell and the medium bathing the cell. It is best measured by means of salt-bridges in contact with these phases. However, in much early work (e.g. Osterhout and Harris 1929; Osterhout 1930; Osterhout and Hill 1938) measurements were made of the p.d. between two different liquid contacts on the cell surface. Microelectrodes inserted into the cell were employed by Umrath (1930, 1934), Studener (1947), and Walker (1955). It was shown that, in *Nitella*, the resting potential arises as a p.d. between the external medium and the cytoplasm, there being no measurable p.d. between the cytoplasm and the vacuolar sap (Walker 1955). The effects of the concentrations of ions in the external medium have been often investigated; increasing concentrations of cations reduce the magnitude of the resting potential, potassium having a greater effect than other cations. Only Osterhout has attempted a quantitative treatment.

The resting potential of the cell has been ascribed to various mechanisms, among them being:

(1) A diffusion potential in one or more cellular membranes which separate phases of different ion concentrations (see Osterhout 1952);

(2) A redox potential in a layer (membrane) allowing electronic conduction and separating phases of different redox potentials (see Lund 1947);

(3) A membrane containing oriented dipoles (Umrath 1942); and

* Plant Physiology Unit, Division of Food Preservation, C.S.I.R.O., and Botany School, University of Sydney.

† Division of Plant Industry, C.S.I.R.O., Canberra; present address: Physiological Laboratory, University of Cambridge.

(4) A Donnan distribution due to the presence of indiffusible ions in the cytoplasm of the cell (Blinks 1940; Hope and Robertson 1953).

Blinks and Pickett (1940) have provided experimental evidence against the redox mechanism (2), and there has been no theoretical or experimental justification advanced for the dipole mechanism (3). The Donnan mechanism (4) seems unable, alone, to account for the different effects of sodium and potassium ions on the resting potential; it may, however, occur in combination with another mechanism such as (1).

Osterhout proposed mechanism (1), and supported it by many experimental studies. There are a number of reasons for re-examining the question. His results were compared with the predictions of Henderson's equation for liquid junction potentials, an equation which is simple, but which is based on an impossible assumption (Johnson, Eyring, and Polissar 1954). His experiments, too, are sometimes of doubtful validity—e.g. the permeability ratio for potassium to sodium was calculated from potentials measured in 10 mN KCl. This concentration of KCl, as Osterhout himself showed, frequently causes an abnormal change in resting potential, which is related to the action potential.

The measurements described in this paper were made with inserted microelectrodes, and gave the p.d. between medium and cytoplasm or medium and vacuole directly. These p.d.'s. are here assumed to be equal. Methods using external contacts, such as that of Osterhout, give only the algebraic sum of two such p.d.'s The present experiments were designed to keep the Donnan potential of the cell wall constant, while keeping the cell under nearly physiological conditions. The Donnan potential of the wall was not involved in the overall p.d. between medium and cytoplasm. The measured p.d.'s are compared with the predictions of modern equations for diffusion potentials across membranes.

The first measurements of membrane resistance in these cells were made by Blinks (1930, 1936), using direct current (d.c.). The highest surface specific resistance he found, 250 kΩ cm^2,* has been widely quoted (Cole 1942; MacRobbie and Dainty 1958) without consideration of the conditions of his experiment. He observed that potassium ions in the external medium reduced the resistance more than sodium, lithium, and other ions. Neither he, nor Umrath (1940), nor Weidmann (1949) offered a quantitative interpretation of their results. Bennett and Rideal (1954) made measurements of resistance in *Nitella* cells using a microelectrode of Ag/AgCl, but since they used alternating current of frequency 1000 c/s the current flow was not homogeneous through the cell surface. Thus their measured resistances and capacitances are difficult to interpret. In none of these experiments is it possible to distinguish the contributions of the tonoplast and plasmalemma to the measured resistance. Walker (1957, 1960) found in *Nitella* that the plasmalemma contributed most or all of the measured membrane resistance. The values of surface specific resistance found by different workers have varied widely between the 250 kΩ cm^2 of Blinks (1930), the 50 kΩ cm^2 of Findlay (1959), and the 5 kΩ cm^2 of Walker (1960).

The present paper reports measurements of membrane resistance as a function of the composition of the external medium and as a function of current density.

* i.e. 250,000 ohms for each cm^2 of cell surface.

These were made concurrently with the measurements of membrane potential. A quantitative treatment of these sets of data is attempted, in terms of the permeabilities to ions of the plasmalemma, and of the ionic activities on each side of this membrane.

II. Experimental Method

The material used was *Chara australis* R.Br. var. *nobilis* A.Br.; it was collected from field ponds and remained viable for some weeks in the laboratory in an artificial pond water.* Cells for experiment were freed from neighbouring cells, and soaked for 2–6 hr, or occasionally overnight (16 hr) in 5·0 mN NaCl solution, and then transferred to 1·0 mN NaCl+0·1 mN KCl to await use. The aim was to remove the exchangeable calcium from the cell wall. During experiments the cells were bathed in a flow of fresh solution of constant normality (1·1 mN or 2·0 mN total). The effects of changes in the ratio of sodium to potassium or in the nature of the anion were measured.

Measurements of membrane potential and resistance were made with two inserted microelectrodes; the method was similar to that of Walker (1960). Two reference electrodes dipped into the external solution; a calomel half-cell and salt-bridge for potential measurements, and a coiled Ag/AgCl electrode for resistance measurements. The microelectrodes were generally inserted into the vacuole a distance of 100–200 μ. Under these conditions the "seal" described by Walker (1955) between the glass of the electrode and the cytoplasm did not form within 6 hr. After the insertion of the electrodes the potential was frequently steady ($\pm$0·5 mV) within 2 min.

The electrical measuring apparatus was similar to that used by Walker (1960). It was capable of measuring p.d. to within $\pm$0·1 mV, but usually the accuracy was $\pm$0·5 mV. Current was measured to within $\pm$2%. The measurement of membrane resistance was accurate to about $\pm$5%.

III. Results

(*a*) *Potential Difference Measurements*

The p.d. between cytoplasm and medium during two types of change in the external medium is shown in Figure 1. In one experiment (Fig. 1(*a*)), the external medium was changed from 0·1 mN KCl to 1·0 mN KCl; in the other (Fig. 1(*b*)), it was changed from 1·0 mN NaCl+0·1 mN KCl to 0·6 mN NaCl+0·5 mN KCl. It would be expected that in the latter case (but not in the former) the Donnan potential of the cell wall would remain constant. Comparison of the time courses in Figure 1 shows a marked difference. The rapid initial rise in curve (*a*) is absent in (*b*), which suggests that it is a change in the wall potential. The monotonic rise in (*b*) is due to a membrane which distinguishes between potassium and sodium; presumably the plasmalemma. The rise in potential has a half-time of 3–5 min, the significance of which is discussed below.

* This contained Na, 1·0 mN; Ca, 0·5 mN; K, 0·1 mN; and Cl, 1·6 mN.

In the following experiments the p.d. was measured at equilibrium after a change in the concentration of potassium (and sodium), *keeping the total concentration constant* as in Figure 1(*b*). On restoring an original concentration after such a change, the p.d. generally returned to a value within 2–4 mV of the original. A typical sequence of external concentrations of potassium chloride (K_o) (with that of sodium (Na_o) in parenthesis) was 0·1 (1·0), 0·25 (0·85), 0·4 (0·7), 0·7 (0·4), 1·0 (0·1), 0·7 (0·4), 0·4 (0·7), 0·25 (0·85), 0·1 (1·0) mN. In such experiments, when the repeatability was as good as mentioned above, the mean of the two determinations of p.d. (E_{oi})* for a given K_o was plotted against $\log_{10} K_o$. Figure 2 shows a typical

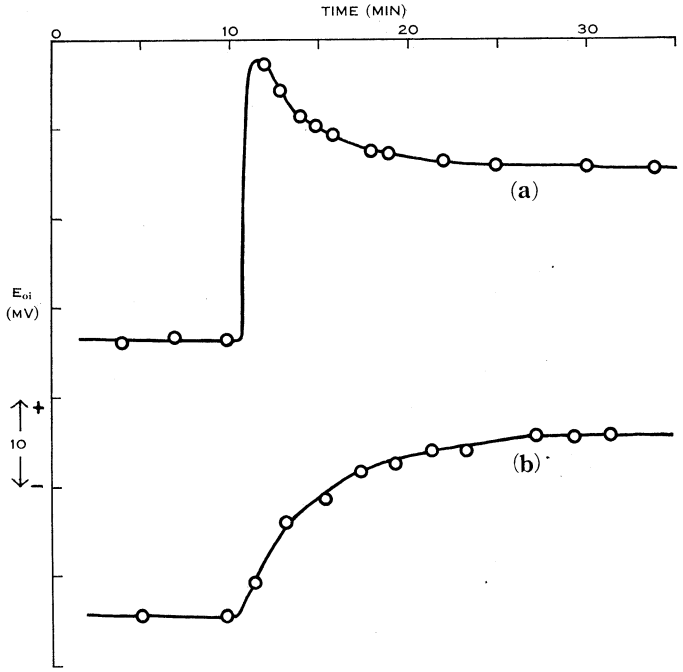

Fig. 1.—Time courses of the potential difference between the interior of the cell and the bathing medium (E_{oi}). In each case the potassium concentration in the bathing medium was changed at 10 min on the time scale. (*a*) Bathing medium changed from 0·1 mN KCl to 1·0 mN KCl. (*b*) Bathing medium changed from 0·1 mN KCl+1·0 mN NaCl to 0·5 mN KCl+0·6 mN NaCl.

result where the external anion was chloride, and the total external cation concentration was 1·1 mN. Similar results were obtained with anions other than chloride, and with a total cation concentration of 2·0 mN (Fig. 3). At higher total cation concentrations, the high potassium end of the range could not be explored, as spontaneous action potentials occurred. In each of Figures 2, 3, and 4 a theoretical curve has been drawn, based on the equation:

$$E_{oi} = (RT/F)\ln[(K_o + \alpha \cdot Na_o)/(K_i + \alpha \cdot Na_i)], \quad (1)$$

where α ($= P_{Na}/P_K$) is the ratio of the permeabilities of the interface between *o* and

* $E_{oi} = E_i - E_o$, and thus has the sign of phase *i* relative to *o*.

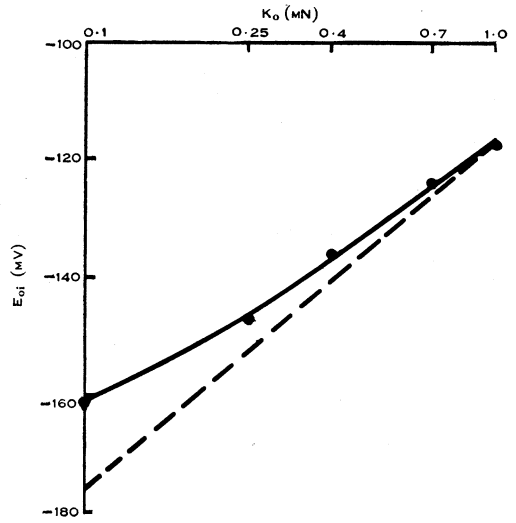

Fig. 2.—Potential difference between the cell interior and the bathing medium, as a function of the potassium concentration in the medium (keeping $K_o+Na_o = 1 \cdot 1$ mN). ● Experimental points (means for one cell). ——— Fitted curve based on equation (1), with $\alpha = 0 \cdot 074$ and $K_i+\alpha \,.\, Na_i = 97$ mN. – – – – Limiting slope of 58 mV per log unit ($\alpha = 0$).

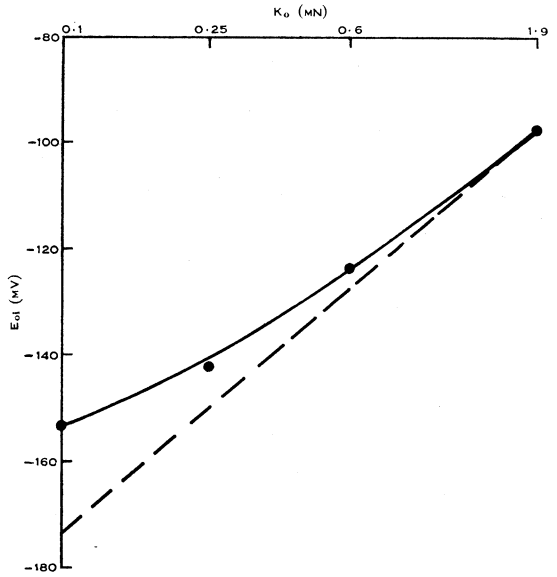

Fig. 3.—Potential difference between the cell interior and the bathing medium, as a function of the potassium concentration in the medium (keeping $K_o+Na_o = 2 \cdot 0$ mN). ● Experimental points (means for one cell). ——— Fitted curve based on equation (1), with $\alpha = 0 \cdot 059$ and $K_i+\alpha \,.\, Na_i = 90$ mN. – – – – Limiting slope of 58 mV per log unit ($\alpha = 0$).

i to sodium and potassium, and ($K_i + \alpha \cdot Na_i$) is an internal concentration parameter. Both α and ($K_i + \alpha \cdot Na_i$) are assumed constant (see Section IV).

Although the behaviour depicted in Figures 2 and 3 was frequently obtained, in some experiments consistent differences between such theoretical curves and the experimental points were noted. Figure 4(*a*) illustrates deviations from the "normal" curve at the high potassium end of the concentration range and Figure 4(*b*) at the high sodium end.

One of the assumptions implicit in equation (1) is that the membrane is not significantly permeable to the anion. In some experiments the anion was changed

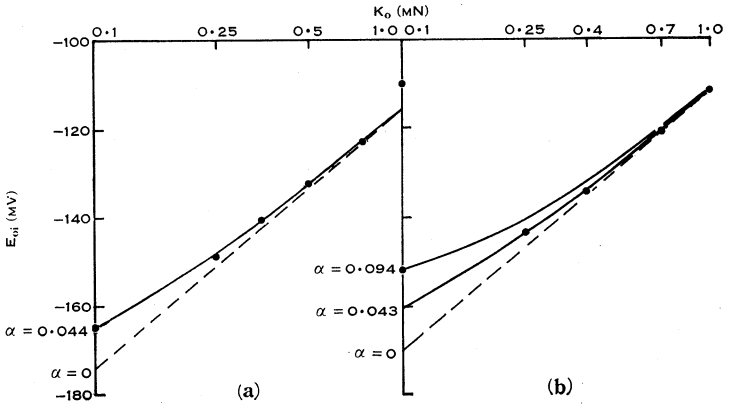

Fig. 4.—Potential difference between the cell interior and the bathing medium as a function of the potassium concentration in the medium ($K_o + Na_o = 1 \cdot 1$ mN). (*a*) and (*b*) are different experiments. ● Experimental points (means for one cell). ——— Fitted curves based on equation (1): values of α used in the calculation are given. – – – – Limiting slope of 58 mV per log unit ($\alpha = 0$).

from chloride to sulphate, nitrate, benzenesulphonate, or glucuronate. A very quick change in p.d. occurred, suggesting a diffusion p.d. in the cell wall. With some anions the p.d. for chloride was reverted to, but with others the change in p.d. persisted for up to 15 min which was the maximum duration of the experiment. The steady values of p.d. are given in Table 1.

In addition, the effect of substituting choline for sodium was examined. The p.d. changed always to a more negative value, with a half-time of 1–2 min. Table 2 lists the results of such experiments.

(*b*) *Resistance Measurements*

During the experiments already described, measurements were made of the membrane resistance of the cells. In some experiments the resistance was measured as a function of current density for each value of the external potassium concentration. In others, the resistance for very small current flows only (both positive and negative) was measured for each concentration. The values obtained must be corrected for the series resistance of the external medium and of the cell sap (Walker 1960); however, the correction for the resistance of the sap was found to be small, and it was neglected.

The corrected values were then converted to membrane resistances (r, in ohm cm^2). As previously, the resistance used was the gross d.c. resistance, given by:

$$R = (\Delta E_{oi})/I, \tag{2a}$$

where I is the current, and not the incremental d.c. resistance given by

$$R' = \partial E_{oi}/\partial I. \tag{2b}$$

TABLE 1

CHANGES IN POTENTIAL DIFFERENCE ON SUBSTITUTING VARIOUS ANIONS FOR CHLORIDE

Total anion concentration = 1·1 mN: Na_o = 1·0 mN and K_o = 0·1 mN in all experiments except experiment 5, where K_o = 1·0 mN and Na_o = 0·1 mN

Expt. No.	External Medium Change	Potential Difference (mV)		
1	Chloride, sulphate	−151,	−151	
2	Chloride, nitrate, chloride	−147,	−149,	−147
3	Chloride, benzenesulphonate, chloride	−156,	−167,	−159
4	Chloride, benzenesulphonate, chloride	−161,	−169,	−162
5	Chloride, benzenesulphonate, chloride	−117·5,	−127·5,	−116·5
6	Chloride, glucuronate, chloride	−182,	−188,	−180
7	Chloride, glucuronate, chloride	−178,	−185,	−180

A typical set of results on one cell is shown in Figure 5. The resistance was highest in high sodium media and lowest in high potassium, and the rise of resistance with negative current (rectification) was greatest in the media with low potassium.

TABLE 2

CHANGES IN POTENTIAL DIFFERENCE ON SUBSTITUTING CHOLINE FOR SODIUM

External Medium	Potential Difference (mV)				
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5
(i) Potassium 0·1 mN, sodium 1·0 mN	−170	−146	−157	−147·5	−144
(ii) Potassium 0·1 mN, choline 1·0 mN	−183	−160	−164	−159	−157
(iii) Potassium 1·0 mN, sodium 0·1 mN	−127	−108	−108	−107	−117
(iv) (ii)—(iii) (see text, p. 38)	−56	−52	−56	−52	−40

All cells gave qualitatively the same picture, with some variation in the actual resistance from cell to cell. Figure 6 shows the membrane resistance at zero current plotted against K_o for five cells.

Table 3 gives the results of some measurements of resistance in media in which other anions were substituted for chloride. For times of up to 15 min, the resting resistance was not changed by the anion substitutions.

In calculating the membrane resistance from the product of the measured resistance (corrected as mentioned above) and the surface area of the cell, it is assumed that the current flow is homogeneous over the cell surface. This depends on the homogeneity of the cell surface. In addition, if the longitudinal resistance of the vacuolar sap were high enough relative to the membrane resistance, most of the current would leave the cell in the neighbourhood of the implanted current-carrying microelectrode. The membrane resistance calculated using total surface

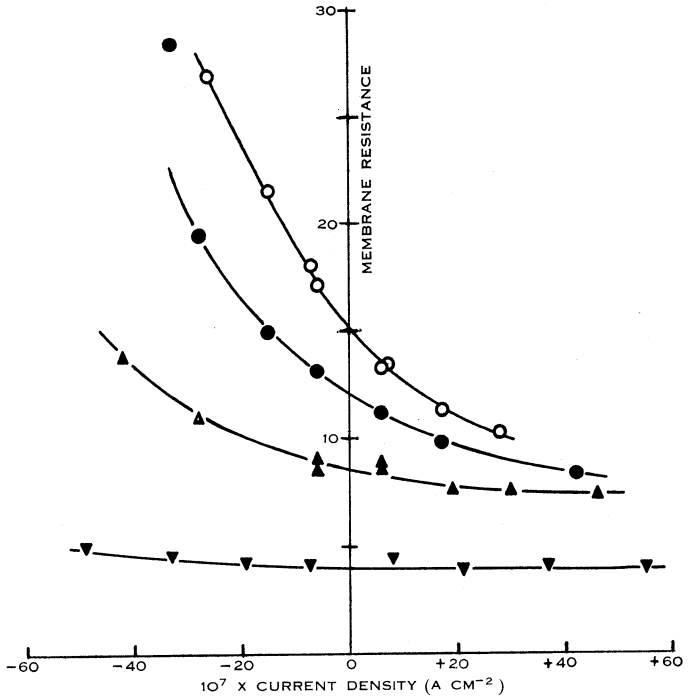

Fig. 5.—Membrane resistance (in kΩ cm^2) between the cell interior and the medium as a function of the current density in various bathing media. Curves connect points in one medium, and are *not* calculated from theory. All measurements made on one cell:

○ $Na_o = 1{\cdot}9$, $K_o = 0{\cdot}1$ mN
● $Na_o = 1{\cdot}6$, $K_o = 0{\cdot}4$ mN
▲ $Na_o = 1{\cdot}2$, $K_o = 0{\cdot}8$ mN
▼ $Na_o = 0{\cdot}1$, $K_o = 1{\cdot}9$ mN

area would then be an overestimate. To confirm that the current flow was nearly homogeneous, the change in potential of a microelectrode just *external* to the cell was measured in response to a small current passed between an implanted microelectrode and the coiled Ag/AgCl electrode lying parallel to the cell, as a function of distance from the current microelectrode. Such a series of measurements is shown in Figure 7, which shows that the current density is nearly independent of the distance from the current-carrying probe. Cells with length greater than 2 cm were seldom used. Very close to the region where the current probe had entered the cell, the current density was sometimes 1·5–2 times the average over the cell length, more particularly immediately after the probe had entered, indicating a local low membrane resistance. Since in the experiment of Figure 7 K_o was 1·0 mN and the membrane

resistance about 5 kΩ cm², the current flow would have a greater tendency towards inhomogeneity than with lower concentrations of potassium and higher membrane

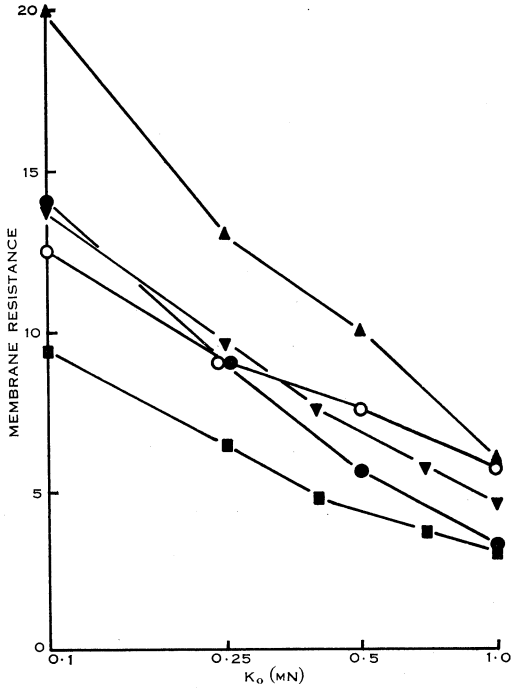

Fig. 6.—Membrane resistance r_o (in kΩ cm²) at zero current as a function of the potassium concentration in the medium. Curves connect points representing one cell, and are not calculated from theory. ○, ●, ▲, ▼, and ■ represent experiments on different cells.

resistances. Little difference was found in the distribution of current through the cell in media containing 0·1 mN and 1·0 mN KCl. Thus the assumption of homogeneous

TABLE 3

MEMBRANE RESISTANCE AFTER ANION SUBSTITUTIONS

Membrane resistance (r_o) in kΩ cm²

External media (mN)*	KCl 0·1 / NaCl 1·0	KCl 0·1 / Na glucuronate 1·0	KCl 0·1 / NaCl 1·0	$C_6H_5SO_3K$ 0·1 / $C_6H_5SO_3Na$ 1·0	KCl 0·1 / NaBr 1·0	KCl 0·1 / NaCl 1·0
Cell 1	13·7	13·0	12·0	12·5	—	12·0
Cell 2	12·0	12·0	11·7	—	—	—
Cell 3	12·5	14·5	14·5	—	15·0	14·6

* In time sequence from left to right.

current flow is sufficiently accurate for the present experiments (where R can be determined to about ±5%).

IV. Theoretical

The theoretical treatment of such results meets with various mathematical difficulties (Goldman 1943). There are further difficulties connected with the choosing of simple models (Johnson, Eyring, and Polissar 1954). Calculations of membrane potentials and resistances are possible only for very simple models, and then only with the aid of various unjustified assumptions. Divergences between the experimental data and the theoretical predictions are therefore difficult to deal with.

In the discussion which follows, models are considered in which a membrane separates two phases. Electric charge is assumed to be carried across the membrane only by the passive diffusion of ions. Such models may represent living cells if the active transport of ions in these cells does not transfer net charge across the membrane.

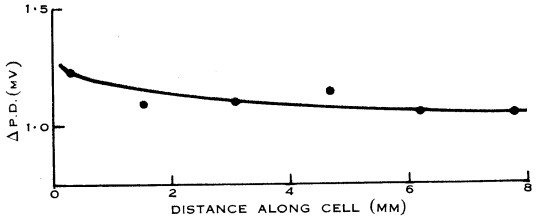

Fig. 7.—Change in the potential difference between a probe near the cell surface and the reference electrode, upon passing current across the cell surface, plotted as a function of the distance of the probe from the inserted micro-electrode.

(*a*) *Membrane Potentials*

In a system of two phases, containing only univalent ions, and separated by a membrane permeable to cations only, one can derive without arbitrary assumptions (Hodgkin and Katz 1949):

$$E_{oi} = (RT/F)\ \ln(\Sigma^{+}_{j} P_j \cdot {}_{o}a_j)/(\Sigma^{+}_{j} P_j \cdot {}_{i}a_j), \tag{3}$$

$$= (RT/F)\ \ln(C^{+}_{o}/C^{+}_{i}), \tag{3a}$$

where

$${}_{o,i}a_j = \text{activity in the } o \text{ and } i \text{ phase of the } j\text{th ion},$$

$$P_j = \text{permeability of the membrane to the } j\text{th ion},$$

and

$$C^{+}_{o,i} = \Sigma^{+}_{j} P_j \cdot {}_{o,i}a_j .$$

Superscript + or − signs indicate that the summation is taken over cations or anions only.

Equation (3a) is the general form of equation (1) already used. The permeability coefficient P_j contains the product of the mobility of the ion in the membrane and its partition coefficient between membrane and solution.

In calculations based on more difficult models, e.g. those in which the membrane is permeable to ions of both signs or to bivalent cations, it is usually assumed that

the partition coefficients are all unity. (A model for which this may be justified is one in which ions penetrate the membrane in regions of high dielectric constant.) Further simplifying assumptions are also necessary. There are three common assumptions:

(i) Henderson's—that all ions have linear concentration gradients in the membrane.

(ii) Goldman's—that the electric field has a linear gradient in the membrane.

(iii) Planck's—that there is everywhere microscopic electroneutrality.

These lead to the following (different) equations:

(i) Henderson's equation

$$E_{oi} = (RT/F)\left[\frac{\sum_j P_j z_j^{-1}({}_i a_j - {}_o a_j)}{\sum_j P_j({}_i a_j - {}_o a_j)}\right]\ln(C_o/C_i). \tag{4}$$

(ii) Goldman's equation

$$E_{oi} = (RT/F)\ln[(C_o^+ + C_i^-)/(C_i^+ + C_o^-)]. \tag{5}$$

(iii) Planck's equation

$$E_{oi} = (RT/F)\ln[(C_o^+ + pC_i^-)/(C_i^+ + pC_o^-)], \tag{6}$$

where

$$p = \left[\frac{\ln(A_i/A_o) - (FE_{oi}/RT)}{\ln(A_i/A_o) + (FE_{oi}/RT)}\right] \times \left[\frac{A_o - A_i[\exp(FE_{oi}/RT)]}{A_o[\exp(FE_{oi}/RT)] - A_i}\right],$$

and

$$A_{o,i} = \sum_j {}_{o,i}a_j.$$

The nature of the expression for p makes the Planck equation insoluble except by trial and error. This in unfortunate, since it is based on a more plausible assumption than the others. The underlying assumption in the Henderson equation is physically impossible. Accordingly, we follow Johnson, Eyring, and Polissar (1954) in discarding the Henderson equation.

If the simple model (of a membrane separating two phases) is modified by the addition of a negatively charged Donnan phase separating phase o from the membrane, we have a model which may more closely represent the plant cell. Equation (5) is then used to find E_{wi}, the p.d. between the wall phase (w) and the i phase, and the following substitutions made:

$$E_{oi} = E_{ow} + E_{wi},$$

and

$${}_w a_j = {}_o a_j \exp(-z_j F E_{ow}/RT).$$

We then obtain:

$$E_{oi} = (RT/F)\ln\left[\frac{C_o^+ + C_i^- \exp(FE_{ow}/RT)}{C_i^+ + C_o^- \exp(FE_{ow}/RT)}\right], \tag{7}$$

where E_{ow} is the Donnan p.d. between the wall and the o phase. The effect of the anions on the overall p.d. is thus reduced by a factor of 10 for each 58 mV of Donnan potential of the wall.

If the anions are assumed to have vanishingly small permeabilities, equation (7) reduces again to equation (3). In this case estimates of α and $(K_i+\alpha \cdot Na_i)$ from equation (1) are independent of the presence of a wall p.d., whether the Donnan system of the cell wall is in contact with the membrane or not.

(*b*) *Membrane Resistances*

Even in the simple case in which only univalent cations are considered, it is necessary to use simplifying assumptions to derive expressions for the membrane resistance. There is thus no simple rigorous equation corresponding to equation (3). The quantities measured in the present work are r and r_o, defined by:

$$r = (E'_{oi}-E_{oi})/J = V/J,$$

and

$$r_o = \lim_{J \to 0} (r),$$

where E'_{oi} is the p.d. when the current density is J, E_{oi} is the p.d. at zero current, and $V = E'_{oi}-E_{oi}$.

Expressions can be derived for r and r_o in terms of A_o, A_i, V, C_o, and either E_{oi} or C_i. Since E_{oi} is also measured in these experiments, its use is convenient. For the model involving permeability to univalent cations only, we find (using Goldman's equation):

$$r_o = \frac{RT[(1/C_o)-(1/C_i)]}{F \ln(C_i/C_o)} \tag{8}$$

$$= \frac{R^2T^2[\exp(FE_{oi}/RT)-1]}{F^3E_{oi}C_o}, \tag{8a}$$

and

$$r = \frac{RTV[1-\exp(FE'_{oi}/RT)]}{F^2E'_{oi}C_o[1-\exp(FV/RT)]}. \tag{9}$$

Using Planck's equation

$$r_o = \frac{RT \ln(A_i/A_o)[A_o-A_i \exp(FE_{oi}/RT)]}{F^2C_o(A_i-A_o)[\ln(A_o/A_i)-(FE_{oi}/RT)]}, \tag{10}$$

and

$$r = \frac{V \ln(A_i/A_o)[A_o-A_i \exp(FE'_{oi}/RT)]}{FC_o(A_i-A_o)[FE'_{oi}/RT+\ln(A_i/A_o)][\exp(FV/RT)-1]}. \tag{11}$$

For the model involving univalent ions of both signs, the Goldman equations (8, 8a, and 9) are simply modified by writing $(C_o^+ + C_i^-)$ for C_o, and $(C_i^+ + C_o^-)$ for C_i.

If, as we assume in treating the experimental results, the important terms in C_o are $P_K \cdot K_o$ and $P_{Na} \cdot Na_o$, we can write

$$C_o = P_K(K_o+\alpha \cdot Na_o).$$

It is then possible, using the Goldman equation, to calculate r for each cell from the parameters derived from the measurements of potential, i.e. E_{oi} and $(K_o+\alpha \cdot Na_o)$, if a value is assumed for P_K. Fitting the experimental values for r then gives a value for P_K. If the Planck equation is to be used, a plausible guess must be made as to the value of A_i.

It is found that the following features distinguish the Goldman equations from the Planck equations:

(i) In the Goldman equation, r_o varies more rapidly with K_o than it does in the Planck equation.

(ii) The addition of a Donnan phase separating the o phase from the membrane reduces the range of r_o in the Goldman equation, but not in the Planck equation.

(iii) The value of P_K calculated for observed values of r_o, α, and $(K_i + \alpha \cdot Na_i)$ using equation (8) is much greater than that using equation (10).

(iv) In the Goldman equation, r varies more rapidly with J or E'_{oi} than it does in the Planck equation, i.e. the rectification effect is greater. However, r varies less rapidly with J or E'_{oi} in the presence of an added Donnan phase. In the Planck equation the rectification does not depend on the presence of a Donnan system.

V. Discussion

(a) *Potential Differences*

(i) *Comparison with Theory.*—The results (cf. Figs. 2 and 3) have been compared with the predictions of equation (1), derived for a very simple model. It is assumed (1) that potassium and sodium have sufficiently large permeabilities or concentrations, compared with the other ions, that they alone determine the membrane potential difference; and (2) that the ratio of their permeabilities, and their activities in the cytoplasm, remain constant. When the two available parameters are selected for best fit, the agreement between results and calculation is generally good. This is not sufficient justification for assuming the correctness of the model, for equation (5) shows that under the conditions of these experiments a non-zero value of $P_{Cl} \cdot Cl_i$ cannot be distinguished from a non-zero value of $P_{Na} \cdot Na_o$. However, the results of experiments in which choline is substituted for sodium (Table 2) indicate that $P_{Cl} \cdot Cl_i$ is negligibly small, and that the model involving only potassium and sodium may be used. In mixtures of potassium and choline chlorides, the difference in p.d. between $K_o = 1 \cdot 0$ and $K_o = 0 \cdot 1$ mN is close to the value of 58 mV predicted for a potassium electrode (line (iv) in Table 2). Whether choline or sodium is present at a concentration of $0 \cdot 1$ mN affects the p.d. little (line (iii) in Table 2) since the product $P_K \cdot K_o$ is so much greater than the corresponding ones for choline or sodium. The different behaviour in sodium and potassium mixtures must then be due to the penetration of sodium, and not to the efflux of internal chloride ions.

The values of the parameters obtained using equation (1) are entirely plausible: $P_{Na}/P_K = \alpha = 0 \cdot 06 \pm 0 \cdot 01$ (mean of seven experiments $\pm$S.E.M.) (MacRobbie and Dainty calculate $0 \cdot 05$ for *Nitellopsis obtusa* from measurements of ionic fluxes into the "protoplasmic non-free space"), and $K_i + \alpha \cdot Na_i = 112 \pm 14$ mN (mean of seven experiments $\pm$S.E.M.) (typical values of the vacuolar concentrations of these ions are 80 mN and 50 mN for K and Na respectively (Hope and Waker 1960)). The concentrations of these cations in the vacuole are supposed by MacRobbie and

Dainty to equal those in the cytoplasm, but this rests on some assumptions. The value under discussion is thus not inconsistent with past findings.

This suggests that the model represents a good approximation to the true state of affairs. Accordingly we conclude that Osterhout was correct in attributing the resting potential to a diffusion potential for which potassium is chiefly responsible.

(ii) *The Cell Wall in Relation to the Membrane.*—The approach of the potential to equilibrium (Fig. 1(*b*)) has in most experiments a half-time of 3–5 min. From this time course one can calculate the time course of the potassium and sodium concentrations just outside the membrane. These are presumably the concentrations in the innermost layer of the cell wall. The half-times for the approach of these concentrations to the values for the bathing medium are found to be also in the region of 3–5 min. Crank (1956) has given graphical solutions for the problem of diffusion into a plane sheet. From his graphs it appears that the half-time for equilibration of the inner face of such a sheet is about twice the half-time for the equilibration of the *average* concentration in the sheet. Thus our measured half-times of 3–5 min for the concentration in the inner region of the cell wall correspond to half-times of $1\frac{1}{2}$–$2\frac{1}{2}$ min for the average concentration of the whole wall. This is very similar to the value of 116 ± 13 sec found by Dainty and Hope (1959) for isotopic sodium exchange in isolated walls of this species, under comparable conditions. We conclude that sodium–potassium exchange in the neighbourhood of the plasmalemma proceeds via the Donnan phase of the cell wall.

Measurements of the overall p.d. (E_{oi}) give no information as to the wall Donnan potential. Gaffey and Mullins (1958) erroneously concluded that the wall Donnan potential was low because they did not detect it during measurements of E_{oi}. The question of a possible intimate contact between the membrane and the Donnan phase of the wall will be discussed below.

(iii) *Deviations from the Model.*—Some results (Fig. 4), particularly those from cells which have been given prolonged pretreatment in sodium chloride, show consistent small differences from the calculations which are difficult to treat. In particular, the p.d. for $K_o = 0{\cdot}1$ ($Na_o = 1{\cdot}0$) is sometimes more positive than is predicted by a constant α and ($K_i + \alpha \,.\, Na_i$) derived from the other p.d. values (Fig. 4(*b*)). Another occasional deviation is a more positive p.d. in $K_o = 1{\cdot}0$ ($Na_o = 0{\cdot}1$), when the change in p.d. upon changing K_o from $0{\cdot}7$ (or $0{\cdot}5$) to $1{\cdot}0$ may exceed the "thermodynamic" value for a perfect potassium electrode (Fig. 4(*a*)).

In principle, the model might plausibly be modified in a number of ways:

(1) K_i or Na_i might vary with K_o or Na_o;

(2) α might vary with E_{oi}, K_o, or Na_o;

(3) P_{Ca} might not be negligibly small; or

(4) P_{Cl} might not be negligibly small.

These possibilities will now be discussed:

(1) Some change in K_i or Na_i might occur during equilibration in solutions of varying K_o. This would give a divergence opposite to that observed in high K_o solutions, but might explain the divergence in low K_o. However, we favour the explanation next discussed.

(2) A small increase in α (i.e. P_{Na}/P_K) would explain the divergence in low K_o solutions; since K_i is likely to be much greater than $\alpha \cdot Na_i$, the p.d. in high K_o will be very insensitive to α. As will be discussed later, an increase of α in low K_o is also suggested by the resistance measurements.

(3) Under the conditions of the present experiments $P_{Ca} \cdot Ca_o$ should be negligible. A moderate value of Ca_i will add a constant term to the quantity $K_i + \alpha \cdot Na_i$ without altering the form of the curve for E_{oi} against K_o.

(4) As has already been discussed, a non-zero value of $P_{Cl} \cdot Cl_i$ has been ruled out by the choline chloride experiments. If P_{Cl} is not zero, there will be a term $(P_{Cl}/P_K) \cdot Cl_o$ to be added to $K_i + \alpha \cdot Na_i$, but it is likely to be negligible under resting conditions. It seems possible, however, that the more positive potential observed in high K_o may be due to an increase in P_{Cl}, so that the term $(P_{Cl}/P_K) \cdot Cl_i$ may no longer be negligible compared with $(K_o + \alpha \cdot Na_o)$. This increase in P_{Cl} as E_{oi} decreases is a reasonable assumption if the initiation of the action potential is indeed due to an increase of P_{Cl} (Gaffey and Mullins 1958).

(iv) *Effect of Pretreatment.*—Almost no change in resting potential was observed when K_o was varied in media also containing 0·5 mN calcium ions. A model which would give this effect is one in which a Donnan phase (negatively charged) is in contact with the membrane, and separates it from the external medium. However, as will be discussed, this model does not fit the data for membrane resistance as a function of current density. The more likely explanation of the action of the pretreatment in allowing the observed changes in p.d. is that exchange tracks with mostly monovalent cations as counterions are established between a region near the plasmalemma and the medium. Before the pretreatment the counterions would have been almost entirely calcium and the exchange of a given number of monovalent cations along these paths would have been more difficult.

(*b*) *Resistances*

(i) *Comparison with Theory.*—When the data for E_{oi} as a function of K_o are fitted by the simple equation (1) in which α and $(K_i + \alpha \cdot Na_i)$ are constant, we can evaluate these two parameters for each cell. It is then possible to calculate for each cell the relative membrane resistance in each experimental solution, using equation (8) or (10) for r_o. Absolute values of r_o or r involve P_K as an additional parameter, which can be chosen for each cell for best fit. It is found that the experimental values for r_o agree qualitatively with the values calculated from Goldman's equation (8), but that the quantitative agreement is not good. For example the range of r_o as K_o goes from 0·1 to 1·0 mN is frequently about 3 : 1 (Fig. 6), while equation (8) predicts about 4·5 : 1 for most cells. Similarly, Planck's equation (10) predicts* a range of 2·5 : 1; again the fit is not exact, although it is better than equation (8).

When calculations are made of r as a function of current density (or, more conveniently, as a function of the change in membrane potential, V) the Goldman

* Assuming a plausible value for A_i.

equation (9) is very often an excellent quantitative fit to the data (Fig. 8). The Planck equation (11) predicts a much smaller change in r with V than is observed (Fig. 8). A similar observation was made by Goldman (1943).

Calculations from the Goldman equation applied to the model containing a Donnan phase resemble those from the Planck equation, as would be expected (Johnson, Eyring, and Polissar 1954). Thus they tend to fit the data for r_o better than the simple model, but are a worse fit to the data for r.

One factor which could reduce the range of r_o with concentration is an increase in P_{Na} (i.e. α) in solutions with relatively high sodium concentration. Indications of such an effect were mentioned above, in discussing the p.d.'s. From the results of two experiments, calculations were made of the variation in α needed to be consistent

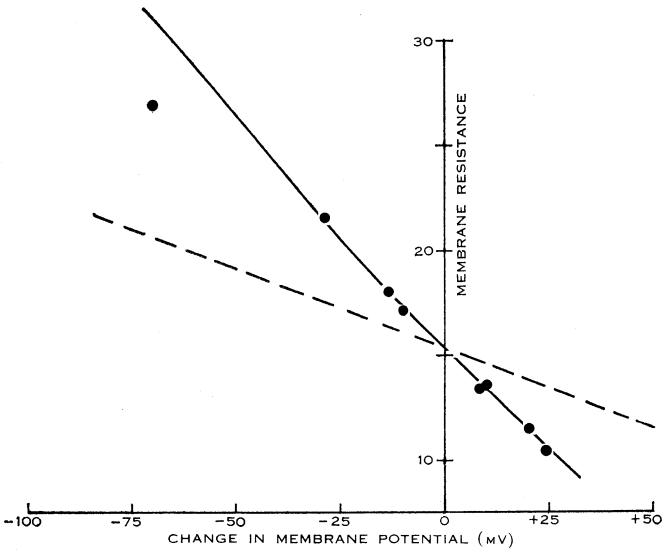

Fig. 8.—Membrane resistance r (in kΩ cm^2) as a function of the change in membrane potential produced by the applied current. ● Experimental points for one cell. ——— Fitted curve based on Goldman's equation (eqn. 8). – – – – Curve based on Planck's equation (eqn. 11), fitted at V = 0 (at the resting potential).

with the observed range of r_o. To reduce this range from 4·5 : 1 to 3 : 1, α would have had to increase from 0·08 to 0·15 as Na_o approached 1·0 mN. In the second experiment the increase in α was from 0·05 to 0·14. The resting potential ($Na_o = 1·0$) would be very little different from that observed, as a result of such a change. Thus, the assumption of a constant α should be regarded as a first approximation only.

It is difficult to explain, on the present basis, the absence of a large increase in resistance on substituting choline for sodium. The increase was less than would be expected if α were as high as here suggested. The choline ion itself may penetrate, but the data for p.d. change on choline substitution (Table 2) suggest that the cell is much less permeable to choline than sodium.

In some experiments, not illustrated here, the resistance (r) observed during the passage of a depolarizing current was higher than that calculated. This may result from an increase in chloride permeability due to the depolarization. A similar explanation has been offered here for the extra depolarization sometimes observed in high potassium solutions.

(ii) *The Meaning of "Membrane Resistance".*—It is apparent from the results that qualitatively the membrane resistance is determined by the concentration of potassium, and, to a lesser extent, sodium. This largely explains the great variation in the published values for "membrane resistance" in the Characeae, since, amongst other things, the potassium concentration varied considerably amongst the published experiments. A comparison of this sort is given by Walker (1960). In these cells, too, there is little doubt that almost the whole of the observed resistance refers to a structure somewhere between the microelectrode in the flowing cytoplasm and the external medium, the contribution of the tonoplast being immeasurably small (see Walker (1960) for experiments bearing on this point made with *C. australis* as well as *Nitella* sp.). The most likely structure with the required properties is the plasmalemma.

(iii) *The Permeability of the Plasmalemma.*—By identifying the observed membrane resistance r_o with the theoretical resistance calculated from equations (8) or (10), and inserting values of α and $K_i + \alpha \cdot Na_i$ from measurements of p.d., it is possible to calculate P_K and P_{Na}. Values from the present studies are $P_K = 10^{-5}$ and $P_{Na} = 10^{-6}$ cm sec^{-1}, if the Goldman equation is adhered to. This would suggest passive fluxes across the plasmalemma of the order of 10 p-equiv. cm^{-2} sec^{-1} for both K and Na, for an external medium containing 0·1 mN K and 1·0 mN Na. Using values of E_{oi} and fluxes of potassium and sodium reported by other workers using cells from the Characeae, the permeabilities to sodium and potassium can be calculated. Using the constant field assumption, the influx into phase i is (for values of E_{oi} greater than about 50 mV):

$$(\phi_j)_{o \to i} \simeq (-FE_{oi}/RT)P_j \cdot {}_o a_j .$$

These values are compared in Table 4 with permeabilities from the present study. The latter are considerably higher. Clearly measurements of fluxes across the plasmalemma need to be made on the present cells, since the agreement of the permeabilities calculated from the two types of experiment would be useful evidence for the "constant field" model.

(iv) *Rectification by the Plasmalemma.*—It is interesting to see that rectification as observed is quite accurately predicted by the simple model of a membrane with unequal concentrations of ions on the two sides. Internal cations at high concentrations are able to carry positive current out of the cell with relative ease compared with external cations at lower concentration, carrying positive current into the cell when the current is reversed. The higher resistance when positive current is carried into the cell is successively reduced as the permeating ion (potassium) is increased relative to sodium (cf. Fig. 5).

Weidmann (1949) obtained somewhat different results with *Nitella*, but Findlay's (1959) data, also for *Nitella* sp., can be fitted qualitatively by the Goldman

relation, although he interprets his results as a sharp change in r as the applied current is reversed in direction.

(v) *Conductance by Anions.*—The experimental results reported here have been interpreted in terms of the permeabilities of the plasmalemma to potassium and sodium ions only. This has been reasonably successful, and it is concluded that the permeability to chloride, or to be precise its product with the chloride concentration, is generally negligible compared with the same quantities for potassium and sodium.

TABLE 4

COMPARISON OF PERMEABILITIES OF THE PLASMALEMMA IN VARIOUS CELLS

Species	Influx (p-equiv/cm² . sec)		External Concentration (mN)		E_{oi} (mV)	Permeability (cm sec^{-1})		Reference
	K	Na	K	Na		K	Na	
Nitellopsis obtusa	4	8	0·65	30	−130	1×10^{-6}	5×10^{-8}	MacRobbie and Dainty (1958)*
Nitella axillaris	0·47	—	0·06		*c.* −150	$1\cdot3\times10^{-6}$	—	Diamond and Solomon (1959)*
Chara globularis (corticated)	2·8	0·7	1·4	10	−150	3×10^{-7}	1×10^{-8}	Gaffey and Mullins (1958)*
Chara australis	—	—	0·1	1·0	−155	10^{-5}	10^{-6}	Present work†

* Fluxes measured. † Resistance measured.

(c) *Conclusions*

We have shown that, under suitable conditions, the p.d. and resistance of the plasmalemma of *C. australis* cells are largely determined by the passive diffusion of sodium and potassium ions across it. The present measurements of p.d. and resistance have given plausible values for the permeabilities of the membrane to sodium and potassium, and for the internal concentration parameter $K_i+\alpha\,.\,Na_i$. The results agree with the predictions of the Goldman equations, which involve the assumption of a linear potential gradient in the membrane and the neglect of phase boundary potential differences at the membrane surfaces. There are (contrary to the opinion of Johnson, Eyring, and Polissar 1954) models for which the neglect of the phase boundary p.d.'s may be justified. However, the linear potential gradient remains an arbitrary assumption, although widely accepted. It is possible that other models, based on a less arbitrary assumption, may yield similar equations, and this is being investigated.

For reasons discussed we suggest that the plasmalemma is in contact with a layer of solution with approximately the same ionic concentrations as the external medium, but is cut off from the latter by the Donnan phase of the cell wall, which governs the rate of approach to equilibrium on changing the external environment.

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