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The (K^+-Na^+)-dependence of the membrane parameters of *Nitella translucens*

It has been shown[1,2] that the plasmalemma potential (E_{co}) of the single cells of the Characeae, under conditions when Ca^{2+} is absent from the external bathing medium, is primarily governed by the passive diffusion of K^+ and Na^+ through the membrane and is adequately described by the equation:

$$E_{co} = \frac{RT}{F} \ln \frac{P_{K^+}[K^+]_o + P_{Na^+}[Na^+]_o}{P_{K^+}[K^+]_c + P_{Na^+}[Na^+]_c} = \frac{RT}{F} \ln \frac{C_o}{C_c} \quad (1)$$

P_{K^+} and P_{Na^+} are the respective K^+ and Na^+ permeability coefficients; $[\ ]_o$ and $[\ ]_c$ denote the appropriate ionic concentrations in the external medium and cytoplasm; R, T and F have their usual significance. This equation can be used to give the values of (a) the permeability ratio, $\alpha = P_{Na^+}/P_{K^+}$, and (b) the internal concentration parameter, $[K^+]_c + \alpha[Na^+]_c$. The individual permeabilities may be determined by electrical means by making the additional measurement of membrane resistance (R_m) and by using the equation:

$$R_m = \frac{RT}{F^2} \frac{(1/C_o - 1/C_c)}{\ln C_c/C_o} \quad (2)$$

The applicability of this equation to the plasmalemma of *Nitella translucens* has yet to be demonstrated. In this communication we describe an experimental test of the equation; the experiments involve the concurrent measurement of membrane potential and resistance in solutions containing different K^+ and Na^+ concentrations.

The methods for measuring the membrane potential and resistance in the present experiments have already been described[3] and in both cases the measurements were made between the vacuole of the cell and the external medium. The cells were subjected to the usual pretreatment in 5 mM NaCl for prolonged periods. The experimental solutions contained only KCl and NaCl and the total KCl + NaCl concentration was always 1.1 mM; the variation in the K^+ concentration in the different solutions was achieved at the expense of the Na^+ concentration[1,2]; the temperature of the solutions was maintained at 20°. The potential and resistance were measured as the solutions were changed in order from $[K^+]_o = 0.1$ mM to $[K^+]_o = 1.0$ mM and then in the reverse order; the average values were taken for each parameter in a given solution. Each cell consistently showed a depolarisation of E_{co} coupled with a fall in R_m with increasing $[K^+]_o$.

The mean values of both parameters for ten cells are given in Table I. The values for the plasmalemma potential were obtained by correcting the measured potential, which is the 'vacuolar' potential, by 15 mV, this being an average value for the tonoplast potential and which is known to be insensitive to external concentration changes[2,4]. The membrane resistance is the total resistance of the plasmalemma and the tonoplast but virtually corresponds to that of the plasmalemma alone[5]; in the present work we make the reasonable assumption that the tonoplast resistance is independent of the external ionic concentration in much the same way as the tonoplast potential.

TABLE I

THE MEAN VALUES OF E_{co} AND R_m FOR TEN CELLS TOGETHER WITH THE COMPUTED VALUES OF α, $[K^+]_c + \alpha[Na^+]_c$, P_{K^+} AND P_{Na^+}

$[K^+]_o$	E_{co} (mV)	R_m ($k\Omega \cdot cm^2$)	α	$[K^+]_c + \alpha[Na^+]_c$	P_{K^+} ($\times 10^6\ cm \cdot sec^{-1}$)	P_{Na^+} ($\times 10^6\ cm \cdot sec^{-1}$)
0.1	−137.5	28.7	0.268	89.3	4.5	1.2
0.25	−133.7	22.0	0.263	88.3	4.1	1.1
0.4	−128.1	16.5	0.257	87.5	3.8	1.0
0.7	−119.1	9.8	0.244	86.6	3.1	0.8
0.85	−116.1	8.4	0.234	86.5	3.1	0.7
1.0	−111.4	7.7	0.221	86.1	3.0	0.7

In Fig. 1 we show a plot of $\exp(E_{co}F/RT)$ *vs.* $[K^+]_o$ taking the mean values of E_{co} for the ten cells. We have drawn the line of best fit and it should be noted that the line shows a small but definite curvature; this might be interpreted as being due to a gradual decrease in α with increasing $[K^+]_o$ (see Table I). Fig. 2 shows a plot of R_m *vs.* $[K^+]_o$ and while the variation between these quantities is in the same direction as that predicted by Eqn. 2, there is clearly a considerable deviation between the experimental results and those computed from Eqn. 2 on the basis of a constant α, P_{K^+} and $[K^+]_c + \alpha[Na^+]_c$. The values of α and $[K^+]_c + \alpha[Na^+]_c$ which were computed to give the line of best fit in Fig. 1 are shown in Table I. The table also gives the values of P_{K^+} and P_{Na^+} calculated from the experimental values of R_m and using the values of α and $[K^+]_c + \alpha[Na^+]_c$ appropriate to the particular value of $[K^+]_o$. These results suggest that, for a 10-fold change in $[K^+]_o$, there is a change in α of about 15 % while the changes in both P_{K^+} and P_{Na^+} are nearer 35 %.

In summary it can be said that Eqns. 1 and 2 are apparently only applicable to the experimental results if the permeability coefficients are concentration dependent. It is not possible at this stage to decide whether this reflects a real situation

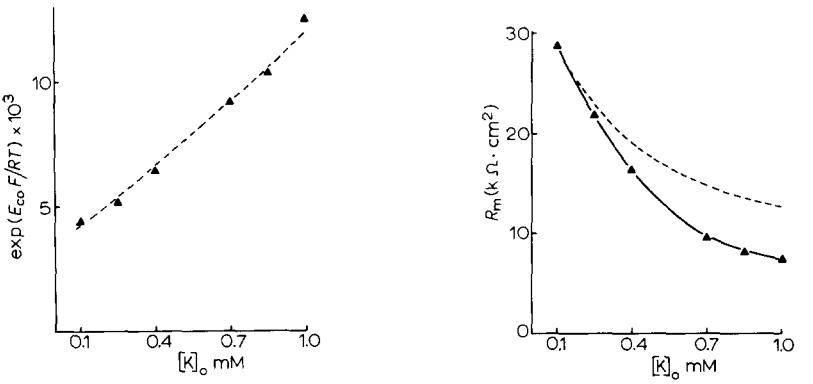

Fig. 1. The plot of $\exp(E_{co}F/RT)$ *vs.* $[K^+]_o$ taking the mean values of E_{co} for 10 cells. ▲, experimental points; the curve drawn is the one giving the best fit and was computed using the method of least squares.

Fig. 2. The plot of R_m *vs.* $[K^+]_o$ taking the mean values of R_m for 10 cells. ▲, experimental points; – – – –, calculated curve from Eqn. 2 using constant values of α (0.268), P_{K^+} ($4.5 \cdot 10^{-6}$ cm·sec^{-1}) and $[K^+]_c + \alpha[Na^+]_c$ (89.3 mM).

in the membrane or a fundamental weakness in the membrane model. It should be noted that the use of Eqn. 2 to describe the membrane resistance implies that the total membrane conductance is simply the sum of the K^+ and Na^+ conductances. This, however, need not be the case since recent experiments with *Nitella clavata*[6] suggests that it is the H^+ conductance which provides the major contribution to the total conductance. If this is also the case in *Nitella translucens* then it follows that the values of P_{K^+} and P_{Na^+} estimated from electrical experiments such as are described herein are grossly overestimated.

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Separation of membrane components produced by anionic detergents and maintained after the latter's removal

In a previous paper[1] it was reported that sodium deoxycholate and sodium dodecyl sulfate produced a high degree of separation of the proteins and the phospholipids of isolated rat-liver plasma membranes that persisted after removal of the detergent by dialysis. This permanent separation was at variance with the results obtained by TERRY *et al.*[2] and by ENGELMAN AND MOROWITZ[3] on surface membranes of *Mycoplasma laidlawii*. According to these authors, the proteins and phospholipids of the mycoplasma membranes, dissociated by sodium dodecyl sulfate, reaggregated to form a homogeneous particle by dialyzing the detergent out.

Rat-liver plasma membranes are rich in cholesterol, whereas the mycoplasma membranes are not; their molar ratios of cholesterol to phospholipid-P amount to 0.65 and 0.1, respectively. In order to explain the aforementioned difference between the two membrane species, it was suggested[1] that by dialyzing detergent-solubilized liver membranes phospholipid–cholesterol complexes might be formed in preference to phospholipid–protein complexes, whereas homogeneous complexes of the latter type might arise from detergent-solubilized membranes containing but little cholesterol.

In the present experiments, the first possibility was studied by comparing the equilibrium distributions of cholesterol and phospholipid-P of isolated rat-liver mem-