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Effect of a Spatially Inhomogeneous Membrane upon the Measured Electrical Properties of *Chara*

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Summary. The electrical properties of radially symmetric, cylindrical cells are considered. It is shown that the presence of spatial inhomogeneities in the membrane conductance, that might occur as a consequence of pH banding in *Chara* and *Nitella*, can greatly influence the "cable" properties of the cell. Thus if current is injected into such a cell at only a single point, calculation of the area-specific conductance of the membrane at that point requires a knowledge of the spatial distribution of the transverse current flow through the membrane. If spatial inhomogeneities are present and are not explicitly allowed for, the estimated area-specific membrane conductance at the point of current injection can be drastically in error. Techniques are discussed whereby the actual area-specific conductance of the membrane in the acid and basic zones in *Chara* can be experimentally determined.

Key Words conductance · *Chara* · pH banding · membrane · cable

Introduction

The long cylindrical internodes of the plants *Chara* and *Nitella* have been used in many electrophysiological studies of plant cells. Experimenters are often interested in the area-specific values of the membrane electrical parameters. Towards this end the effective area through which an applied current flows can be estimated by two main methods. One technique (i.e. space-clamping) employs an electrode geometry that ensures a spatially uniform membrane potential difference (PD). The other involves the injection of current into the interior of the cell at only a single point. The spatial distribution of the transverse current flow through the membrane is then calculated using the theory of a leaky homogeneous cable.

This latter approach has received wide-spread usage since its first applications to cylindrical plant cells (e.g. Walker, 1960; Williams, Johnston & Dainty, 1964). This is presumably because both the electrodes involved and their insertion into the cell are simpler than that required for space-clamping. If the cell section under study is short in comparison with the cable length, or if the resultant PD is measured 0.42 times the length of the cell section distant from the point of current injection (Hogg, Williams & Johnston, 1968), the correction for the cable properties is simple. Few experimenters have, however, attempted to measure the cable length or checked whether the model of a simple homogeneous cable is applicable.

Hope and Walker (1961) determined that the current flow through the membranes was reasonably homogeneous in their experiments on *Chara*. Williams et al. (1964), Bradley and Williams (1967) and Hogg, Williams and Johnston (1969) measured the response of the membrane PD at two points along internodes of *Nitella translucens*, and found a difference consistent with a cable length of 25 to 30 mm. This length agreed with that calculated from reasonable values of the transverse and longitudinal impedances of the cell. Volkov and Platonova (1970) found a shorter cable length (13 mm) for *Nitella flexilis*. In a very large number of studies, however, the model of a homogeneous cable has been assumed applicable without any attempt at justification.

The presence of spatial inhomogeneity in the membrane of *Chara* under some conditions has been known for some time. Thus the membrane can, upon illumination, make the external medium adjacent to the cell alternately acidic and basic (e.g. Spear, Barr & Barr, 1969; Lucas & Smith, 1973). It has also been shown that large electric currents can circulate between these regions (Walker & Smith, 1977). In *Chara* the membrane has been found to become markedly more conductive when the pH of the bathing solution approaches that expected in the basic regions (Bisson & Walker, 1980, 1981). This suggests that the conductance of *Chara* will be spatially inhomogeneous during

pH banding. Indeed recent experiments on *Chara* internodes suspended in air have shown that the impedance[1], as well as the area-specific capacitance and conductance (Chilcott, Coster, Ogata & Smith, 1981[2]), are markedly inhomogeneous, the basic zones being more conductive.

Thus there is good evidence that under some conditions a large degree of spatial inhomogeneity in its electrical properties is present in the membrane of *Chara*. In this paper some consequences of this are studied and it is shown how they can markedly affect the interpretation of experimental results. In an accompanying paper (Smith and Walker, 1983), the theory developed herein is used to estimate the area-specific conductance of the acid and basic zones in *Chara* from appropriate experiments.

Theory

For the correct interpretation of experimental results, a knowledge of the relationships between the measured electrical parameters and the electrical properties of individual regions in an electrically inhomogeneous membrane is necessary. These relationships are now examined for the two commonly used experimental techniques. The treatment in this paper is confined to one-dimensional, radially symmetric, cylindrical cells.

The complex AC impedance Z can be defined by

$$Z = V/I \tag{1}$$

where V is the AC voltage (or PD) developed across the membrane and I is the transverse AC current passing through it. Defining the admittance Y as the reciprocal of the impedance, the capacitance C and conductance G are then defined by

$$G + j\omega C = Y = 1/Z \tag{2}$$

where ω is the angular AC frequency and j is $(-1)^{\frac{1}{2}}$. The conductance $G(x)$ of a membrane element at position x is thus related to the real part of the ratio of the current $I(x)$ through the membrane at that point to the PD $V(x)$ developed across the membrane at that point, i.e.

$$G(x) = \text{Real}\,(I(x)/V(x)). \tag{3}$$

In experiments it is usual to measure the total current I_T flowing through the membrane and the membrane PD at one point ($=V^*$) on the cell surface. The measured conductance G^* is thus defined by

$$G^* = \text{Real}\,(I_T/V^*). \tag{4}$$

The two main experimental techniques by which such conductance measurements are usually performed are now treated.

Space-Clamping: Homogeneous Membrane Potential

When current is passed between an internal wire electrode which extends longitudinally for the length of the cell and an external electrode which effectively covers the outer cell surface, then the resultant PD should be effectively uniform over the entire membrane. If an inhomogeneous membrane is thought of as a mosaic of different regions, each of fractional area Q_i and admittance Y_i, with a transverse current I_i flowing through each region, then the total current is given by

$$I_T = \Sigma I_i = V_m \Sigma Y_i Q_i \tag{5}$$

and consequently

$$Y^* = \Sigma Y_i Q_i. \tag{6}$$

The measured admittance Y^* is thus simply the sum of the admittances of the individual regions (multiplied by their fractional areas), and consequently any variations in the admittance of individual regions will be reflected in the total.

If the DC limit is considered then the area-specific conductance G^o averaged over the total membrane area A is given by

$$G^o = G^*/A = \Sigma G_i Q_i/A. \tag{7}$$

Point Current Injection: Inhomogeneous Membrane Potential

In this technique current is passed into the interior of the cell at only a single point. The resultant change in membrane PD is then not uniform, but will decrease monotonically with increasing distance from the point of current injection at a rate dependent upon the relative magnitudes of the transverse and longitudinal impedances of the cell. To calculate this rate of decay the cell is usually treated as a radially symmetric homogeneous cable (e.g. Hodgkin & Rushton, 1946). An effective area (i.e. the equivalent area through which the current would flow if the membrane PD were uniform) can then be derived and the area-specific conductance calculated. The variant of this approach applied to plant cells (e.g. Williams et al., 1964) usually has several implicit assumptions, some of which are:

(a) Both ends of the cell are assumed to be terminated by infinite transverse impedances. Measurements of the time dependence of the voltage response to a current pulse in *Nitella* (Hogg et al., 1969) indicate that this assumption is reasonable.

(b) The cytoplasm is assumed to possess negligible longitudinal conductivity. Theoretical calculations (J.R. Smith, *unpublished*) show that for measurements of the PD between the vacuole and the external solution this assumption will usually produce a negligible error for *Chara* and *Nitella*.

(c) The intrinsic membrane electrical properties are assumed homogeneous.

(d) The voltage dependence of the membrane electrical parameters must be negligible over the range of membrane PD experienced. Otherwise the inhomogeneous PD over the membrane will produce inhomogeneous electrical properties.

Solutions to the Cable Equations. A suitable equivalent circuit for a region in the membrane of a plant cell is given in Fig. 1. The transverse impedance of the membrane (times unit length) is denoted by z_m, whereas the longitudinal resistance per unit length of the internal and outside media are defined by r_i and r_o, respectively. Following the standard approach (e.g. Williams et al., 1964), the application of Kirchoff's Laws to the equivalent circuit then gives

[1] Ogata, K., Chilcott, T.C., Coster, H.G.L. Spatial variation of the electrical properties of *Chara*: I. Electrical potentials and membrane conductance. (*Submitted* to *Aust. J. Plant Physiol.*)

[2] Chilcott, T.C., Coster, H.G.L., Ogata, K., Smith, J.R. Spatial variation of the electrical properties of *Chara*: II. Membrane capacitance and conductance as a function of frequency. (*Submitted* to *Aust. J. Plant Physiol.*)

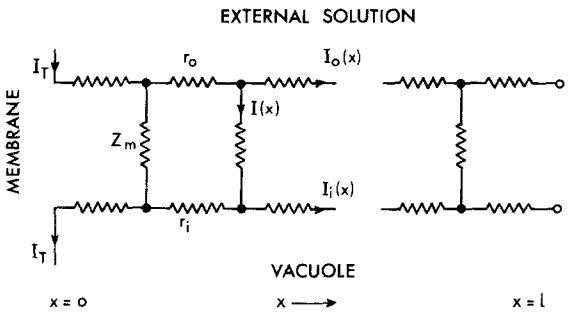

Fig. 1. A suitable equivalent circuit for the membrane of a plant cell

$$dV/dx = I_i r_i - I_o r_o \tag{8}$$

$$dI_o/dx = -I \tag{9}$$

$$dI_i/dx = I \tag{10}$$

where I_i and I_o are the longitudinal interior and outside currents, respectively. All current flow (I) through the membrane is assumed to be perpendicular to the longitudinal axis, whereas the current flow through the interior and external solution are assumed to be entirely longitudinal. From Ohm's Law

$$V = Iz_m. \tag{11}$$

Differentiating Eq. (8) and substituting Eqs. (9–11) then gives the following second order differential equation usually referred to as the cable equation:

$$\lambda^2 d^2V/dx^2 - V = 0 \tag{12}$$

where λ is the characteristic or cable length and is defined by

$$\lambda = (z_m/(r_o + r_i))^{\frac{1}{2}}. \tag{13}$$

The general solution to this equation is

$$V(x) = \alpha \exp(x/\lambda) + \beta \exp(-x/\lambda) \tag{14}$$

where α and β are constants to be determined from the boundary conditions. From Eqs. (9), (11) and (14) the longitudinal current is then

$$I_o(x) = -(\alpha\lambda/z_m)\exp(x/\lambda) + (\beta\lambda/z_m)\exp(-x/\lambda). \tag{15}$$

Thus for each region in the membrane with distinct electrical properties, two boundary conditions are necessary to determine $V(x)$ and $I_o(x)$.

Homogeneous Cable. For a section of homogeneous cable of length l terminated by an infinite impedance at $x = l$, and with a current of magnitude I_T injected at $x = 0$, the two required boundary conditions are

$$I_o(0) = I_T \tag{16}$$

$$I_o(l) = 0. \tag{17}$$

Combining Eqs. (15–17) gives

$$\beta = \alpha \exp(2l/\lambda) \tag{18}$$

$$\alpha = I_T z_m/(\lambda((\exp(2l/\lambda) - 1))). \tag{19}$$

Substituting Eqs. (18) and (19) into Eq. (14) gives the following relationship between the total current I_T injected at $x = 0$ and the resultant membrane PD change as derived by Williams et al. (1964):

$$V(x) = \frac{z_m \cosh((l-x)/\lambda)}{\lambda \sinh(l/\lambda)} I_T. \tag{20}$$

To relate the measured admittance Y^* (and hence the conductance G^*) to the actual area-specific admittance Y and conductance G, the following relationship is needed:

$$z_m = (Y\pi d)^{-1} \tag{21}$$

where d is the diameter of the cell. If the membrane PD V^* is measured at $x = 0$, then the measured admittance Y^* is given by

$$Y^* = I_T/V^* = I_T/V(0) = Y\pi\lambda d \tanh(l/\lambda). \tag{22}$$

In the low frequency limit $\omega \to 0$, $Y^* \to G^*$, $Y \to G$. Thus the DC conductance is given by

$$G = G^*/\pi\lambda d \tanh(l/\lambda). \tag{23}$$

For cell sections where $l \gg \lambda$, then $\tanh(l/\lambda) \sim 1$ and Eq. (23) reduces to

$$G = G^*/\pi\lambda d. \tag{24}$$

In this case the effective area of the cell is thus only the area up to a distance λ away from the point of current injection.

For cell sections with $l \ll \lambda$, then $\tanh(l/\lambda) \to l/\lambda$ and Eq. (23) simplifies to

$$G = G^*/\pi d l. \tag{25}$$

In this situation the effective area is thus the entire surface area of this cell section because the membrane PD is spatially uniform.

For cell sections with length comparable to λ, the following equation must be solved (unless of course λ is known by independent means):

$$G^{\frac{1}{2}} \tanh\,(l^2 G\pi d(r_o + r_i))^{\frac{1}{2}} = G^*(r_o + r_i)^{\frac{1}{2}}/(\pi d)^{\frac{1}{2}}. \tag{26}$$

Thus providing that the constants l, d, r_o and r_i are known (or that reasonable estimates are possible), the membrane conductance can be accurately calculated from measurements of the total current I_T injected into the cell, and the resultant membrane PD at a single point (here at $x = 0$). Indeed an ingenious technique proposed by Hogg et al. (1968) considerably simplifies the algebra of Eq. (26) by measuring the membrane PD at a point 0.42 l distant from the point of current injection.

Inhomogeneous Cable. The electrical properties of a cell section composed of three regions with different electrical properties are now discussed. The first region (subscript 1) has admittance Y_1 and extends from the point of current injection at $x = 0$ to $x = l_1$. The second and third regions have admittances Y_2 and Y_3, respectively, and extend from $x = l_1$ to l_2 and from $x = l_2$ to l. In each region (i.e. for $j = 1, 2, 3$) the membrane PD V and the longitudinal current I_o in response to an injected current I_T will be given by (from Eqs. 14 and 15):

$$V_j(x) = \alpha_j \exp(x/\lambda_j) + \beta_j \exp(-x/\lambda_j) \tag{27}$$

$$I_{oj}(x) = -(\alpha_j\lambda_j/z_{mj}) \exp(x/\lambda_j) + (\beta_j\lambda_j/z_{mj}) \exp(-x/\lambda_j) \tag{28}$$

where z_{mj} and λ_j can be calculated from Eqs. (21) and (13). There are now six constants to be determined (i.e. α_{1-3}, β_{1-3}). The boundary conditions at each end of the cable are as for the homogeneous cable, i.e.

$$I_{o1}(0) = I_T \tag{29}$$

$$I_{o3}(l) = 0. \tag{30}$$

The other four boundary conditions come from the continuity of V and I_o at $x = l_1$ and l_2. Thus

$$V_1(l_1) = V_2(l_1) \tag{31}$$

$$V_2(l_2)=V_3(l_2) \tag{32}$$

$$I_{o1}(l_1)=I_{o2}(l_1) \tag{33}$$

$$I_{o2}(l_2)=I_{o3}(l_2). \tag{34}$$

Substituting Eqs. (27) and (28) into the boundary conditions (29–34) then respectively give

$$-\alpha_1 K_1+\beta_1 K_1=I_T \tag{35}$$

$$-\alpha_3 \exp(l/\lambda_3)+\beta_3 \exp(-l/\lambda_3)=0 \tag{36}$$

$$\alpha_1 \exp(l_1/\lambda_1)+\beta_1 \exp(-l_1/\lambda_1)-\alpha_2 \exp(l_1/\lambda_2) -\beta_2 \exp(-l_1/\lambda_2)=0 \tag{37}$$

$$\alpha_2 \exp(l_2/\lambda_2)+\beta_2 \exp(-l_2/\lambda_2)-\alpha_3 \exp(l_2/\lambda_3) -\beta_3 \exp(-l_2/\lambda_3)=0 \tag{38}$$

$$-\alpha_1 K_1 \exp(l_1/\lambda_1)+\beta_1 K_1 \exp(-l_1/\lambda_1) +\alpha_2 K_2 \exp(l_1/\lambda_2)-\beta_2 K_2 \exp(-l_1/\lambda_2)=0 \tag{39}$$

$$-\alpha_2 K_2 \exp(l_2/\lambda_2)+\beta_2 K_2 \exp(-l_2/\lambda_2) +\alpha_3 K_3 \exp(l_2/\lambda_3)-\beta_3 K_3 \exp(-l_2/\lambda_3)=0 \tag{40}$$

where $K_j=\lambda_j/z_{mj}$. Solution of the six simultaneous Eqs. (35–40) will then give the six constants in terms of I_T. Substitution back into Eq. (27) then gives the behavior of $V(x)$ in terms of I_T. The relationship between the measured conductance and the area-specific conductance is then specified by Eq. (4). Extension of this approach to cells with different numbers of electrically distinct regions is obvious.

Results

As discussed in the Introduction, it appears that the conductance of the basic zones in illuminated internodes of *Chara* can be markedly higher than that of the acid zones. The effect of the presence of such inhomogeneities upon the relationship between the measured conductance and the actual area-specific conductance of the zone into which the current is injected is now examined. The theoretical plots were obtained via numerical solutions of simultaneous Eqs. (35–40). The parameters involved were ascribed values thought to be suitable for *Chara* (*see* Figure captions for values). All results shown are for very low frequencies where the contribution of reactive elements is negligible (i.e. at DC).

Current Injection into a Basic Zone

The situation shown in Fig. 2(*a*) is considered first. Current is injected at $x=0$ into a cell (or cell section) of length l. The zone between $x=0$ and $x=b$ is assumed to possess an area-specific conductance G_B which is greater than or equal to that of the rest of the cell ($=G_A$). Figure 3 shows the theoretical relationship between the measured conductance G^* and the area-specific conductance G_B of the region into which current is injected for different ratios of the conductance of the two regions (i.e. G_B/G_A). The membrane PD was calculated at

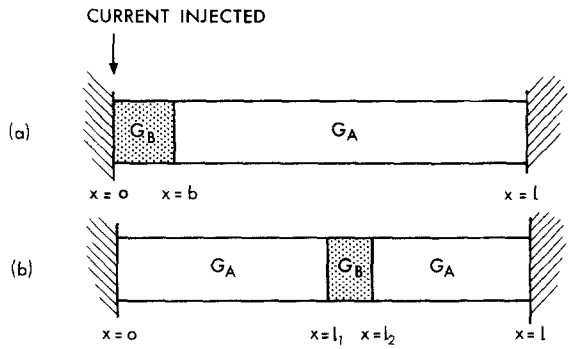

Fig. 2. Schematic diagrams showing idealized situations corresponding to current injection into (*a*) a basic zone or (*b*) an acid zone of an illuminated *Chara* cell

the point of current injection ($x=0$). The curve for $G_B/G_A=1$ corresponds to the normal assumption of a homogeneous cable. It is immediately apparent, however, that large errors will occur in the estimation of G_B if the membrane is spatially inhomogeneous without this being recognized. For example if $G_B=4$ S/m^2 and the ratio $G_B/G_A=10$, then Fig. 3 shows that the measured conductance of a cell with the parameters given would be 40 μS. If the membrane were assumed homogeneous however, G_B would be erroneously interpreted as only 1 S/m^2.

Figure 4 shows the theoretical relationships for the commonly used technique of Hogg et al. (1968), whereby the membrane PD in response to the injected current is measured at a distance 0.42 l away. It is apparent that the effects of membrane inhomogeneities are enhanced in this situation. For example, a measured conductance of 53 μS would correspond to an area-specific value of 4 S/m^2 for an inhomogeneous membrane with $G_B/G_A=10$. Assumption of a homogeneous membrane would only yield a value of 0.8 S/m^2. The enhanced effect of any inhomogeneity in this technique results from the current flow through the membrane being predominantly through a region electrically different than that where the PD response is measured.

Figure 5 shows the theoretical dependence of the relationship between G^* and G_B for different widths (b) of the high conductance zone into which the current is injected and the membrane PD measured at $x=0$. It is apparent that the dependence of the relationship upon the width of the high conductance zone is not particularly strong. Thus any error in estimating b produces a correspondingly smaller error in estimating G_B. Essentially the cable properties of the cell under these circumstances are dominated by those of the region into which current is injected.

The results presented show that spatial inho-

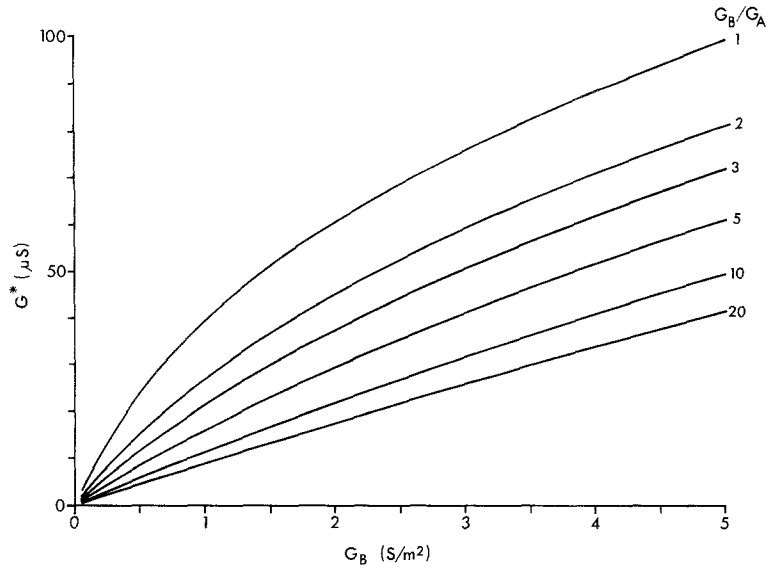

Fig. 3. Theoretical relationship between the measured conductance G^* and the actual area-specific conductance G_B of the region into which current was injected (*see* Fig. 2(*a*)). The relationship is shown for varying degrees of spatial inhomogeneity (i.e. the ratio G_B/G_A), where G_A is the conductance of the remainder of the cell. In the calculations it was assumed that $(r_o+r_i)=5/\pi$ MΩ/m, $l=20$ mm, $b=2$ mm, $d=1$ mm and that V^* was measured at $x=0$

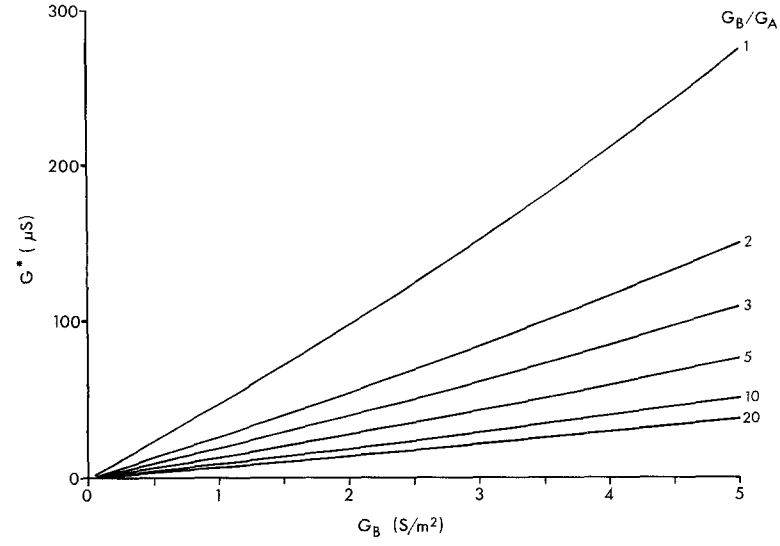

Fig. 4. As for Fig. 3, except that the membrane PD was measured 8.4 mm distant from the point of current injection (i.e. at $x=0.42\,l$) as proposed by Hogg et al., 1968.The nearly linear relationship between G_B and G^* that makes this such a useful technique for homogeneous cables (i.e. $G_B/G_A=1$) is apparent

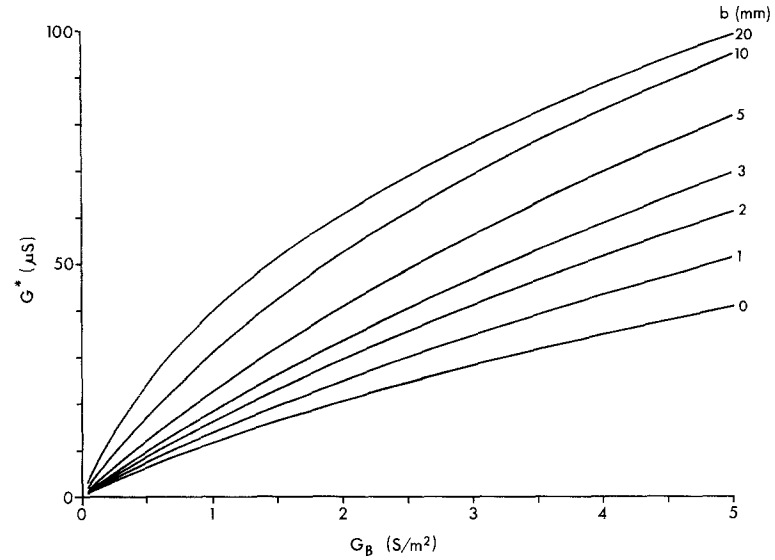

Fig. 5. Theoretical relationship between the measured conductance G^* and the area-specific conductance G_B of the region into which current is injected and the PD measured for different widths (b) of this region (*see* Fig. 2(*a*)). Parameters had the same values as Fig. 3, except $G_B/G_A=5$

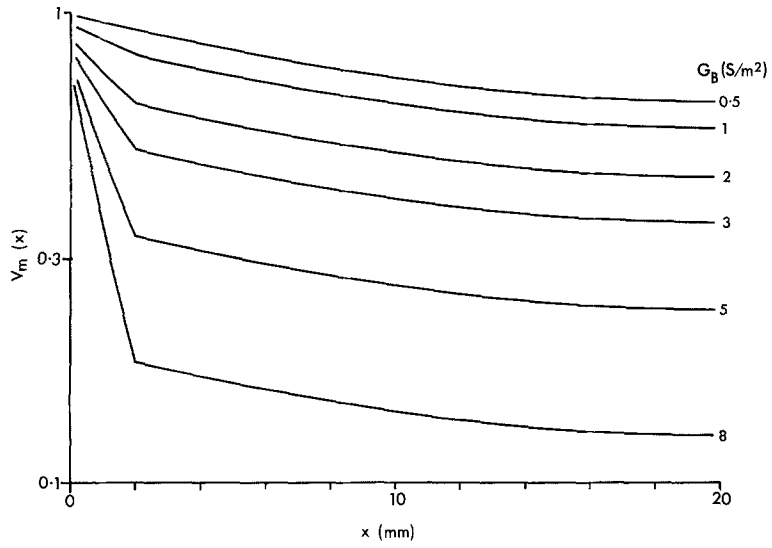

Fig. 6. Theoretical spatial dependence of the change in membrane PD $V_m(x)$ produced by current injection at $x=0$ (*see* Fig. 2(*a*)) for different values of G_B, the area-specific conductance at the point of current injection. The dependences of $V_m(x)$ are plotted on a logarithmic scale and are normalized with respect to $V_m(0)$ calculated for a homogeneous cable. The other parameters had the values $b=2$ mm, $l=20$ mm, $d=0.7$ mm, $G_A=0.5$ S/m², $\lambda_A=20$ mm

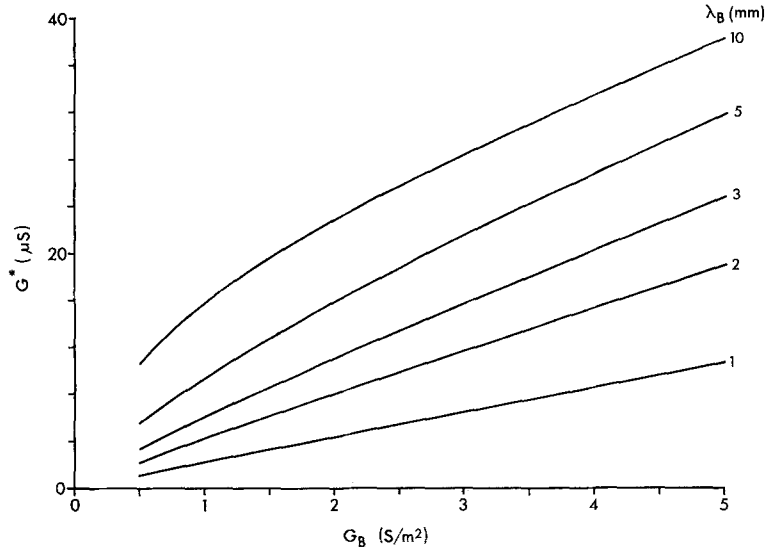

Fig. 7. Theoretical relationship between the measured conductance G^* and the area-specific conductance G_B of the zone into which current was injected (*see* Fig. 2(*a*)), for different values of λ_B, the cable length at the point of current injection. The parameter values used were $G_A=0.5$ S/m², $b=2$ mm, $l=20$ mm, $d=0.7$ mm

mogeneities in the membrane conductance can produce massive errors in the calculated area-specific values, if they are not correctly accounted for. The problem that remains is how to determine the degree of spatial inhomogeneity present.

Towards this it is instructive to examine how the membrane PD (and transverse current – *see* Eq. 11) decreases with increasing distance away from the point of current injection. This is illustrated in Fig. 6, where the results are shown normalized with respect to that PD present for a homogeneous cable at $x=0$. The relationships are shown for $\lambda_A=20$ mm with varying G_B. As the degree of inhomogeneity increases (i.e. as G_B increases), the presence of two distinct characteristic lengths in the decay of the membrane PD becomes evident. These correspond to the two cable lengths λ_A and λ_B present as a consequence of the two conductances G_A and G_B.

Measurement of these two cable lengths can thus provide information upon the degree of inhomogeneity present. The relationship between G^* and G_B can then be deduced from the spatial dependence of the transverse current flow. Figure 7 shows the results of such calculations for varying cable lengths λ_B at the point of current injection. The relationships were found to be insensitive to variations in λ_A. Thus measurement of λ_B in conjunction with G^* will permit the evaluation of G_B.

Current injection into an Acid Zone

The situation of Fig. 2(*b*), which could reflect that of current injection into an acid zone in an illumi-

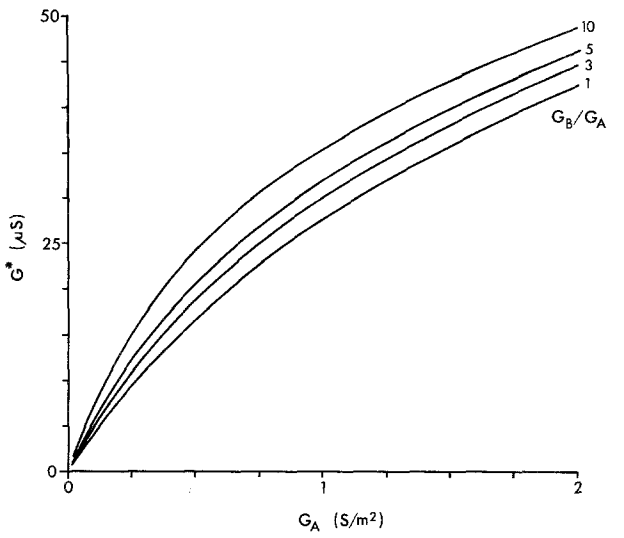

Fig. 8. Theoretical relationship between the measured conductance G^* and the area-specific conductance G_A of the zone into which current was injected (*see* Fig. 2(*b*)). The relationship is shown for different ratios (G_B/G_A) of the conductance of a distant narrow zone to that of the rest of the membrane. The parameter values used were $(r_o+r_i)=2.25$ MΩ/m, $b=2$ mm, $l_1=10$ mm, $d=0.7$ mm, $l=20$ mm

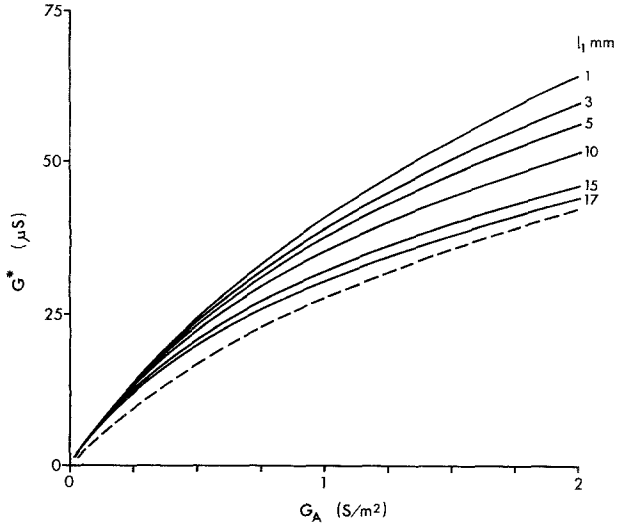

Fig. 9. Effect of the proximity of the high conductance zone upon the theoretical relationship between the measured conductance G^* and the area-specific conductance G_A of the zone into which current was injected (*see* Fig. 2(*b*)). Parameter values were as for Fig. 8, except $G_A/G_B=5$. The dashed line indicates the relationship in the absence of the high conductance zone

nated *Chara* cell, is now considered. Current is injected into a cell section of length l at $x=0$. The membrane is assumed to possess a conductance G_A, except for a narrow zone of conductance G_B between $x=l_1$ and l_2.

Figure 8 demonstrates how the relationship between G^* and G_A is affected by the presence of

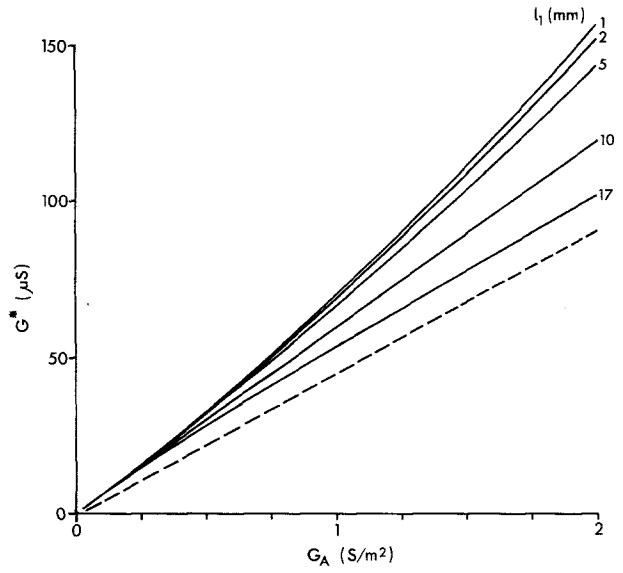

Fig. 10. As for Fig. 9, except that the membrane PD was measured 8.4 mm distant from $x=0$ (i.e. $x=0.42\,l$)

a narrow zone of increasingly higher conductance (G_B). The effect of this high conductance region is seen to be relatively slight. Thus in this situation a 1000% increase in the conductance of a zone 10 mm distant will only affect the measured conductance by ~30%.

Figure 9 shows how the relationship between G^* and G_A is affected by the proximity of the high conductance zone. Providing that the high conductance zone is at least 10 mm distant, the conductance measured at $x=0$ is altered by only 15 to 20% if $G_B/G_A=5$. Figure 10 shows a similar relationship to that of Fig. 9 if the PD is measured at $x=0.42\,l$ rather than 0 (i.e. the method of Hogg et al., 1968). In this situation the effect of a highly conductive region upon the measured conductance is somewhat increased.

Thus as a first approximation a cylindrical cell can be treated as effectively homogeneous, providing that the high conductance region is approximately a cable length distant from the point of current injection.

Discussion

The theoretical calculations presented clearly demonstrate that the presence of highly conductive zones in the membrane of cylindrical cells like *Chara* can drastically alter the interpretation of experimental conductance measurements, if current injection at a single point is used. Measurements in which current is injected into an acid zone will typically not be greatly affected. However, experiments where current is injected into a highly con-

ductive illuminated basic zone can greatly underestimate the true area-specific conductance of that region. This is because the effective area of the cell membrane through which the current actually flows is much less than that predicted by using the larger cable length of the low conductance regions.

Examination of Fig. 6 reveals that measurement of the response in membrane PD at just two positions remote from the point of current injection (as used by Williams et al., 1964; Bradley & Williams, 1967; Hogg et al., 1969) is unlikely to measure the important cable length in a spatially inhomogeneous cell, i.e. that at the point of current injection. As it appears likely that the membranes of illuminated cells could be spatially inhomogeneous, it is necessary to study the actual spatial distribution of injected current through the membrane to accurately determine the individual conductances. Without a detailed knowledge of this distribution, Figs. 3 and 4 show that a high area-specific conductance associated with the basic zones will not usually be evident if the membrane is treated as homogeneous. This error will be enhanced if the widely used technique of Hogg et al. (1968) is used whereby the resultant membrane PD is measured a distance 0.42 l away from the point of current injection (e.g. Figs. 4 and 10).

As discussed in the Theory, measurements which ensure a homogeneous membrane PD (i.e. those employing space-clamping) will reveal the presence of high conductance regions because the measured conductance is simply the spatial average over the entire membrane. Thus if high conductance regions are present under some conditions it might be expected that measurements involving space-clamping would produce higher area-specific conductances than those utilizing point current injection. An examination of the literature suggests that this is indeed the case (*see* Smith & Walker, 1983).

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