SHORT COMMUNICATIONS

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A simplified method for measuring membrane resistances in *Nitella translucens*

It has been shown[1] that there is a close similarity between the electrical properties of the internodal cells of *Nitella translucens*, near the resting state, and those of a short, leaky, coaxial cable. A quantitative analysis shows that if a pulse of constant current I_o is injected into a cell of length $2\,l$ at its midpoint, then the voltage response, V_x, at any distant point x from the current-injecting electrode is given by:

$$\frac{V_x}{I_o} = \frac{r_m}{2\lambda} \cosh \frac{(l-x)}{\lambda} \Big/ \sinh \frac{l}{\lambda} \tag{1}$$

r_m is the resistance times unit length of the cell membrane ($1/r_m$ is the conductance per unit length of the cell) and λ is the space constant of the cell. λ is a measure of the attenuation of the voltage response along the length of the cell and is related to r_m by the expression $\lambda = [r_m/(r_i + r_o)]^{1/2}$, where r_i and r_o are the resistances per unit length of the internal and external solutions of the cell. The resistance of unit area of the membrane (in $\Omega \cdot \mathrm{cm}^2$) for a cell of diameter d, is given by $R_m = \pi\, d\, r_m$, and this quantity can thus be evaluated if d and r_m are known. The value of r_m can be obtained from known values of λ and $(r_i + r_o)$ but, as Eqn. 1 implies, the values of these two parameters can only be calculated if the voltage responses to applied current are measured at two points along the cell length. This means that three electrodes have to be inserted into the cell—one current-injecting electrode and two voltage-recording electrodes. However, the following simple analysis suggests that R_m can be determined simply and directly if the voltage response is measured by a single voltage electrode positioned at a critical distance from the current-injecting electrode; there is then no further need for a second voltage electrode and the computations of λ and $(r_i + r_o)$ are eliminated.

Eqn. 1 can be rewritten in the form:

$$\frac{V_x}{I_o} \cdot 2\pi dl = \frac{r_m 2\pi dl}{2\lambda} \cosh (L - X)/\sinh (L) \tag{2}$$

where $L = l/\lambda$ and $X = x/\lambda$. The expression on the left side of the equation is the membrane resistance, R_m', uncorrected for the space constant. Thus Eqn. 2 simplifies to:

$$R_m' = R_m[L \cosh (L - X)/\sinh (L)] \tag{3a}$$

or

$$R_m' = kR_m \tag{3b}$$

where

$$k = L \cosh (L - X)/\sinh (L) \tag{3c}$$

The quantity k is a correction factor the significance of which is most easily understood from a plot of its numerical value over a range of values of L and X. Such computations are displayed in Fig. 1. From these graphs it can be seen that at the point $X = 0.42\ L$, *i.e.* where $x = 0.42\ l$, k is unity and is remarkably insensitive to λ over a wide range. Exactly the same conclusion can be arrived at algebraically by expanding the $\cosh(L-X)$ and $\sinh(L)$ terms in Eqn. 3c and equating k to unity.

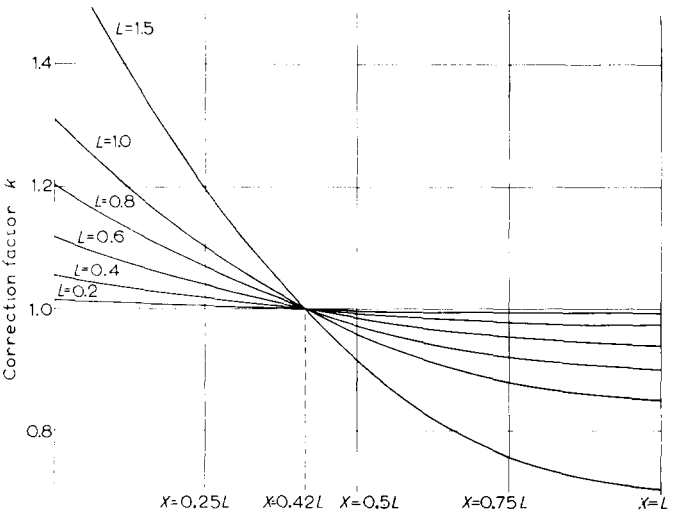

Fig. 1. The graphs of the correction factor k as a function of X and L.

The experimental procedure is, then, to pass current into the cell at its midpoint and to pick up the voltage response at a distance of $0.42\ l$ from the current electrode. The total resistance of the cell membrane can thus be obtained by Ohm's law, and by multiplying this value by the surface area of the cell the resistance of unit area of the membrane can be obtained. The accuracy of the method in a given experiment can readily be seen from Fig. 1. Taking a specific example: suppose l is 3 cm and that the only knowledge of the value of λ is that it lies between 6 and 2 cm; the corresponding values of L are then 0.5 and 1.5. The graphs show that at the point $X = 0.42\ L$ the values of k are, respectively, 1.00 and 0.98 for these limits of L. The maximum error in R_m is thus only 2 %.

In our present experiments, both the current-injecting electrode and the internal voltage electrode were conventional 3 M KCl-filled glass microelectrodes each having an impedance of a few MΩ. The external current electrode was a length of silver wire laid parallel to the length of the cell and close up to it, while the external voltage electrode was a calomel reference electrode placed as close as possible to the internal voltage electrode. The internal electrodes were inserted into the cell transversely to its length, and the electrode tips were located in the cell vacuole. Thus the value of R_m obtained in these experiments is the combined value for both the tonoplast and the plasmalemma. Square pulses of current, of duration 0.2–0.3 sec, were fed into the cell through a 100 MΩ resistance with a low shunt capacitance. The maximum current density used in any experiment was 0.2 $\mu A \cdot cm^{-2}$, and at these levels the voltage responses were invariably capacitative[2]. In all the experiments the cells

were bathed in a standard solution of 1.0 mM NaCl, 0.1 mM KCl and 0.1 mM $CaCl_2$.

The results of measurements made on 12 cells are summarised in Table I. The mean value of 26.4 $k\Omega \cdot cm^2$ for R_m compares favourably with previously published values of 21.4 $k\Omega \cdot cm^2$ and 24.8 $k\Omega \cdot cm^2$ (refs. 1, 3).

TABLE I

THE RESULTS OF RESISTANCE MEASUREMENTS ON 12 CELLS

d is the cell diameter and l is the half-length of the cell. I_o is the amplitude of the current pulse injected into the cell at its midpoint and V is the voltage response to this current at a distance 0.42 l from the current electrode. R_m is the resistance of unit area of the cell membrane (tonoplast and plasmalemma in series).

Cell No.	*d (cm)*	*l (cm)*	*V (mV)*	*I_o (μA)*	*R_m ($k\Omega \cdot cm^2$)*
1	0.07	3.5	2.81	0.20	21.6
2	0.08	3.1	2.93	0.20	22.9
3	0.07	3.1	2.79	0.20	19.0
4	0.08	3.7	2.87	0.20	26.7
5	0.09	3.7	4.01	0.25	33.5
6	0.08	3.8	3.34	0.20	31.9
7	0.08	3.4	3.05	0.20	26.1
8	0.07	3.1	2.96	0.20	20.1
9	0.08	3.1	4.91	0.30	25.5
10	0.09	3.6	3.39	0.20	34.4
11	0.08	3.6	2.69	0.20	24.3
12	0.09	3.9	2.78	0.20	30.6
Mean	0.08	3.5	3.21	0.21	26.4

The method described herein provides a quick and accurate means of determining the membrane resistance of cells such as *N. translucens*. It is particularly useful in experiments designed to examine the functional relationship between resistance and any other variable of the system, *e.g.* temperature. In addition, the elaborate procedures which are usually employed to overcome the complications due to the space constant are eliminated. Nevertheless, any systematic study of a species of cell having a cylindrical geometry should include, as a preliminary measure, an experimental determination of the space constant for the cells of that species.

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