THE POTENTIAL DISTRIBUTION AND THE SHORT-CIRCUITING FACTOR IN THE SUCROSE GAP

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ABSTRACT The sucrose gap technique, though widely employed in many tissues, could not be used for quantitative measurements of the membrane potential, because the value of the short-circuiting factor and the influence of junction potential on the recorded potential difference were unknown. The formula that relates the recorded potential to the true resting membrane potential was found by application of the cable equations to a core conductor placed in a system with three different media, e.g. Ringer, sucrose, and KCl. The formula shows that the potential difference recorded over the sucrose insulator depends on the extracellular and the intracellular longitudinal resistances, the membrane resistance and the membrane potentials in each region, and on the junction potentials between the different media. The true membrane potential in the Ringer region can be calculated from the potential difference recorded after complete depolarization by KCl on one side of the preparation, if the longitudinal resistances, the membrane resistances, the extracellular potential in the sucrose, and the junction potential between Ringer and sucrose are determined by separate measurements.

INTRODUCTION

For structures in which the use of intracellular electrodes is difficult or impossible, a number of techniques have been described which permit the measurement of the membrane potential with extracellular electrodes. Some of these methods are based on the fact that after depolarization of part of the membrane, a current flows between the depolarized and the polarized region of the tissue. This current causes, in the extracellular medium, a potential difference, which depends on the membrane potential. The potential difference is recorded with electrodes placed on the depolarized and the intact regions of the cell.

The measurement of the membrane potential by this method is possible only if the mathematical relation between the recorded and the membrane potential is known. The relation usually utilized is:

$$V_m = \frac{R_e + R_i}{R_e} U, \qquad (1)$$

where R_e and R_i are the longitudinal resistances per unit length of the external and internal medium, respectively, and U and V_m are the measured and the membrane potential. The coefficient of equation 1 is the well-known short-circuiting factor.

From equation 1 it is clear, that when $R_e \gg R_i$, the short-circuiting factor approaches unity, and the potential measured with external electrodes will then approximate the true value of the membrane potential. These conditions were realized by Stämpfli (1954) by immersing the region between the recording electrodes in isotonic sucrose solution of high resistance. In the apparatus developed by Stämpfli, a continuous flow of the sucrose solution was maintained, so that a decrease in specific resistance by diffusion of ions from the preparation was avoided.

The method originally described for myelinated nerve fibers was subsequently applied to non-myelinated (*C*) fibers, (Ritchie and Straub, 1957), smooth muscle (Burnstock and Straub, 1958; Bülbring and Burnstock, 1960), striated muscle (König, 1962), and heart muscle (Rougier, Vassort, and Stämpfli, 1968), as well as to giant nerve fibers (Julian, Moore, and Goldman, 1962 *a*), and is extensively used in these tissues.

A number of technical realizations have been published, e.g. the use of rubber membranes (Berger, 1963) or of Vaseline insulators (Merrem, Küchler, and Isenberg, 1968) at either side of the sucrose region, the use of two sucrose insulators (Schmidt, 1962; Stämpfli, 1963), and the application of voltage-clamp conditions (Julian, Moore, and Goldman, 1962 *b*; Rougier et al., 1968).

In all these cases, however, the extracellular medium is divided into regions of different extracellular resistances, so that the simple formula of equation 1 is not applicable, for it was derived for a system in which the intracellular and the extracellular resistances were uniform with respect to distance (see Hodgkin and Rushton, 1946). For the conditions of the sucrose gap a different formula ought to be used. The derivation of this formula, which is necessary for quantitative measurements of the membrane potential with the sucrose gap method, is described in this paper. A preliminary communication on this subject has already appeared elsewhere (Jirounek and Straub, 1969).

FUNDAMENTAL EQUATIONS

A number of mathematical theories of transient and steady-state electrical properties of core conductors have been developed and discussed (Hermann, 1879; Hodgkin and Rushton, 1946; Rashevsky, 1960; Taylor, 1963; Clark and Plonsey, 1966; Hellerstein, 1968). In the system considered here, steady-state conditions are assumed for the electrical variables. The potential distribution can then be derived by application of one-dimensional cable equations (see Cole, 1968).

$$\frac{dV_e}{dx} = -R_e i_e,$$

$$\frac{dV_i}{dx} = -R_i i_i,$$

$$\frac{di_e}{dx} = -\frac{di_i}{dx} = i_m = \frac{(V_e - V_i)2\pi r}{R_m}. \qquad (2)$$

For a system which is homogeneous with respect to longitudinal distance, the solutions of these equations for V_i and V_e can be written in the following way:

$$V_i(x) = V_i(0)e^{-\alpha x},$$
$$V_e(x) = -\beta V_i(0)e^{-\alpha x}, \tag{3}$$

where

$$\alpha = \sqrt{\frac{2\pi r R_i(\beta + 1)}{R_m}}, \tag{4}$$

$$\beta = \frac{R_e}{R_i}, \tag{5}$$

and where $V_i(x)$ and $V_e(x)$ are the intracellular and extracellular potentials, respectively, x is the distance along the system, $V_i(0)$ the potential in the core at $x = 0$, r the radius of the core, R_m the membrane resistance per unit area, and R_i and R_e are the resistances per unit length of the internal and external media, respectively.

Let us now consider a system with two different extracellular media I and II (see Fig. 1). The membrane potential in the two regions will be designated by E_m^{I} and E_m^{II}, the junction potential between the two media by J. The distribution of

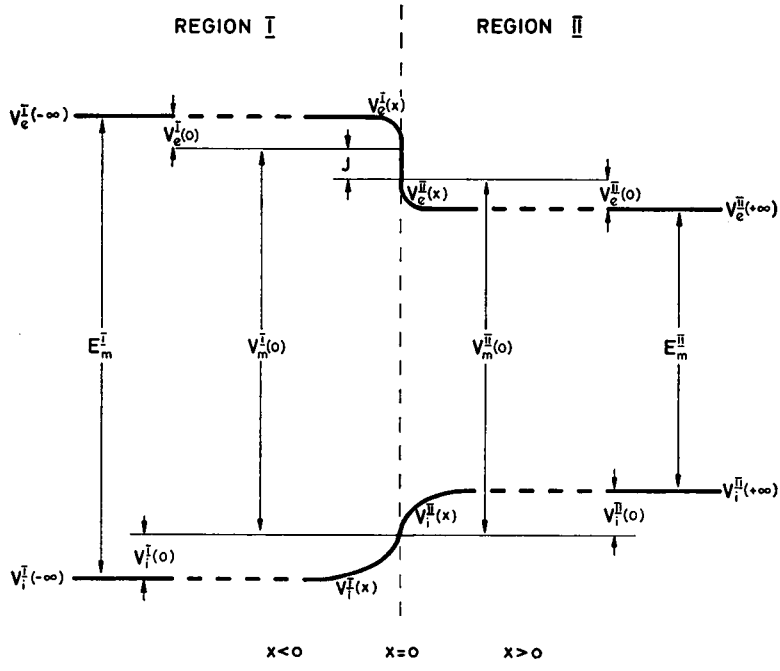

FIGURE 1 Distribution of extracellular and intracellular potentials in two regions with different extracellular media.

$V_i(x)$ and $V_e(x)$ is then given by Fig. 1, where the origin of x lies at the interphase between the two regions.

From Fig. 1 it can be seen that the resting membrane potential in region I is:

$$V_m^{\mathrm{I}}(x) = E_m^{\mathrm{I}} + V_e^{\mathrm{I}}(x) - V_i^{\mathrm{I}}(x). \tag{6}$$

Introducing equation 3 into equation 6,

$$V_m^{\mathrm{I}}(x) = E_m^{\mathrm{I}} - V_i^{\mathrm{I}}(0)e^{-\alpha x}(\beta^{\mathrm{I}} + 1). \tag{7}$$

Similarly in region II the membrane potential is:

$$V_m^{\mathrm{II}}(x) = E_m^{\mathrm{II}} + V_e^{\mathrm{II}}(x) - V_i^{\mathrm{II}}(x). \tag{8}$$

Introducing equation 3 into equation 8,

$$V_m^{\mathrm{II}}(x) = E_m^{\mathrm{II}} - V_i^{\mathrm{II}}(0)e^{-\alpha x}(\beta^{\mathrm{II}} + 1). \tag{9}$$

From equations 7 and 9 the intracellular potential at $x = 0$ in region I will be

$$V_i^{\mathrm{I}}(0) = -[V_m^{\mathrm{I}}(0) - E_m^{\mathrm{I}}]\frac{1}{\beta^{\mathrm{I}} + 1}, \tag{10}$$

and in region II,

$$V_i^{\mathrm{II}}(0) = -[V_m^{\mathrm{II}}(0) - E_m^{\mathrm{II}}]\frac{1}{\beta^{\mathrm{II}} + 1} = -[V_m^{\mathrm{I}}(0) - J - E_m^{\mathrm{II}}]\frac{1}{\beta^{\mathrm{II}} + 1}, \tag{11}$$

where

$$J = V_m^{\mathrm{I}}(0) - V_m^{\mathrm{II}}(0). \tag{12}$$

We can now write the equations for the longitudinal distribution of the extracellular and intracellular potentials in regions I and II. From Fig. 1 and equation 3,

$$\left.\begin{aligned} V_i^{\mathrm{I}}(x) &= V_i^{\mathrm{I}}(-\infty) + V_i^{\mathrm{I}}(0)e^{\alpha^{\mathrm{I}}x}, && (13)\\ V_e^{\mathrm{I}}(x) &= V_e^{\mathrm{I}}(-\infty) - \beta^{\mathrm{I}}V_i^{\mathrm{I}}(0)e^{\alpha^{\mathrm{I}}x}, && (14)\end{aligned}\right\} x < 0$$

$$\left.\begin{aligned} V_i^{\mathrm{II}}(x) &= V_i^{\mathrm{II}}(+\infty) + V_i^{\mathrm{II}}(0)e^{-\alpha^{\mathrm{II}}x}, && (15)\\ V_e^{\mathrm{II}}(x) &= V_e^{\mathrm{II}}(+\infty) - \beta^{\mathrm{II}}V_i^{\mathrm{II}}(0)e^{-\alpha^{\mathrm{II}}x}. && (16)\end{aligned}\right\} x > 0$$

Introducing equation 10 into equations 13 and 14,

$$V_i^{\mathrm{I}}(x) = V_i^{\mathrm{I}}(-\infty) + [E_m^{\mathrm{I}} - V_m^{\mathrm{I}}(0)]\frac{1}{\beta^{\mathrm{I}} + 1}e^{\alpha^{\mathrm{I}}x}, \tag{17}$$

$$V_e^{\mathrm{I}}(x) = V_e^{\mathrm{I}}(-\infty) - [E_m^{\mathrm{I}} - V_m^{\mathrm{I}}(0)] \frac{\beta^{\mathrm{I}}}{\beta^{\mathrm{I}} + 1} e^{\alpha^{\mathrm{I}} x}, \tag{18}$$

and equation 11 into equations 15 and 16,

$$V_i^{\mathrm{II}}(x) = V_i^{\mathrm{II}}(+\infty) - [V_m^{\mathrm{I}}(0) - J - E_m^{\mathrm{II}}] \frac{1}{\beta^{\mathrm{II}} + 1} e^{-\alpha^{\mathrm{II}} x}, \tag{19}$$

$$V_e^{\mathrm{II}}(x) = V_e^{\mathrm{II}}(+\infty) + [V_m^{\mathrm{I}}(0) - J - E_m^{\mathrm{II}}] \frac{\beta^{\mathrm{II}}}{\beta^{\mathrm{II}} + 1} e^{-\alpha^{\mathrm{II}} x}. \tag{20}$$

Equations 17–20 describe the longitudinal extracellular and the intracellular potential in the proximity of the interface between the two regions. From these equations and from Fig. 2, which explains the symbols used in the following formula, we now find the fundamental equations for the longitudinal potential distribution in the three regions of the sucrose gap:

(*a*) in the Ringer region,

$$V_i^R(x) = V_i^R(-\infty) + [E_m^R - V_m^R(0)] \frac{1}{\beta^R + 1} e^{\alpha^R x}, \tag{21}$$

$$V_e^R(x) = V_e^R(-\infty) - [E_m^R - V_m^R(0)] \frac{\beta^R}{\beta^R + 1} e^{\alpha^R x}; \tag{22}$$

(*b*) in the sucrose region,

(i) in the proximity of the Ringer region,

$$V_i^S(x) = V_i^S(+\infty) - [V_m^R(0) - J_1 - E_m^S] \frac{1}{\beta^S + 1} e^{-\alpha^S x}, \tag{23}$$

$$V_e^S(x) = V_e^S(+\infty) + [V_m^R(0) - J_1 - E_m^S] \frac{\beta^S}{\beta^S + 1} e^{-\alpha^S x}; \tag{24}$$

(ii) in the proximity of the region of the test solution,

$$V_i^S(x) = V_i^S(-\infty) + [E_m^S - V_m^S(0)] \frac{1}{\beta^S + 1} e^{\alpha^S x}, \tag{25}$$

$$V_e^S(x) = V_e^S(-\infty) - [E_m^S - V_m^S(0)] \frac{\beta^S}{\beta^S + 1} e^{\alpha^S x}; \tag{26}$$

(iii) in the region of the test solution,

$$V_i^T(x) = V_i^T(+\infty) - [V_m^S(0) - J_2 - E_m^T] \frac{1}{\beta^T + 1} e^{-\alpha^T x}, \tag{27}$$

$$V_e^T(x) = V_e^T(+\infty) + [V_m^S(0) - J_2 - E_m^T] \frac{\beta^T}{\beta^T + 1} e^{-\alpha^T x}. \tag{28}$$

THE POTENTIAL RECORDED BETWEEN RINGER AND TEST SOLUTION

From Fig. 2 we see that the over-all recorded potential is

$$U = [E_m^R - V_m^R(0)]\frac{\beta^R}{\beta^R + 1} + J_1 + [V_m^R(0) - J_1 - E_m^S]\frac{\beta^S}{\beta^S + 1}$$
$$+ [E_m^S - V_m^S(0)]\frac{\beta^S}{\beta^S + 1} + J_2 + [V_m^S(0) - J_2 - E_m^T]\frac{\beta^T}{\beta^T + 1}. \quad (29)$$

This formula applies, as shown by Fig. 2, only in cases where the widths of the three regions are many times larger than the corresponding space constants and the recording electrodes placed sufficiently far away from the interphases. If, for example, the width of the sucrose region is near the length of the space constant in this region, an interaction between the three potentials appears. This interaction has been studied experimentally by Blaustein and Goldman (1966).

The potential changes in the intracellular medium must be continuous; for this

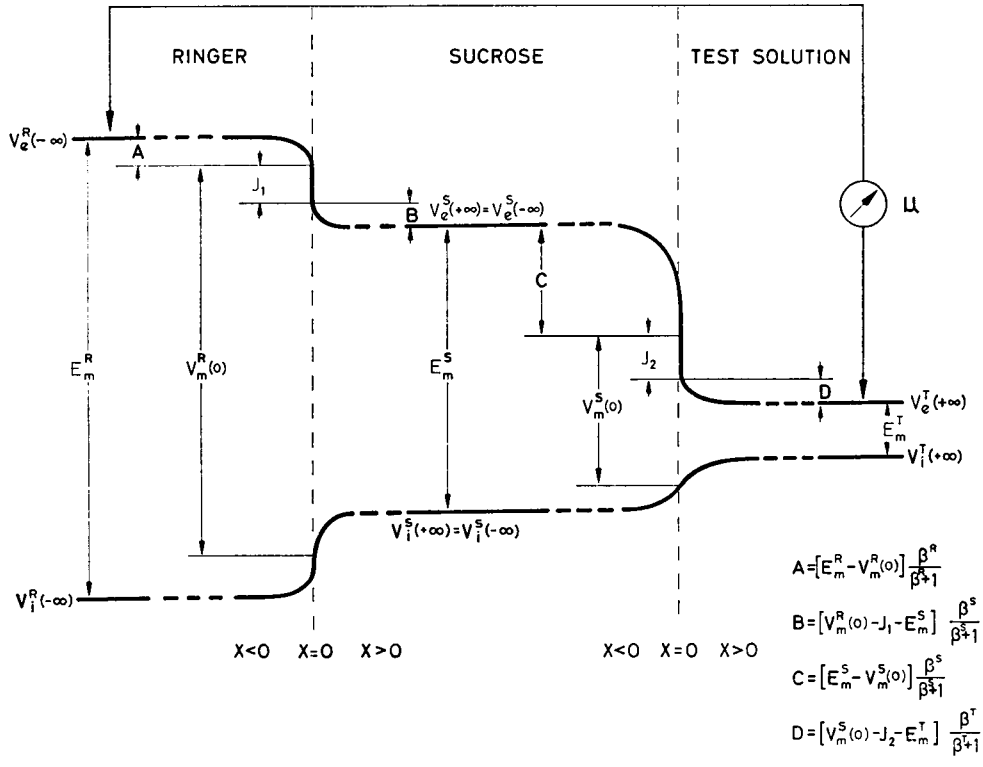

FIGURE 2 Distribution of extracellular and intracellular potentials in the sucrose gap apparatus.

reason we can write at the interphase between Ringer and sucrose:

$$\left[\frac{dV_i^R(x)}{dx}\right]_{x=0} = \left[\frac{dV_i^S(x)}{dx}\right]_{x=0}, \tag{30}$$

and at the interphase between sucrose and test solution,

$$\left[\frac{dV_i^S(x)}{dx}\right]_{x=0} = \left[\frac{dV_i^T(x)}{dx}\right]_{x=0}. \tag{31}$$

Differentiating equations 21, 23, 25, and 27 we find,

$$\frac{dV_i^R(x)}{dx} = [E_m^R - V_m^R(0)]\frac{\alpha^R}{\beta^R + 1} e^{\alpha R_x}, \tag{32}$$

$$\frac{dV_i^S(x)}{dx} = [V_m^R(0) - J_1 - E_m^S]\frac{\alpha^S}{\beta^S + 1} e^{\alpha S_x}, \tag{33}$$

$$\frac{dV_i^S(x)}{dx} = [E_m^S - V_m^S(0)]\frac{\alpha^S}{\beta^S + 1} e^{\alpha S_x}, \tag{34}$$

$$\frac{dV_i^T(x)}{dx} = [V_m^S(0) - J_2 - E_m^T]\frac{\alpha^T}{\beta^T + 1} e^{\alpha T x}. \tag{35}$$

Introducing equations 32 and 33 into equations 30, and equations 34 and 35 into equation 31 we have:

$$[E_m^R - V_m^R(0)]\frac{\alpha^R}{\beta^R + 1} = [V_m^R(0) - J_1 - E_m^S]\frac{\alpha^S}{\beta^S + 1}, \tag{36}$$

$$[E_m^S - V_m^S(0)]\frac{\alpha^S}{\beta^S + 1} = [V_m^S(0) - J_2 - E_m^T]\frac{\alpha^T}{\beta^T + 1}, \tag{37}$$

and solving these equations with respect to $V_m^R(0)$ and $V_m^S(0)$ we find:

$$V_m^R(0) = \frac{J_1 B^S \alpha^S + E_m^S B^S \alpha^S + E_m^R B^R \alpha^R}{B^R \alpha^R + B^S \alpha^S}, \tag{38}$$

$$V_m^S(0) = \frac{J_2 B^T \alpha^T + E_m^S B^S \alpha^S + E_m^T B^T \alpha^T}{B^T \alpha^T + B^S \alpha^S}, \tag{39}$$

where

$$B^R = \frac{1}{\beta^R + 1}; \qquad B^S = \frac{1}{\beta^S + 1}; \qquad B^T = \frac{1}{\beta^T + 1}.$$

Introducing equations 38 and 39 into equation 29 we obtain, after rearrangement, the

final expression for the potential recorded between the Ringer and the test solution:

$$U = J_1\left(1 - \frac{C^R B^S \alpha^S + C^S B^R \alpha^R}{B^S \alpha^S + B^R \alpha^R}\right) + J_2\left(1 - \frac{C^S B^T \alpha^T + C^T B^S \alpha^S}{B^S \alpha^S + B^T \alpha^T}\right)$$
$$+ E_m^R \frac{C^R B^S \alpha^S + C^S B^R \alpha^R}{B^S \alpha^S + B^R \alpha^R} - E_m^T \frac{C^S B^T \alpha^T + C^T B^S \alpha^S}{B^S \alpha^S + B^T \alpha^T}$$
$$+ E_m^S \left[\frac{B^S \alpha^S}{B^R \alpha^R + B^S \alpha^S}(C^S - C^R) - \frac{B^S \alpha^S}{B^S \alpha^S + B^T \alpha^T}(C^S - C^T)\right], \quad (40)$$

where

$$C^R = \frac{\beta^R}{\beta^R + 1}; \qquad C^S = \frac{\beta^S}{\beta^S + 1}; \qquad C^T = \frac{\beta^T}{\beta^T + 1}.$$

DISCUSSION

According to equation 40, the recorded potential difference is the sum of five expressions, which correspond to the contributions of the membrane potentials in the three regions, and the contributions of the two junction potentials.

Since the formula is rather complicated, the importance of the different factors is probably best explained by a numerical example. In order to do so, the coefficients of the five potentials will be designated by K_{J1}, K_{J2}, K_{ER}, K_{ET} and K_{ES}, so that equation 40 can be written in the form:

$$U = J_1 K_{J1} + J_2 K_{J2} + E_m^R K_{ER} - E_m^T K_{ET} + E_m^S K_{ES}. \quad (40\,b)$$

Fig. 3 illustrates the values of these factors in function of the longitudinal extracellular resistance in the sucrose solution (R_e^s), all other resistances being kept constant. The values of these resistances were chosen so as to correspond to those of a single myelinated fiber mounted in a sucrose gap apparatus and depolarized on one side.

It is evident from this calculation that nearly the full value of the membrane potential is recorded, if the extracellular resistance of the sucrose solution is sufficiently large, and that in this case, the contributions of the junction potentials become negligibly small. For lower values of R_e^s the appropriate correcting factors have to be used. For comparison the corresponding values of the "classical" short-circuiting factor are also shown in Fig. 3.

Fig. 3 also illustrates that relatively small changes in the resistance of the sucrose solution may easily affect the recorded potential: e.g. if the R_e^s is increased from 4 to 5 Ωcm^{-1} the coefficients change by approximately 5%.

In the case of complete depolarization of the membrane in the test region by KCl, E_m^T is equal to zero and the junction potential J_2 is negligibly small, so that equation 40 simplifies to the sum of three expressions. In addition to the recorded potential difference (U), the determination of the membrane potential (E_m^R) then requires the

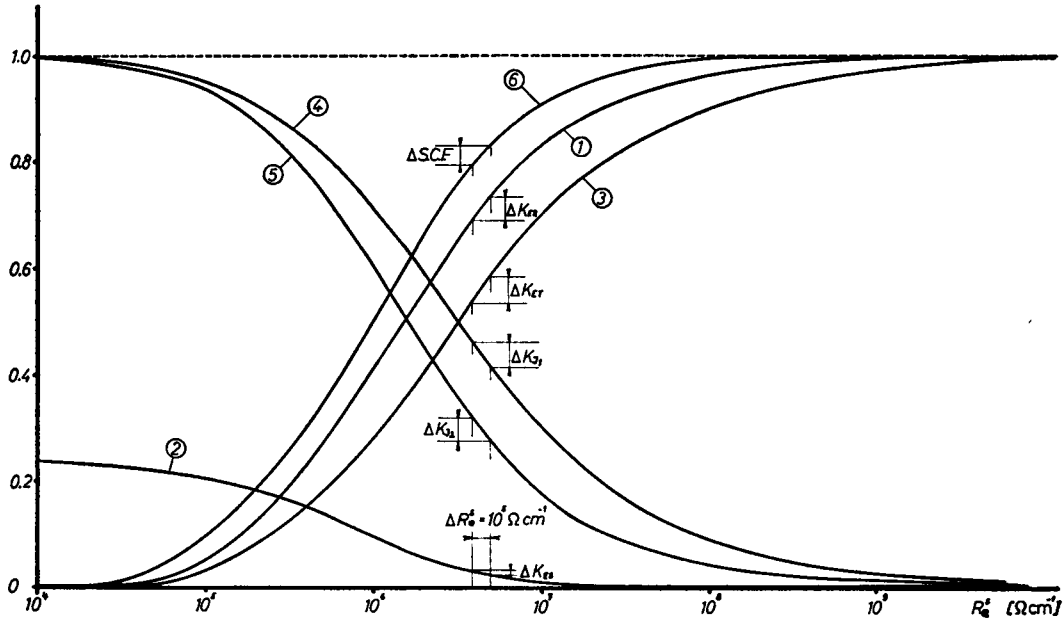

FIGURE 3 Relation between the values of the coefficients of equation 40 *b* and the resistance of sucrose. Curve 1 corresponds to the coefficient K_{ER}, curve 2 to K_{ES}, curve 3 to K_{ET}, curve 4 to K_{J1}, and curve 5 to K_{J2}. For comparison the corresponding values of the "classical" short-circuiting factor (S.C.F.) are also shown (curve 6). The values of the other resistances were chosen so as to correspond to those of a single myelinated fiber.

determination of the junction potential between Ringer and sucrose (J_1), as well as measurements of the extracellular and intracellular longitudinal resistances and of the membrane resistances in the three regions, and the determination of the resting potential in the sucrose (E_m^S). Knowledge of this value is fortunately not of great importance, for if the extracellular, the intracelluar, and the membrane resistance in Ringer are approximately equal to those in the test solution, C^R is close to C^T, B^R close to B^T, and α^R close to α^T, resulting in the coefficient of E_m^S to approach zero.

The classical sucrose gap apparatus does not allow measurements of the junction potentials, and of the membrane potential and the space constant in the sucrose region. However, these parameters can be measured in a modified apparatus which will be described elsewhere. Measurements of the space constants and of the sum of the extracellular and intracellular resistances in the Ringer and test region are also possible in this apparatus; further, knowledge of the different conductivities and of the geometrical factors then allows calculation of the extracellular resistances.

In many cases, the importance of the different factors can be estimated from known values of the membrane resistance and the resistance of the axoplasm; moreover, values for the junctions potentials can be found in the literature. Tables of standard numerical solution of the different factors of equation 40 would then permit rapid estimations of the membrane potential; such tables are now being prepared;

an example is plotted in Fig. 3. The equation for the recorded potential, though complicated, can thus be used in practice for the determination of the membrane potential by the sucrose gap.

Other techniques, apart from the sucrose gap apparatus, also make use of an increase in extracellular resistance, e.g. the air gap technique, or the division of the extracellular medium by small bore spacers (see Stämpfli; 1952); further, in preparations *in situ*, an inhomogeneity of the extracellular medium is often found. Application of equation 40 in these cases should also allow a better correlation between the membrane potential calculated from extracellular measurements and the true membrane potential.

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