THE CORRECTION FACTORS FOR SUCROSE GAP MEASUREMENTS AND THEIR PRACTICAL APPLICATIONS

P. JIROUNEK, G. J. JONES, C. W. BURCKHARDT, AND R. W. STRAUB, *Département de Pharmacologie, Ecole de Médecine, 1211 Genève 4, and Institut de Microtechnique, Ecole Polytechnique Fédérale de Lausanne, 1025 St. Sulpice, Switzerland*

ABSTRACT The distribution of extracellular and intracellular potential in the sucrose gap apparatus, previously established for a single fiber using the cable equations for a core conductor model (Jirounek and Straub, *Biophys. J.*, **11**:1, 1971), is obtained for a multifiber preparation. The exact equation is derived relating the true membrane potential change to the measured potential differences across the sucrose gap, the junction potentials between sucrose and physiological solution, the membrane potential in the sucrose region, and the electrical parameters of the preparation in each region of the sucrose gap. The extracellular potential distribution has been measured using a modified sucrose gap apparatus for the frog sciatic nerve and the rabbit vagus nerve. The results indicate a hyperpolarization of the preparations in the sucrose region, of 60–75 mV. The hyperpolarization is independent of the presence of junction potentials. The calculation of the correction terms in the equation relating the actual to the measured potential change is illustrated for the case of complete depolarization by KCl on one side of the sucrose gap. The correction terms in the equation are given for various experimental conditions, and a number of nomographic charts are presented, by means of which the correction factors can be rapidly evaluated.

INTRODUCTION

In an earlier study, the theoretical distribution of the extracellular and intracellular potentials in the sucrose gap was described, as well as the relationship between the electrical parameters of the preparation and the potential recorded by extracellular electrodes (Jirounek and Straub, 1971). The equations turned out to be rather complicated, even though the study was based on a number of simplifying assumptions, such as the use of one-dimensional cable equations, and was confined to the case of a single fiber preparation.

In the present paper, the study is extended to a multifiber preparation. The equations thus derived are tested experimentally to judge whether the use of the one-dimensional equations is justified. In addition, the hyperpolarization observed in the region of the sucrose gap adjacent to the sucrose partition (see Blaustein and Goldman, 1966) is examined both theoretically and experimentally: it appears to be caused by a large hyperpolarization of the preparation in the sucrose solution.

Finally, to simplify the utilization of the derived equations, a number of nomographic charts are presented. These allow the rapid quantitive estimation of the correction factors, for different experimental conditions. In this way, measurements of high precision can be made using the sucrose gap method.

MATERIALS AND METHODS

Recordings of the extracellular potential were made on sciatic nerves of the frog (*Rana temporaria*) and on rabbit vagus nerves. The nerves were desheathed and mounted in a modified sucrose gap apparatus, where they were superfused with Ringer's (frog sciatic) or Locke (rabbit vagus) solution in the two lateral regions (*R* and *T*, Fig. 1). The central regions were superfused with isotonic sucrose solution (*S*, Fig. 1). All experiments were at room temperature (20–22°C), and at pH 7.4.

Ringer's solution contained: 115 mM NaCl, 2.5 mM KCl, 1.0 mM $CaCl_2$, 1 mM Tris. Locke solution contained: 154 mM NaCl, 5.6 mM KCl, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, 1 mM Tris. K-rich solutions were obtained by replacing NaCl with KCl. Isotonic sucrose was 230 or 315 mM. The resistivity of the sucrose solution was periodically controlled with a conductivity bridge.

The modified sucrose gap apparatus, which consists of a system of channels cut in a Perspex plate, is shown schematically in Fig. 1. Measurements of the extracellular potential along the preparation were made with a glass microelectrode, continuously moved along the preparation by means of a micromanipulator. The electrodes were made from Clark Electromedical GC 200F–4, pulled to ~10 μm tip diameter, and filled with 3 M KCl. The position of the electrode was observed with a binocular microscope, and determined to a tenth of a millimeter using the micromanipulator scale. The potential was recorded with respect to a KCl-AgCl-Ag electrode, placed in one of the lateral regions, amplified by a conventional differential amplifier and recorded with a pen recorder.

Whenever possible, results are expressed as mean ± SD.

THEORY

The Validity of the One-dimensional Cable Equations

The equations describing the distribution of extracellular and intracellular potentials in the sucrose gap apparatus were derived in a previous paper (Jirounek and Straub, 1971) using the basic one-dimensional cable equations. This simplified model is expected to be valid for most sucrose gap preparations, which are generally used when the diameters of the cells of interest are not large enough to permit the use of intracellular microelectrodes. In these cases, radial currents are only a small fraction of the longitudinal, axial currents, and the general electrostatic equation reduces to the one-dimensional cable equations (Clark and Plonsey, 1966; Hellerstein, 1968; Eisenberg and Johnson, 1970). This condition is equivalent to assuming that the electrical length constant of the cells is much greater than the diameter

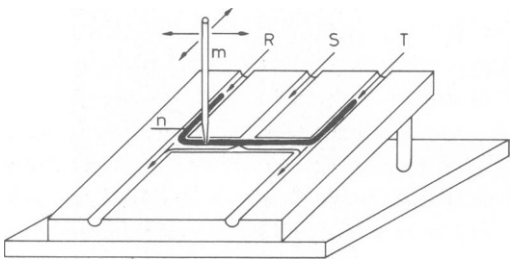

FIGURE 1 Schematic drawing of the modified sucrose gap apparatus, which allows recording of the extracellular potential along the nerve. *R*, *S*, and *T* designate the regions of Ringer's, sucrose, and test solution, respectively, *n* the nerve, and *m* the microelectrode which is moved along the preparation with a micromanipulator.

(Eisenberg and Johnson, 1970), a condition which is well satisfied for the nerve fibers used in our studies (Jirounek, 1978), and for many other preparations.

Junction potentials are present in the sucrose gap apparatus (Jirounek and Straub, 1971) and could present external sources of current. These potentials, however, can be considered to have radial symmetry, and, to a first approximation, will introduce negligible corrections to the solutions of the one-dimensional cable equations.

Extension to Multifiber Preparations

In the previous analysis, only a single fiber was considered (Jirounek and Straub, 1971). For a preparation containing a whole nerve with n fibers, assuming steady-state conditions, and homogeneity of the extracellular medium, the one-dimensional cable equations are:

$$\begin{aligned} \frac{dV_e}{dx} &= -R_e i_e \\ \frac{dV_i}{dx} &= -R_i i_i \\ -\frac{di_e}{dx} &= n\frac{di_i}{dx} = \frac{V_e - V_i}{R_m} 2\pi an, \end{aligned} \tag{1}$$

where V_e and V_i are the extracellular and intracellular potentials, respectively; i_e and i_i the total extracellular current and the intracellular current for a single fiber, respectively; x is the distance along the longitudinal axis of the cells; R_m the membrane resistance times unit area, R_e and R_i the resistance per unit length of the external medium and internal medium (for one fiber), respectively; and a is the radius of the fibers, taken to be an average value for the whole nerve.

The solution of Eq. 1, for a system which is homogeneous with respect to longitudinal distance, is:

$$V_i(x) = V_i(0) \cdot e^{-\alpha x} \tag{2}$$

$$V_e(x) = -n\frac{R_e}{R_i} \cdot V_i(0)e^{-\alpha x}, \tag{3}$$

where

$$\alpha = \sqrt{\frac{2\pi a(nR_e + R_i)}{R_m}}. \tag{4}$$

Eq. 2, 3, and 4 are formally identical to the solution for a single fiber, the only difference being that the inverse of the length constant (4) and the coefficient in Eq. 3 contain in addition the number of fibers, n.

Calculation of the Change in Membrane Potential

The one-dimensional cable equations were, in an earlier paper (Jirounek and Straub, 1971), used for the derivation of the relation between the measured potential in the sucrose gap

apparatus and the electrical parameters of the preparation. The result is now used to calculate an expression for the change in membrane potential in the test region, in terms of the measured potential differences in the sucrose gap.

In the beginning of an experiment with the sucrose gap, the two lateral regions R and T are perfused with the same physiological solution. When the solutions have diffused into the extracellular space, the potential U recorded by the extracellular electrodes becomes stable and is given by a sum of five expressions (Jirounek and Straub, 1971, Eq. 40), which can be rearranged and simplified so that the following equation is obtained:

$$U = E_m^R K_{RS} + E_m^S (K_{ST} - K_{RS}) - E_m^T K_{ST} - J_1(1 - K_{RS}) - J_2(1 - K_{ST}), \quad (5)$$

where:

$$K_{RS} = \frac{B^S \alpha^S C^R + B^R \alpha^R C^S}{B^R \alpha^R + B^S \alpha^S}, \quad (6)$$

$$K_{ST} = \frac{B^T \alpha^T C^S + B^S \alpha^S C^T}{B^S \alpha^S + B^T \alpha^T}. \quad (7)$$

and:

$$\begin{aligned} B^R &= \frac{R_i^R}{nR_e^R + R_i^R}; \quad B^S = \frac{R_i^S}{nR_e^S + R_i^S}; \quad B^T = \frac{R_i^T}{nR_e^T + R_i^T} \\ C^R &= \frac{nR_e^R}{nR_e^R + R_i^R}; \quad C^S = \frac{nR_e^S}{nR_e^S + R_i^S}; \quad C^T = \frac{nR_e^T}{nR_e^T + R_i^T}. \end{aligned} \quad (8)$$

J_1 and J_2 are the junction potentials between the solutions in regions R and T and the sucrose solution, respectively, and E_m is the membrane potential. The superscripts R, S, and T now refer to the values of the coefficients in the three different regions of the sucrose gap.

From Eq. 5 the membrane potential in the region of the test solution can be calculated:

$$E_m^T = -\frac{U}{K_{ST}} + E_m^R \frac{K_{RS}}{K_{ST}} + E_m^S\left(1 - \frac{K_{RS}}{K_{ST}}\right) - J_1\left(\frac{1}{K_{ST}} - \frac{K_{RS}}{K_{ST}}\right) - J_2\left(\frac{1}{K_{ST}} - 1\right). \quad (9)$$

After the substitution of the physiological solution in the region T by a test solution, the membrane potential in this region will tend to a new value, $E_m^{T\prime}$. At the same time, R_e^T, R_m^T, and R_i^T could also be modified, as well as the junction potential J_2. Their new values are designed $R_e^{T\prime}$, $R_m^{T\prime}$, $R_i^{T\prime}$, and J_2'. The recorded potential will now be:

$$U' = E_m^R K_{RS} + E_m^S(K'_{ST} - K_{RS}) - E_m^{T\prime} K'_{ST} - J_1(1 - K_{RS}) - J_2(1 - K'_{ST}), \quad (5a)$$

where K'_{ST} is obtained from Eqs. 8 and 7 by substituting $R_e^{T\prime}$, $R_i^{T\prime}$, and $R_m^{T\prime}$ for R_e^T, R_i^T, and R_m^T. The new value of the membrane potential in the T region, calculated from Eq. 5a is therefore:

$$E_m^{T\prime} = -\frac{U'}{K'_{ST}} + E_m^R \frac{K_{RS}}{K'_{ST}} + E_m^S\left(1 - \frac{K_{RS}}{K_{ST}}\right) - J_1\left(\frac{1}{K'_{ST}} - \frac{K_{RS}}{K'_{ST}}\right) - J_2'\left(\frac{1}{K'_{ST}} - 1\right). \quad (10)$$

From Eqs. 9 and 10, the modification of the membrane potential in the T region by the test solution then becomes:

$$\Delta E_m^T = E_m^T - E_m^{T\prime} = -\frac{\Delta U}{K'_{ST}} + U\left(\frac{1}{K'_{ST}} - \frac{1}{K_{ST}}\right) + (E_m^R - E_m^S)\left(\frac{K_{RS}}{K_{ST}} - \frac{K_{RS}}{K'_{ST}}\right) + J_1\left(\frac{1}{K'_{ST}} - \frac{1}{K_{ST}} + \frac{K_{RS}}{K_{ST}} - \frac{K_{RS}}{K'_{ST}}\right) + J_2\left(1 - \frac{1}{K_{ST}}\right) + J'_2\left(\frac{1}{K'_{ST}} - 1\right), \quad (11)$$

where $\Delta U = U - U'$.

Effect of the Test Solution on the Electrical Parameters of the Test Region

Eq. 11 expresses the fact that the membrane potentials E_m^R, E_m^S and E_m^T, as well as the junction potentials J_1 and J_2 contribute to the potential measured by the extracellular electrodes in a different manner before and after introduction of the test solution. The complexity of this equation is due, at least partially, to this phenomenon. However, if the test solution alters only the membrane potential E_m^T, without affecting significantly the other electrical parameters of the preparation and the surrounding medium in the test region, then:

$$K_{ST} = K'_{ST} = K \neq K_{RS},$$

and Eq. 11 simplifies to:

$$\Delta E_m^T = -\frac{\Delta U}{K} + J_2\left(1 - \frac{1}{K}\right) + J'_2\left(\frac{1}{K} - 1\right). \quad (11a)$$

If furthermore the junction potential J_2 does not change after the introduction of the test solution, then $J_2 = J'_2$, and Eq. 11 becomes simply:

$$\Delta E_m^T = -\frac{\Delta U}{K}. \quad (11b)$$

Effect of an Initial Symmetry of the Sucrose Gap

On the other hand, when the resistances R_e, R_i, and R_m of the region R are equal to the corresponding resistances of the region T before the application of the test solution, and if the junction potentials J_1 and J_2 are equal, a simplification of Eq. 11 is obtained even if the test solution influences the value of K_{ST}. In this case $K_{RS} = K_{ST} = K \neq K'_{ST}$, and $J_1 = -J_2 = J \neq J'_2$, and Eq. 11 now becomes:

$$\Delta E_m^T = -\frac{\Delta U}{K'_{ST}} + U\left(\frac{1}{K'_{ST}} - \frac{1}{K}\right) + (E_m^R - E_m^S)\left(1 - \frac{K}{K'_{ST}}\right) + J\left(\frac{1-K}{K'_{ST}}\right) + J'_2\left(\frac{1}{K'_{ST}} - 1\right). \quad (11c)$$

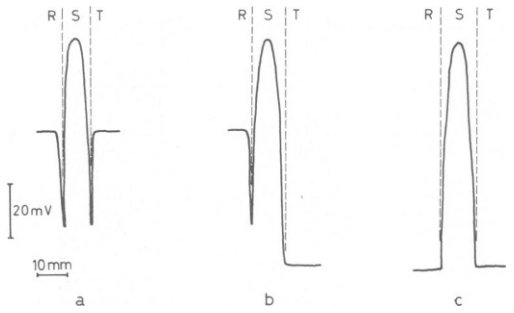

FIGURE 2 Recordings of the extracellular potential, measured along a frog sciatic nerve using the modified sucrose gap apparatus shown in Fig. 1. In *a*, the two lateral regions were perfused with Ringer's. In *b*, the Ringer's in region *T* was replaced by K^+-rich Ringer's (0 Na, 115 mM KCl), and *c*, the two lateral regions were perfused with K^+-rich Ringer's. The central region was perfused with isotonic sucrose. Note the junction potential between the Ringer's and sucrose solution, and the hyperpolarization of the preparation in the sucrose region. The hyperpolarization is independent of the junction potentials.

If, in addition, the test solution does not alter the junction potential J_2:

$$\Delta E_m^T = -\frac{\Delta U}{K'_{ST}} + U\left(\frac{1}{K'_{ST}} - \frac{1}{K}\right) + (E_m^R - E_m^S)\left(1 - \frac{K}{K'_{ST}}\right) + J\left(1 - \frac{K}{K'_{ST}}\right), \quad (11d)$$

where now $J_1 = -J_2 = -J'_2 = J$.

EXPERIMENTAL RESULTS

Typical records of the distribution of the extracellular potential along the sucrose gap preparation are shown in Fig. 2, for the frog sciatic nerve, and in Fig. 3 for the rabbit vagus nerve. In these experiments, the central regions were perfused with isotonic sucrose and the

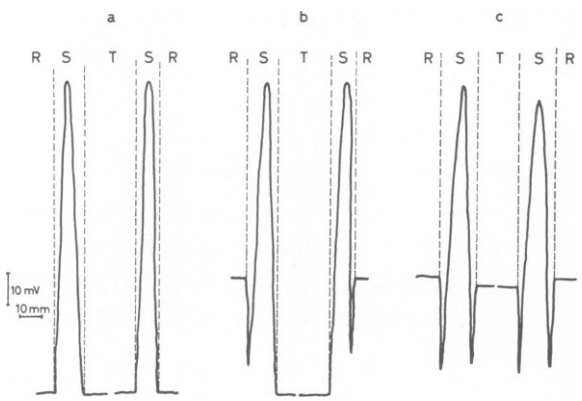

FIGURE 3 Recordings of the extracellular potential along a rabbit vagus nerve under sucrose gap conditions. In this experiment the potential was first measured in one direction, then repeated in the reverse direction. In *a*, both lateral regions were perfused with K^+-rich Locke solution (0 Na, 150 mM KCl). In *b*, the region *R* was perfused with Locke and the region *T* with K^+-rich Locke solution. In *c*, both lateral regions were perfused with Locke solution. Note the junction potential between the Locke and sucrose solutions, and the hyperpolarization in the sucrose region (cf. Fig. 2).

lateral regions with Ringer's or Locke solution or, to depolarize the preparation, with K^+-rich solutions. The records clearly show the junction potential at the interface between the Ringer's or Locke solution and the sucrose solution. On the other hand, in agreement with the experimental results of Blaustein and Goldman (1966), and with theoretical predictions, no measurable junction potential was observed between the K^+-rich and the sucrose solutions.

Besides the junction potentials, an important hyperpolarization in the sucrose region appears in all our records. The hyperpolarization restricted to the sucrose region, is present even in the absence of junction potentials (Fig. 2, record *c*, and Fig. 3, record *a*). The form of the hyperpolarization was studied in a series of experiments where the microelectrode was displaced in a "point-to-point" manner. The hyperpolarized portion of the nerve was lengthened by perfusing the two neighboring regions S and T with isotonic sucrose. The results are shown in Fig. 4 *a*, for the frog sciatic nerve.

Eq. 3 shows that there should be an exponential relation between the steady extracellular potential and the distance along the nerve. Hence, if Eq. 3 is obeyed, a straight line should be obtained when the logarithm of the potential is plotted against distance. Fig. 4 *b* shows that this is indeed the case. The length constant ($1/\alpha$) obtained from this figure is 1.70 ± 0.14 mm ($N = 5$). In a separate series of experiments, a similar analysis for the rabbit vagus nerve gave a value for the length constant in the sucrose region of 1.14 ± 0.09 mm ($N = 5$).

The finding that the extracellular potential follows closely an exponential curve provides experimental evidence for the use of the one-dimensional cable equations to describe the steady potential distribution in the sucrose gap. In addition, the magnitude of the hyperpolarization in the sucrose region, and the size of the correction factors in Eq. 11 can be calculated. The hyperpolarization is found from the following equation, obtained by combining Eqs. 24 and 38 of Jirounek and Straub (1971):

$$E_m^S - E_m^R = -J - A/C^S\left(1 + \frac{B^S\alpha^S}{B^R\alpha^R}\right),$$

where now A is the measured external potential drop in the sucrose region, α^S is the reciprocal

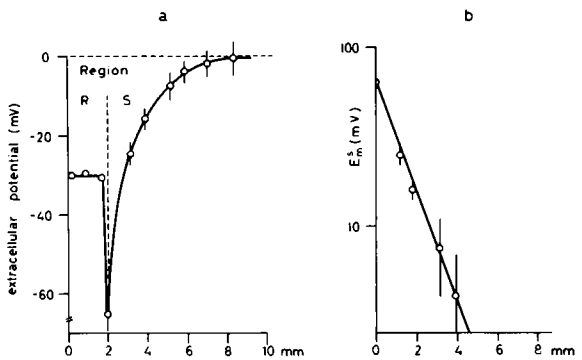

FIGURE 4 Analysis of the extracellular potential measured in the sucrose region with a frog sciatic nerve in the sucrose gap apparatus. In *a*, the potential was measured successively at different points on each side of the Ringer's-sucrose solution junction. In *b*, the logarithm of the external potential in the sucrose region is plotted against distance. Each point is the mean and standard deviation of results from five separate experiments. The space constant obtained from *b* is 1.70 ± 0.14 mm.

of the measured space constant in the sucrose region, and the other constants are as previously defined. For the results shown in Fig. 4, and using the published data (see Hodgkin, 1967), the hyperpolarization is found to be 61 ± 5 mV for the frog sciatic nerve ($N = 5$). For the rabbit vagus nerve, A was measured as 85 ± 12 mV, and, using data from Straub (1963), the hyperpolarization was found to be 75 ± 11 mV ($N = 5$).

The use of Eq. 11 is illustrated by calculation of the correction terms for the experiment of Fig. 2. In this experiment, the sucrose gap was initially symmetrical, and the initial potential difference was zero (Fig. 2 *a*; $U = 0$, Eq. 5, since $K_{RS} = K_{ST}$, $J_1 = -J_2$, $E_m^R = E_m^T$). On introduction of K^+-rich solution in the test region, the measured potential change was 51 mV (Fig. 2 *b*). This potential change is related to the change in membrane potential by Eq. 11c, where $U = 0$ and $J_2' = 0$. Since, for this experiment, $K = 0.68$ and $K_{ST}' = 0.78$ (see legend to Fig. 5), the correction due to the hyperpolarization in the sucrose region, the third term in Eq. 11c, is equal to $(-61) \cdot (1 - 0.68/0.78) = -7.8$ mV. The correction term due to the junction potential is $(+34) \cdot (1 - 0.68)/0.78 = +13.9$ mV. The calculated change in membrane potential is thus $51/0.78 = 65$ mV, to which a net correction of +5.1 mV should be added.

The size of the correction terms in the above calculation are 12% and 21% of the change in membrane potential calculated using a simple "short circuiting factor" (K_{ST}'). The correction terms calculated for the experiment of Fig. 2 are higher than in other sucrose gap experiments, since our modified sucrose gap apparatus is not optimal, the external resistance in the sucrose region being necessarily lower than is usual to permit the extracellular recordings. However, it should be noted that the size of the corrections vary considerably depending on the experimental conditions, and, even with an optimal sucrose gap, can be up to 40% of the change in membrane potential calculated using a simple short circuiting factor (Jirounek, 1978). Calculation of the correction factors for different experimental situations using the coefficients K_{RS}, K_{ST}, and K_{ST}' can be much simplified using nomographic methods. Nomographic charts for calculation of these factors for the frog sciatic nerve, the rabbit vagus nerve, and the rat diaphragm are presented in the Appendix.

DISCUSSION

The Hyperpolarization in the Sucrose Solution

One of the disagreements between measurements of membrane potential with intracellular electrodes and measurements with the sucrose gap technique has been attributed to a hyperpolarization observed in the neighborhood of the sucrose region of the sucrose gap. As mentioned by several authors (Julian et al., 1962; Stämpfli, 1963; Naharashi et al., 1964), resting membrane potentials as well as action potentials measured under the sucrose gap may be larger than the corresponding potentials recorded by intracellular electrodes in the absence of a sucrose gap. Blaustein and Goldman (1966), studying this phenomenon for the lobster giant axon, concluded that loop currents, generated by the junction potentials, are at least partly responsible for the membrane hyperpolarization. To evaluate the importance of this hyperpolarization, and consequently its possible effect on measured potentials under sucrose gap conditions (see Eqs. 5 and 5a) we have used a modified sucrose gap apparatus to measure the distribution of extracellular potential along the preparation.

The hyperpolarization, as measured in our experiments, appears in the sucrose region. Our

experiments extend those of Blaustein and Goldman (1966), who observed the hyperpolarization in a short seawater region immediately adjacent to a sucrose region. We find that the hyperpolarization is present in the whole of the sucrose region, and will spread into adjacent regions due to the cable properties of the fibers. Measurements made in these adjacent regions can be expected to depend on the presence of local, loop currents. The hyperpolarization in the sucrose region does not depend on the presence of junction potentials since it occurs, and is of the same magnitude, when the junction potentials change or even in the absence of junction potentials. In addition, the hyperpolarization develops progressively on introduction of the sucrose solution, with a time constant of ~7 min for the frog sciatic nerve (unpublished results) corresponding to the time needed for the washing out of ions from the extracellular space, while the junction potentials appear immediately. The origin of the hyperpolarization has not yet been studied in detail.

Simplification of the Formulae for Different Experimental Conditions

The essential equation, which allows the calculation of the change of membrane potential ΔE_m^T from the measured difference of potential ΔU, is represented by Eq. 11, which contains five terms whose importance depends on the experimental conditions.

The first term, $\Delta U/K'_{ST}$, corresponds to the measured potential difference ΔU, corrected by a factor dependent on the electrical parameters of the preparation and of the surrounding solutions. The optimal experimental conditions would be obtained if $K_{ST} = K'_{ST} = 1$. In this case, all other terms in Eq. 11 vanish, and Eq. 11 would be simply $\Delta E_m^T = -\Delta U$, indicating that the change of E_m^T corresponds directly to the measured potential difference ΔU. Experimentally, this situation can only be approached, since the external resistance in the sucrose region cannot be increased indefinitely. The other terms in Eq. 11 must therefore be taken into account, when the ΔE_m^T is calculated.

The second term in Eq. 11 is the product of the potential U, measured before the application of the test solution, and of the difference of two constants, $1/K_{ST} - 1/K'_{ST}$. It is clear that if either U, or $1/K_{ST} - 1/K'_{ST}$ approach zero, the whole term becomes negligible. As we can see from Eq. 5, the potential U will be zero for an electrically symmetric sucrose gap apparatus, since then $J_1 = -J_2$, $K_{RS} = K_{ST}$ and before the introduction of the test solution $E_m^T = E_m^R$; the difference $1/K_{ST} - 1/K'_{ST}$ will become zero in all cases where the test solution does not affect the electrical parameters of the region T (except of course the membrane potential, E_m^T). These symmetry conditions are fulfilled relatively frequently in experiments with the sucrose gap.

The third term in Eq. 11 represents the correction due to the hyperpolarization of the preparation in the sucrose region. This term may be neglected only if $K_{ST} = K'_{ST}$. It signifies that, whenever the test solution modifies the electrical properties of the region T, the hyperpolarization will affect the results.

The same conclusion holds for the next term of Eq. 11, which expresses the influence of the junction potential J_1; only if $K_{ST} = K'_{ST}$ is this term negligible.

The last two expressions of Eq. 11 correct the effects of J_2 and J'_2 on the measured potential. Separately, they can be neglected only when these two junction potentials approach zero. However, if we consider the sum of these two terms, we see that they will cancel each other if $J_2 = J'_2$ and $K_{ST} = K'_{ST}$.

In summary, we can say that there are three important conditions which must be considered when experiments with a sucrose gap are performed: the symmetry of the two lateral regions, the effects of the test solution on the resistances R_e^T, R_i^T, and R_m^T, and the modification of the junction potential J_2 by the test solution. In the optimal case, when the apparatus can be considered as electrically symmetrical, and when no effects of the test solution on either the resistances of the test region or on the junction potential are to be expected, then the essential sources of errors in the sucrose gap measurements are eliminated, and the method allows measurements of high precision.

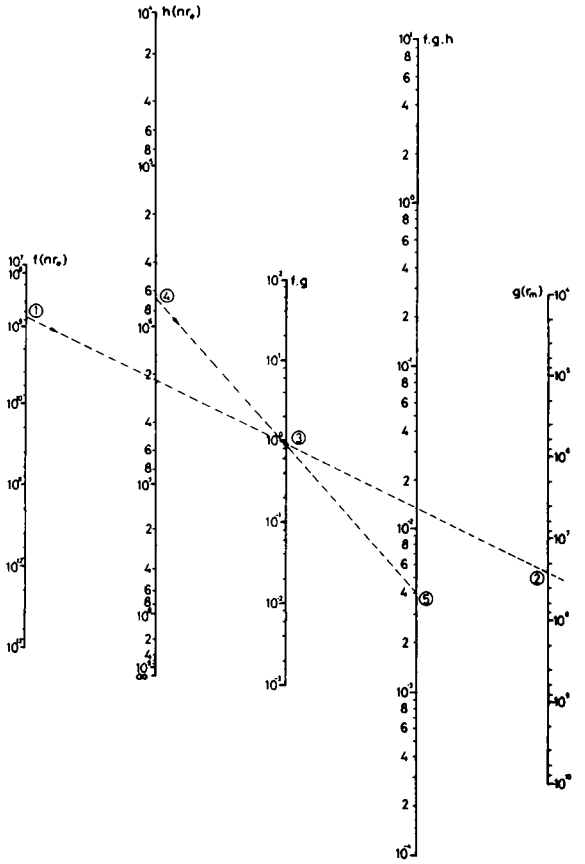

FIGURE 5 Nomogram for the calculation of the coefficients K_{RS}, K_{ST}, and K'_{ST} for the frog sciatic nerve, according to Eqs. A1 and A2. The units are Ω and cm; and the specific resistances are per, or times, unit length. The use of the chart is illustrated for the calculation of K_{RS} for the experiment of Fig. 2. The first point (*1*) on the axis $f(nr_e)$ corresponding to nr_e^S (7.5×10^8 Ωcm^{-1}) is joined to point *2* on the axis $g(r^m)$ corresponding to r_m^S (2.6×10^7 Ωcm, calculated from Eq. 4, using the known value of the space constant in the sucrose region; see Results). Point *3* is obtained on the axis $f \cdot g$ and is the value of $f(nr_e^S) \cdot g(r_m^S) = 0.9$. Point *3* is then joined to point *4*, on the axis $h(nr)$ corresponding to nr_e^R (6.8×10^5 Ωcm^{-1}), and extrapolation to the axis $f \cdot g \cdot h$ gives the value of $f(nr_e^S) \cdot g(r_m^S) \cdot h(nr_m^R) = 0.004$. The procedure is then repeated starting with nr_e^R joined to r_m^R (10^7 Ωcm) to obtain $f(nr_e^R) \cdot g(r_m^R) = 3.7$; followed by extrapolation from nr_e^S to obtain $f(nr_e^R) \cdot g(nr_e^R) \cdot h(nr_e^S) = 3.1$. Hence, from Eq. A1, $K_{RS} = 0.68$. $K'_{ST} = 0.78$ is determined in the same way, with the assumption that the membrane resistance in K^+-rich solution is reduced to 10% of its value in Ringer's.

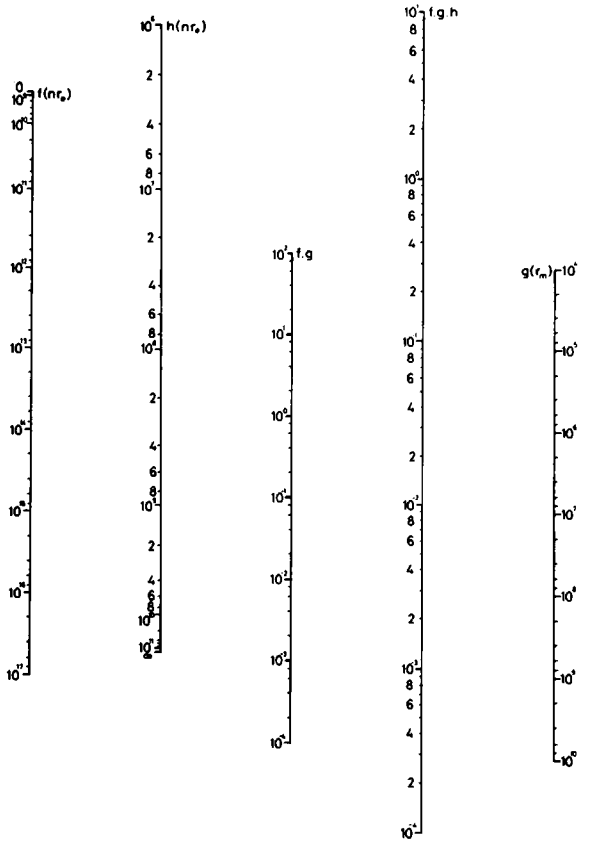

FIGURE 6 Nomogram for the determination of the correction coefficients, Eq. A1, for the rabbit vagus nerve. The units are Ω and cm. For details of the use of the chart, see legend to Fig. 5.

APPENDIX

Nomograms for the Correction Factors

Eqs. 11 and 11 *a–d*, which allow the calculation of changes of the membrane potential from the potential difference measured in the sucrose gap by the extracellular electrodes, all contain one or more of the coefficients K_{RS}, K_{ST}, and K'_{ST}. Calculation of the value of these coefficients from Eqs. 8, 6, and 7 is somewhat complicated and difficult in practice. To simplify their determination, we have, on the basis of equations 8, 6 and 7, established a nomographic chart method. Introducing Eq. 8 into Eqs. 6 and 7, we obtain two identical formulae, which have the following form:

$$K_{uv} = \frac{f(nr_e^u) \cdot g(r_m^u) \cdot h(nr_e^v) + f(nr_e^v) \cdot g(r_m^v) \cdot h(nr_e^u)}{f(nr_e^u) \cdot g(r_m^u) + f(nr_e^v) \cdot g(r_m^v)}, \tag{A1}$$

where:

$$f(nr_e) = \frac{r_i}{\sqrt{nr_e + r_i}},$$

$$g(r_m) = \frac{1}{\sqrt{r_m}}, \tag{A2}$$

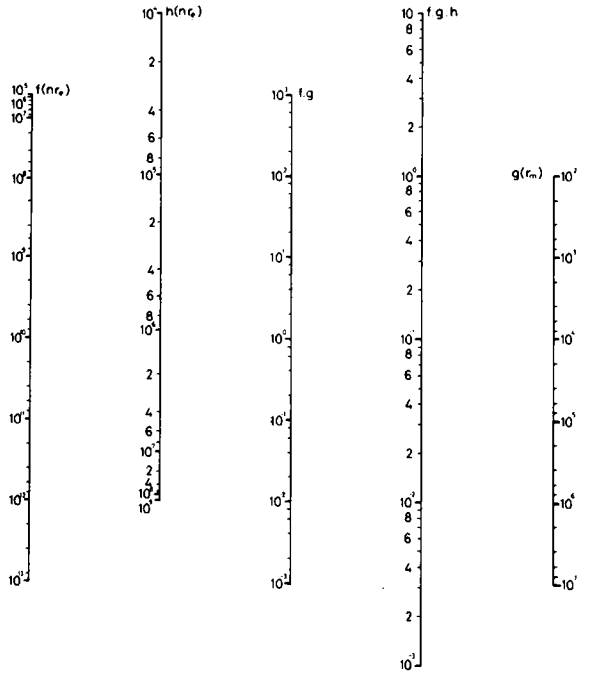

FIGURE 7 Nomogram for the determination of the correction coefficients, Eq. A1, for the rat diaphragm. The units are Ω and cm. For details of the use of the chart, see legend to Fig. 5.

and

$$h(nr_e) = \frac{nr_e}{nr_e + r_i}.$$

In Eq. A1, u and v represent R and S, or S and T, or S and T', when K_{RS}, K_{ST}, or K'_{ST}, respectively, are to be determined. Note that r_m is now expressed as specific resistance times unit length, and r_e and r_i are specific resistances per unit length. The nomographic charts presented in Figs. 5, 6, and 7 were established for the factor: $f(nr_e) \cdot g(r_m) \cdot h(nr_e)$, together with the intermediate factor: $f(nr_e) \cdot g(r_m)$.

Thus, two successive readings of the nomogram allow the determination of the coefficients. The charts presented are for three preparations commonly used in sucrose gap experiments. A different chart is presented for the frog sciatic nerve, the rabbit vagus nerve, and the rat diaphragm, corresponding to the different internal longitudinal resistances of the fibers of these preparations. An example of the utilization of the charts is given in the legend to Fig. 5.

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