THE INTERPRETATION OF CURRENT-VOLTAGE RELATIONS RECORDED FROM A SPHERICAL CELL WITH A SINGLE MICROELECTRODE

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ABSTRACT   An analysis is presented of the displacement of potential recorded when one microelectrode is used both to apply current to and record potential from a spherical cell. There are three significant components of the displacement in potential: a component produced inside the microelectrode, a time-independent component representing the spatially nonuniform flow of current in the immediate vicinity of the microelectrode, and a time-dependent spatially uniform component representing the average potential across the cell membrane. The second component describes changes in the potential across the cell membrane as well as potential drops in the interior of the cell, the importance of each factor being dependent on the location of the electrode. Simple expressions, derived by a theoretical treatment, are given for each component of potential. The implications for the interpretation of experimental results determined with the "single-electrode bridge" technique are discussed and an optimal balancing procedure is suggested.

INTRODUCTION

Many cells with interesting electrical properties are small and inaccessible and thus cannot be penetrated with two microelectrodes. The electrical properties of such cells must be studied by passing current through the same microelectrode which records potential (Fig. 1). Because the microelectrode has a high impedance, usually much higher than that of the cell, specialized electronic circuits, often called a single-electrode bridge (Araki and Otani, 1955; Frank and Fuortes, 1956; Kandel et al., 1961[1]), are used to separate the electrical properties of the cell from those of the microelectrode. The implementation of the electronics causes some problems but there are indications (Schanne et al., 1966; Tupper et al., 1970) from experiments which compared results determined with the single-probe technique and

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[1] These are references to some of the early papers using the technique; many laboratories now use the method routinely.

TWO PROBES
Four Electrodes

SETUP   EQUIVALENT CIRCUIT

Potential
Recording

Cell

Bath
Electrode   ONE PROBE
                          Two Electrodes
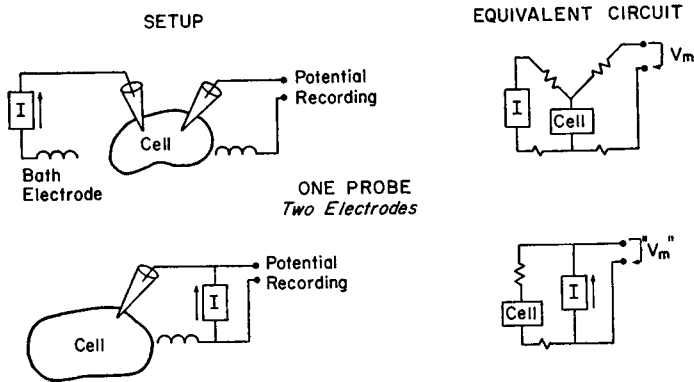
Potential
Recording

Cell

FIGURE 1 A sketch of two setups for recording the electrical properties of cells. The upper
setup uses two microelectrodes. The equivalent circuit to the right shows that measure-
ments made with this technique are not too sensitive to the properties of the microelectrodes.
The lower setup, often called a single-electrode bridge, uses only one microelectrode both to
pass current and record potential. The equivalent circuit shows that the measurements de-
pend critically on the property of the microelectrode and so this technique is used only in
cases where two electrodes cannot be inserted into the cell. The text analyzes the potential
recorded by the one-probe technique.

the usual two-probe technique, that there are other difficulties in using the single-
probe method. It seemed likely that these difficulties might be connected with the
complicated pattern of current flow in the immediate vicinity of the microelectrode
(Falk and Fatt, 1964; Eisenberg and Johnson, 1970; Pickard, 1971). Therefore,
we have analyzed the potential recorded by a single-electrode bridge using the
equations which define the flow of current in three dimensions.

The theoretical relation between the potential observed with the single-probe
technique (Fig. 1) and the resistance and capacitance of cell structures has not pre-
viously been derived because the expressions for the potential near a small source
of current are cumbersome. Recently, these expressions have been simplified by the
development of a mathematical identity (Eisenberg and Engel, 1970; see also
Pickard, 1971) and by the application of the mathematical technique of singular
perturbation (Barcilon et al., 1971). The mathematical identity applies to a restricted
problem and is exact, whereas the perturbation analysis applies to a general case
and is approximate (although accurate enough for our purposes). Furthermore, the
perturbation analysis shows that the properties of any finite cell are similar to those
of a spherical cell. Therefore, we have computed the potential recorded by the
single-probe technique for the case where the probe is inserted into a spherical cell
and expect that the qualitative features of this computation will be generally appli-
cable.

The analysis produces a pleasingly simple result, and the implications of this

result for experimental measurements of cell properties can be neatly summarized: the single-probe technique measures the sum of the displacement of membrane potential which would occur in an isopotential cell (which we will call the spatially uniform potential) and an extra potential associated with the steep gradient of potential which invariably surrounds a small source of current, such gradients being necessary to force the current out of the source. This extra, spatially nonuniform potential reaches steady state much more quickly than the spatially uniform potential, and contributes to the total potential in a simple way, without changing the time course of the spatially uniform component of membrane potential. Our analysis thus provides some support for a method commonly used to determine the spatially uniform component of potential, namely the subtraction of the quickly established, time-independent potential from the total observed potential. We show, however, that part of the subtracted potential represents a true transmembrane potential, not a drop in potential within the cell interior, and that this fact can alter the interpretation of the properties of the cell. For instance, the time-independent component of true membrane potential probably accounts for the different results obtained during the steady state with the two-probe and single-probe techniques.

Our analysis shows that there is not a simple criterion for separating the time-independent component of potential (the spatially nonuniform component) from the "time-independent" potential produced by changes in the electrode resistance.[2] This problem, combined with the difficulty in determining the precise location and size of the microelectrode, will complicate an experimental verification of our results.

The bulk of this paper is devoted to a quantitative statement of the results of our analysis and to a discussion of their physiological implications; the derivation is presented in the Appendix.

RESULTS

We analyze the single-probe method by considering the steps involved in the practical implementation of the technique.

The first step in the single-probe technique is to connect the microelectrode to the electronics, and place the electrode into the bathing solution outside the cell. A step function of current is then applied and the resulting displacement in potential is recorded. This displacement in potential is the sum of the potential drop within the microelectrode and the potential drop in the bathing solution from the tip of the

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[2] Professor J. D. Cole has recently shown that the spatially nonuniform component of potential (loosely referred to in this paper as the time-independent component of potential outside the microelectrode) is itself established on two different time scales. The component representing the potential drop within the cell interior is established "instantaneously" (that is, according to the relaxation time of the solution filling the interior of the cell), whereas the spatially nonuniform component of membrane potential is established on a time scale some 10,000 times faster than the membrane time constant (on a time scale of $\epsilon R_m C_m = a R_i C_m$, symbols defined below). Thus, in principle, the spatially nonuniform component of membrane potential can be separated from the potential drops within the microelectrode and within the cell interior.

microelectrode to the "indifferent" bath electrode. While it is common and sensible to define the "microelectrode resistance" operationally as the sum of these potentials divided by the applied current, this is *not* the convention we have used here; it is easier for our analysis if we define microelectrode resistance R_v to mean only the resistance *within* the microelectrode. Following that convention, we can derive the potential V_e recorded outside the cell

$$V_e = IR_v + \frac{IR_e}{s} \frac{4}{3\pi^2},$$ (1)

where the potential outside the electrode, the second term, has been calculated from equation A 18 of the Appendix.[3] Typical values might be $R_v = 20$ MΩ; R_e, the resistivity of the bathing solutions $= 100$ ohm-cm; s, the radius of the microelectrode $= 0.1$ μ; then 0.135 $R_e/s = 1.35$ MΩ. At this stage it is usual to adjust the electronics to read zero output during the current pulse (except for a nasty transient artifact caused by imperfect behavior of the electronics at short times). This procedure is usually called "balancing the circuit." In our analysis we will *not* balance the circuit at this stage, but will perform the equivalent subtraction later on.

Electrode Just under the Cell Membrane

We next consider the electrode to be inserted into the cell just under the cell membrane. A steady potential, the resting potential, is observed, which potential is uniform both spatially and temporally (except for the effects of damage to the cell). We therefore will only consider the displacement in potential from this resting potential produced by the current applied to the cell, and from now on we use the phrase "membrane potential" as an abbreviation for the phrase "the displacement of the potential immediately across the membrane produced by applied current." We use the word "potential" to mean the displacement in potential within the cell produced by current, noting that the potential so defined will include potential drops within the cytoplasm as well as across the membrane.

In the special case where the electrode is just under the membrane there is no cytoplasm between the tip of the electrode and the cell membrane and there can be no potential drops between the tip of the electrode and the immediately adjacent membrane. The electrode potential is thus a measure of the true transmembrane potential near the microelectrode (if we assume that potential drops in the solution outside the cell are not important; see Rall, 1969). Our analysis (see equation A 19 of the Appendix) shows that the potential measured will then be

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[3] The coefficient of R_e differs slightly (by 8%) from the equivalent expression in Gray et al. (1922), equation 15, p. 143, and from that in Rush et al. (1968), p. 84. The difference arises because our treatment does not allow current to flow from the (infinitely thin) edge of the disc source whereas the solutions derived by the other authors allow such current flow.

$$V_i = IR_v + I\frac{8R_i}{3\pi^2 s} + \frac{IR_m}{4\pi a^2}\{1 - e^{-t/R_m C_m}\}, \tag{2}$$

where the first term represents the potential drop within the electrode and the other terms represent the potential drop across the cell membrane.

V_i = the potential recorded inside the cell after the application of a step function of current (volts),

I = the amount of current applied (amperes),

R_v = the resistance within the microelectrode (ohms),

R_m = the resistance of 1 square centimeter of membrane (ohm-square centimeters),

C_m = the capacitance of 1 square centimeter of membrane (farads per square centimeter),

R_i = the resistivity of the solution filling the cell (ohm-centimeters),

a = the radius of the cell (centimeters), and

s = the inner radius of the opening of the microelectrode (centimeters).

The meaning of the terms which represent potential drops outside the electrode (the last two terms in equation 2) is of considerable interest. The exponential term
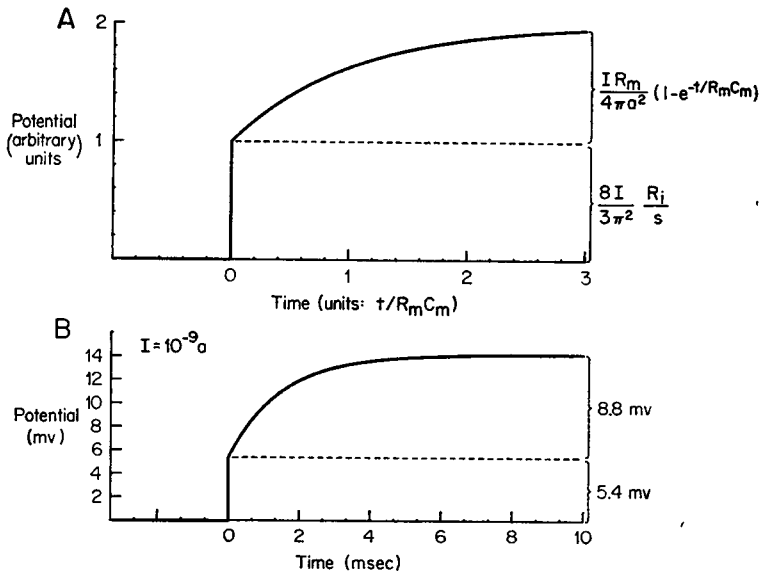

FIGURE 2 The potential outside the microelectrode measured by the single-probe technique when a step function of current is applied to a spherical cell. The upper figure shows a general case, corresponding to equation 2 of the text. The lower figure represents a particular case, with cell parameters C_m = 1.5 μF/cm²; R_m = 1000 ohm-cm²; R_i = 200 ohm-cm; a, the radius of the cell = 30 μ; s, the radius of the microelectrode = 0.1 μ. Note the initial jump produced by the time-independent component of potential, followed by the RC rise representing the charging of the membrane capacitance.

represents the spatially uniform component of membrane potential, i.e. the average potential, the potential being independent of position around the cell and corresponding to the usual assumption that a spherical cell is "isopotential." The other term represents the nonuniform spatial variation of membrane potential produced by the convergence of current, and the concomitant steep gradient of potential, which invariably exist near a small source of current. This extra component of membrane potential reaches steady state much more quickly than the spatially uniform component (quicker by a factor of $R_m/aR_i \simeq 10,000$; see Appendix 2 of Eisenberg and Engel, 1970) and so we can call it the time-independent term. The physical origin of this term is examined in the Discussion section; the rigorous derivation is in the Appendix.

Graphs of the two terms which represent the potential drops outside the electrode are shown in Fig. 2 A (the general case) and Fig. 2 B (a specific case). The latter shows the response computed for a cell of radius 30 μ, membrane resistance 1000 ohm-cm², internal resistivity 200 ohm-cm, membrane capacitance 1.5 μF/cm², and electrode radius 0.1 μ. Similar experimental records can be found, for instance, in Tupper et al. (1970), Fig. 2.

Electrode in the Center of the Cell

So far our analysis has been restricted to the case where the electrode is inserted just under the membrane of the cell; we now consider the case when the electrode is in the center of the cell. Then the potential across the membrane will be uniform and will not vary with position around the cell, but the current will flow across the resistance of the cell interior, producing a potential drop in the interior of the cell. The expression for the potential V_c recorded in the middle of the cell ($r = 0$) will then be different from the potential immediately across the membrane. Indeed,

$$V_c = IR_v + IR_i \left\{ \frac{4}{3\pi^2 s} - \frac{1}{4\pi a} \right\} + \frac{IR_m}{4\pi a^2} \{1 - e^{-t/R_m C_m}\},$$

$$\simeq IR_v + \frac{4IR_i}{3\pi^2 s} + \frac{IR_m}{4\pi a^2} \{1 - e^{-t/R_m C_m}\}, \tag{3}$$

where we have assumed the electrode radius is much smaller than the cell radius and that no balancing or rebalancing has been done. This expression has been determined from equation A 19 in the Appendix.

We see again that there is a time-independent component of potential, composed of the potential drop within the microelectrode and a potential drop outside the electrode, and a spatially uniform component, the average potential across the membrane. The time-independent term which describes potential drops outside the electrode has a different physical significance than the analogous term which occurs when the electrode is just under the cell membrane. In the latter case the term represented a change in membrane potential, whereas in the present case, with the elec-

trode in the middle of the cell, this time-independent term represents only a drop in potential in the resistive material filling the cell.

One further point should be made before we proceed with our analysis. It is interesting to note the similarity between equation 1 and the time-independent terms in equation 3. In particular the terms representing the potential drops associated with current leaving the electrode are similar. These terms are familiarly, if imprecisely, called the "convergence resistance" in the physiological literature. It is evident from the above analysis (see equation 2) that this name is only appropriate to the case where there is a great deal of symmetry in the flow of current, when the electrode is either in a uniform resistive material or in the center of a spherical cell. When current flow is not symmetrical, for instance if the electrode is not in the center of the cell, the time-independent potential has two components, one representing the nonuniform component of membrane potential and the other representing the potential drop within the resistive material filling the cell. According to common usage, all the time-independent components of potential (except those caused by changes in the electrode resistance) are called effects of the convergence resistance. This usage of the phrase "convergence resistance" to describe all the time-independent components of potential is unfortunate since it lumps together quantities with different physical significance; for instance it lumps together potential drops in the cytoplasm and spatially nonuniform components of true membrane potential.

Variation of Potential within the Cell Interior

In this section we consider the general expression for the potential anywhere within the cell. The expression for the total potential recorded (assuming no balancing outside the cell or rebalancing inside) is then (see equation A 19 of the Appendix)

$$V_i = IR_v + \frac{4I}{3\pi^2} \frac{a}{s} \frac{R_i}{a} \left\{ 1 + \mathcal{P}(d/a;\ s/a) \right\} + \frac{IR_m}{4\pi a^2} \{1 - e^{-t/R_m C_m}\}, \qquad (4)$$

where $\mathcal{P}(d/a;\ s/a)$, a function of the distance from the center of the cell d and the microelectrode radius s, is tabulated in Table I and shown in Fig. 3. When $d/a = 1$, and the expression simplifies to the expression given above (equation 2). When the electrode is fairly deep in the cell (say $d/a = 0.9$), the $\mathcal{P}(d/a;\ s/a)$ term is tiny and the equation is quite simple.

Using equation 4, we can describe in a qualitative way (see Fig. 3) the effect of advancing the electrode into the cell. Close to the membrane the effect of advancing the electrode is quite pronounced, reducing the time-independent term by a factor of 2. After that, advancing the electrode hardly changes the size of the time-independent term. This effect occurs because, just under the membrane, current can only leave the electrode by flowing downwards, away from the electrode and the membrane, whereas deeper in the cell current can leave the electrode in all directions.

TABLE I

THE VARIATION OF POTENTIAL WITH DEPTH: A TABLE OF THE
TIME-INDEPENDENT TERM $\mathcal{P}(d/a; s/a)$

Electrode position d/a	Electrode radius s/a			
	0.002	0.004	0.008	0.016
0.1	0.0000	0.0000	0.0000	0.0001
0.2	0.0000	0.0001	0.0002	0.0004
0.3	0.0001	0.0002	0.0005	0.0009
0.4	0.0002	0.0004	0.0009	0.0018
0.5	0.0004	0.0008	0.0016	0.0031
0.6	0.0007	0.0013	0.0027	0.0053
0.7	0.0011	0.0023	0.0045	0.0091
0.8	0.0021	0.0042	0.0084	0.0168
0.9	0.0050	0.0100	0.0201	0.0401
0.91	0.0057	0.0113	0.0227	0.0453
0.92	0.0065	0.0130	0.0259	0.0517
0.93	0.0075	0.0151	0.0301	0.0600
0.94	0.0089	0.0179	0.0357	0.0710
0.95	0.0109	0.0218	0.0435	0.0863
0.96	0.0138	0.0277	0.0552	0.1089
0.97	0.0187	0.0374	0.0744	0.1456
0.98	0.0285	0.0569	0.1122	0.2140
0.99	0.0577	0.1139	0.2169	0.3773
1.00	0.9988	0.9976	0.9954	0.9909

Furthermore, the physical significance of the term changes considerably, as the electrode is lowered into the cell. When the electrode is close to the surface, the time-independent term reflects a change in membrane potential, whereas when the electrode is further in the cell it reflects both the potential drops inside the cell and an extra, spatially nonuniform component of membrane potential. Fig. 4 shows the nonuniform component of membrane potential (the coefficient of $R_i/4\pi a$ in equation A 2) produced by a point source of current at a distance q from the center of the cell. This figure was calculated from equation A 2, with $p = a$. Note that the term is significant even when the electrode is quite deep within the cell.

Balancing and Rebalancing the Single-Electrode Bridge

The single-electrode bridge technique measures the sum of the potential drop within the microelectrode and the potential drop within the cell and across the cell membrane (see Fig. 1). Since the potential drop within the microelectrode is not usually of interest, a method is needed for separating this component of potential from the total potential recorded. Two methods have been used historically, each for sound experimental reasons. In one method the electronics are adjusted, with the microelectrode in the bathing solution, so that there is no voltage displacement seen when cur-
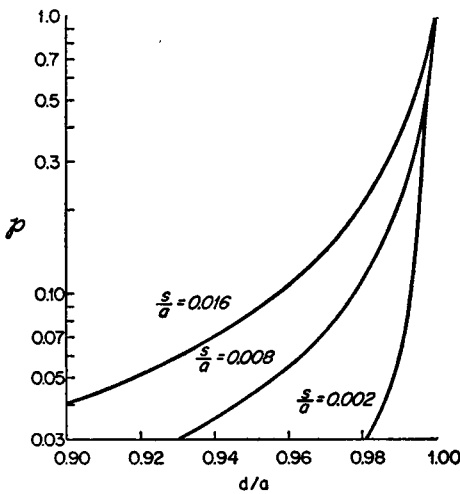
FIGURE 3

FIGURE 4

FIGURE 3   The radial variation of the potential recorded with a single microelectrode bridge. The function $\mathcal{P}(d/a; s/a)$ describes the variation of the time-independent component of potential, as the microelectrode is advanced into the cell. $\mathcal{P}$ is in dimensionless units, d is the radial position of the microelectrode, a is the radius of the cell, and s is the radius of the microelectrode.
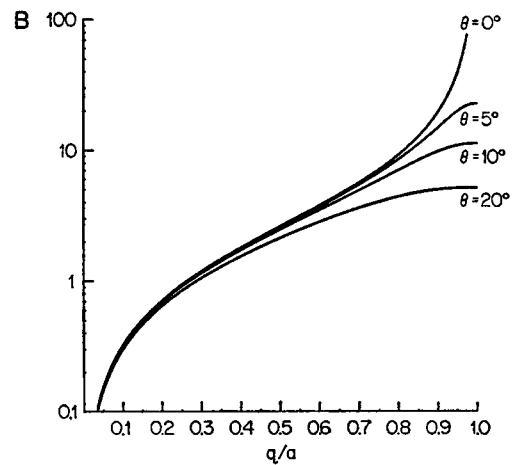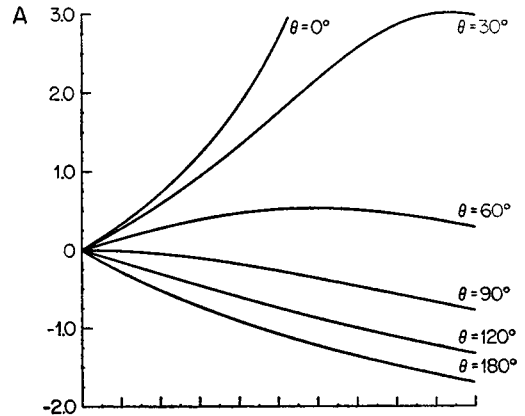
FIGURE 4   The change in membrane potential produced by current applied within the cell. The ordinate of the plot shows the spatially nonuniform component of potential, the sum of the terms which multiply $R_i/4\pi a$ in equation A 2. The plot gives a component of membrane potential at an angular separation θ produced by a current source at radial position q. Because of the symmetry of the situation the plot also gives the component of potential at radial position q, angular position θ, produced by a current source just under the membrane.

rent is passed. The microelectrode is then inserted into the cell and records are taken without further readjustment of the electronics (Schanne et al., 1966; Tupper et al., 1970). This procedure corresponds to subtracting the external response (equation 1) from the internal response (equation 2),

$$V_i - V_e = I\Delta R_v + \frac{4I}{3\pi^2 s}\{R_i(1 + \mathcal{P}(d/a; s/a) - R_e\}$$

$$+ \frac{R_m}{4\pi a^2}\{1 - e^{-t/R_m C_m}\} \quad (5)$$

where ΔR_v represents the change in the resistance within the microelectrode when the electrode is inserted into the cell. The resulting potential has the same shape as that shown in Fig. 2, but the physical meaning of the components of potential is different, since the time-independent component will now reflect the resistivity of the external solution, the properties of the interior of the cell, and, of course, changes in the electrode resistance.

There are some interesting qualitative features of this balancing procedure. Note that if the electrode were inserted just under the membrane of a cell (thus, the function $\mathcal{P}(d/a; s/a)$ in equation 5 equals 1) which has identical internal and external solutions (i.e., $R_e = R_i$), the time-independent term would be just twice the analogous term in equation 1 where the electrode was supposed to be outside the cell. This factor of 2 arises because when the electrode is outside the cell, current flows in all directions, whereas when the microelectrode is just under the cell membrane, current flows almost exclusively away from the membrane, in half the directions previously available. Thus, if a microelectrode is inserted just under the membrane of a cell, one would expect to see a jump in potential (produced by the time-independent term), even if the internal and external resistivity were the same and the microelectrode resistance had not changed. If the microelectrode were then advanced further into the cell, to a point where the function $\mathcal{P}$ were negligible, then the jump in potential would progressively disappear.[4] When the electrode is deep in the cell, the jump in potential appears if the electrode resistance is changed, or if the internal resistivity is different from the external resistivity.

In the usual experimental situation, however, a jump in potential appears when the electrode is inserted into the cell, and the cause is not known. In this case another procedure is often used: the electronics are readjusted (we call this rebalancing) so that the time-independent components of potential are invisible; that is to say, there is no visible jump in potential. This procedure is equivalent to removing the first two terms of equation 4. The resulting potential, the spatially uniform potential, will be an exponential function of time, and can be analyzed to give the parameters (R_m, C_m) of the membrane. On the other hand, it must be remembered that some of the time-independent potential rendered invisible by this rebalancing procedure is a true potential across the membrane and as such can have physiologically interesting effects, such as producing nonlinear changes in membrane properties.

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[4] Note the jump vanishes only with this balancing procedure, when $R_i = R_e$, and when $\Delta R_v = 0$.

DISCUSSION

Approximations Used in the Analysis

There are several approximations that have been used in this analysis. The important approximation, not precisely discussed up to now, is our representation of the microelectrode as a source of current, shaped as an infinitely thin one-sided disc of diameter equal to the inner diameter of the micropipette. The actual situation is much more complicated and requires a specification of the equations which describe current flow within the microelectrode, at the boundary between the microelectrode and the cytoplasm of the cell, at the wall of the microelectrode, in the membrane (presumably damaged) immediately surrounding the microelectrode, as well as the equations we have used to describe the flow of current within the cytoplasm and across the membrane. In our case a consideration of the size of the parameters involved allows us to reduce the complexity of the problem. The essential consideration is that the potential drop within the microelectrode (and within the source impedance of the device connected to the microelectrode) is much greater than the potential drops within the cell and across the membrane; in other words the impedance of the microelectrode (and current source) is much greater than the input impedance of the cell. In that case the current flowing out of the microelectrode is not changed by the potential within the cell or across the membrane, and the microelectrode can be represented as a source of current. The potential produced by a source of current can then be calculated as shown in the Appendix, assuming that the parameter $\epsilon = aR_i/R_m$ is sufficiently small. This latter assumption is of little consequence since the parameter ϵ is of the order of 0.001 or less under most physiological conditions, whereas a value of even 0.03 would not cause serious errors in the analysis (Eisenberg and Engel, 1970).

The representation of the source of current as a one-sided disc, ignoring the wall of the microelectrode and the damaged region of membrane immediately around the electrode, is much harder to justify in a quantitative manner. The effect of leakage around the microelectrode, through the damaged region of the cell, can be approximately determined since the size of the leak can be evaluated from the depression of resting potential produced by insertion of the microelectrode (Adrian et al., 1969, p. 239). The leakage can be represented by a resistance in parallel with the input impedance of the cell, and is usually little enough so that the effects are not important for the analysis presented here.

The microelectrode is represented as a resistance in this analysis and the shunt capacitance from the interior of the microelectrode into the cell interior is neglected. This approximation seems quite sound since the capacitance from the inside of the microelectrode to the cell interior must be of the order of 10^{-14} farads, assuming that 10 μ of the electrode is within the cell and that the capacitance of the microelectrode is some 10^{-12} farads/mm of electrode length (Nastuk and Hodgkin, 1950; Rush et al., 1968). Since most of the resistance of the electrode is within this 10 μ, an upper bound

on the effect of the capacitance into the cell interior can be determined by considering all the resistance of the electrode to be in parallel with the capacitance of 10^{-14} farads. The time constant of such a circuit would be (for an electrode of 20 MΩ resistance) 0.2 μsec, a time much shorter than we are interested in here. Thus, at the times of interest the current flowing from the microelectrode into the cell is in phase with the voltage on the electrode, and the electrode can be considered as a resistor. An attempt to check this point experimentally is currently being made in our laboratory by Dr. R. Valdiosera.

The approximations involved in our analysis should be evaluated in view of the purpose of this paper; namely, this paper seeks to analyze the physical factors which determine the potential recorded when the microelectrode recording potential is also a source of current. While approximations are involved in the representation of the microelectrode as a disc source of current, the further analysis is precise, terms being dropped only when they can be shown to be negligible. An analysis which represents the microelectrode in a more realistic manner must await detailed information about the electrical properties of the region within and immediately around the microelectrode.

Mathematical Derivation and Physical Meaning of the Components of Potential

The results presented in this paper are derived from our previous analysis of the spatial variation of potential by repeatedly integrating the formula which gives the potential produced by a point source. In order to perform these integrals either numerically, with reasonable computing cost, or analytically, the expression which gives the potential produced by a point source must be known in a simple form. The original expressions for potential in a spherical cell, and indeed the only expressions available now for the potential in a cylindrical cell, are quite complicated (Eisenberg and Johnson, 1970) and certainly could not be integrated four times as is necessary in our present analysis. Eisenberg and Engel (1970) were able to simplify these expressions, without introducing approximations, in one particular case, namely the case where the microelectrode is inserted just under the membrane of a spherical cell. For this special case, our present result (essentially equation 2) can be derived from the exact analysis.

The exact analysis was not completely satisfactory since it gave expressions whose physical meaning was often obscure. For instance, it was not clear from the exact analysis why the term which describes the spatially nonuniform potential should be virtually independent of time (that is to say, this term reached steady state much more quickly than the uniform component of membrane potential). It was not clear why an equation describing membrane potential should contain a term which is independent of membrane parameters. Finally it seemed possible that these puzzling properties might depend on the assumption concerning the location of the microelec-

trode just under the membrane. (These features of the exact solution are illustrated by the second term in equation 2.)

In order to meet these criticisms the problem was reanalyzed using singular perturbation theory (Barcilon et al., 1971; see also Pickard, 1971). Briefly, the analysis showed that the total potential could be written as

$$V = \frac{V^{(0)}}{\epsilon} + V^{(1)} + \epsilon V^{(2)} + \cdots ,$$

where $\epsilon = aR_i/R_m$. Each of the terms $V^{(0)}, V^{(1)}, \cdots$ represent the solutions to electric field problems related to our original problem but of simpler form. In the case of the spherical cell, the term $V^{(0)}$ represents the spatially uniform potential $(V^{(0)}/\epsilon) = (IR_m/4\pi a^2)(1 - e^{-t/R_m C_m})$. The $V^{(1)}$ term represents the potential produced by a point source of current located within a hypothetical spherical membrane which is constrained to have a spatially uniform density of current crossing it; that is to say the flux of current across the boundary of the structure is independent of spatial position. $V^{(2)}$ and higher order terms represent the potential produced by a redistribution of current across the cell membrane, current entering some regions of the cell and leaving other regions, there being no net source of current. In our analysis of the physiological case it is only necessary to consider the first two potentials $V^{(0)}$ and $V^{(1)}$ (and quantities derived from them) since the other terms are negligible everywhere in the cell at all times of interest.

Using the result of the perturbation analysis, we can see the physical meaning of the various components of potential. In particular the term in our equations which seemed most troublesome (for instance the second term in equation 2) is derived from $V^{(1)}$ and thus represents properties of the electric field produced by a point source of current, in the case where uniform current flux crosses the membrane. Since the term arises from a problem with a point source, where the current lines are forced to squeeze into a vanishingly small area, we expect that the potential near the source will be large. Furthermore, the problem which specified this component of potential $V^{(1)}$ does not include any membrane parameters, and therefore it is not surprising that the corresponding terms in our equations are independent of membrane properties. Finally, it is now clear why one of the terms is independent of time, that is to say has reached steady state by the times of interest. The only time-dependent properties in our description of the electrical properties of a cell are in the membrane, namely the membrane capacitance; but the $V^{(1)}$ term is independent of all membrane properties, and thus is independent of time, and appears as a constant term in our equations. The higher order terms, $\epsilon V^{(2)}$, etc., are quantitatively unimportant but will depend on membrane properties and time, thus modifying the "constant" terms of our equations under extreme conditions or at very short times.

The above physical analysis is based on results obtained with perturbation theory and it may be interesting to see if it is possible to justify this analysis with physical

arguments alone. It seems unlikely that such *post hoc* physical arguments are unique, but they serve a useful purpose in showing why perturbation theory gives the results it does. On physical grounds the spatially uniform component of potential $(V^{(0)}/\epsilon)$ might be expected to be the largest term; there would be little decrement of potential along distances of one cell radius in a cylindrical cell with the same size and properties as the spherical cell and so a spherical cell must have a quite uniform potential. The correction term $V^{(1)}$ is needed to describe how the electric field forces all the current to flow out of the microelectrode. The term might be expected to be independent of membrane properties (and thus time) since this component of potential is determined primarily by the properties of the cell interior and the microelectrode. Higher order terms might be expected to refine these approximations.

Implications for the Evaluation of Experimental Results

A major result of this analysis is that the time-independent component of potential produced by a step of current is not simply caused by changes in electrode resistance, but also includes components of true membrane potential and potential drops within the cytoplasm of the cell. The time-independent component of potential is seen as a jump in potential immediately after the application of a step function of current. Thus, measurements with the single-probe technique which are made during the steady state, and which ignore the jump in potential (Schanne et al., 1966; Tupper et al., 1970), will include an extra component of potential and will lead to peculiar values of the membrane parameters.

Furthermore, the procedure of balancing the circuit before penetration with the microelectrode and then measuring the response without rebalancing will usually give misleading results. Indeed there is direct experimental evidence of problems with this procedure. Table IV of Schanne et al. shows that the "input resistance" measured with a single electrode applying current and recording potential is some 7.9 times greater than the input resistance measured with two microelectrodes. Tupper et al. (1970) found a similar result and concluded (p. 189) that the discrepancy between the results recorded with two microelectrodes and one microelectrode was caused by "a consistent imbalance . . . which is not dependent on membrane resistance but which is additive to the depolarization produced by the membrane resistance."

Equation 5 shows the origin of this problem: namely, there is indeed an extra additive component of potential. If the electrode is located just under the cell membrane this component reflects differences in the resistivity of the solution inside and outside the cell, and also reflects the changed pattern of current flow, current flowing only in half the directions available when the electrode is outside the cell. If the electrode is located deeper in the cell (where the $\mathcal{P}$ $(d/a; s/a)$ term in equation 5 is negligible) the presence of a time-independent component of potential reflects differences in the resistivity of the cytoplasm and bathing solution.

In spite of the extra time-independent component of potential, linear membrane parameters can be determined by a simple rebalancing procedure. If the electronics are arranged so that the time-independent component of potential is removed, either by electronic or analytical subtraction, the resulting potential is that which would occur in an isopotential cell, one without spatial variation of potential, and the membrane parameters can be calculated by the simple equations which apply to that case. Measurements of the internal resistivity R_i using the time-independent component of potential are possible in principle, but require knowledge of the location and diameter of the microelectrode and assurance that the resistance R_v of the microelectrode has not changed when the electrode was inserted into the cell.

Other procedures for removing the time-independent component of potential are possible. For instance, in a condition in which the membrane impedance is low and current is applied with the single-probe technique the potential recorded will be the time-independent component. Since the action potential represents a state in which the membrane has low impedance (both because the voltage is changing rapidly and because the membrane resistance is low), current applied during the action potentia can be used to determine and remove the time-independent component of potential (Johnson and Tille, 1960, 1961; Martin and Pilar, 1963). These procedures, which also can be applied to records taken with double-barreled microelectrodes (Eisenberg and Johnson, 1970), depend on a detailed analysis of the time course of potential changes. In practice, the limiting factor in this analysis will be the ability of the electronics to faithfully reproduce rapid transients. The reliability of quantitative results determined with the single-electrode bridge, or double-barreled microelectrodes, thus depends critically on the electronics used in the implementation of the techniques.

It must be remembered, moreover, that either of the above procedures results in records which do not reflect the large value of the membrane potential near the microelectrode. This extra potential produced by the divergence of the lines of current flow from the small source can change the properties of the membrane, producing an action potential or even, in an extreme case, dielectric breakdown of the membrane. Thus, measurements of threshold using the single-probe technique would be expected to give low values. Furthermore, the spatial variation of potential near the source means that it is not possible to hold all the membrane at uniform potential, and therefore voltage clamp experiments using a microelectrode as a source of current would be expected to be difficult to interpret. A full discussion of this problem can be found in part 2 of Eisenberg and Johnson (1970).

APPENDIX

In this Appendix we derive approximate expressions for the potential recorded by a microelectrode inserted in a spherical cell, the electrode being used to apply current as well as record potential (see Fig. 1). In this approximation the tip of the microelectrode is treated as an infinitely thin, one-sided disc which, in conjunction with the associated electronics, acts
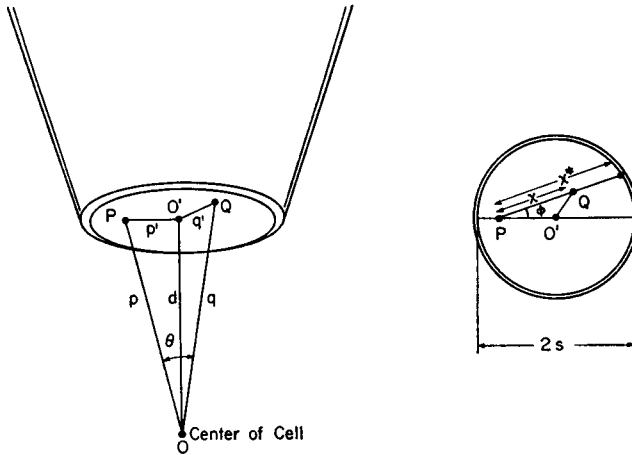
FIGURE 5   (*Left*) A side view of the microelectrode in the cell interior. The microelectrode is treated as a disc in our analysis, the point O' being the center of the disc, the point Q being the location of a point source of current, and the point P being the location at which potential is computed. (*Right*) A face-on view of the microelectrode tip. New coordinates, used in the integrations performed in the text, are shown.

as a current source; that is to say, the current leaving the electrode is independent of the po tential within the cell. Furthermore, the potential within the microelectrode is supposed to be independent of radial position and thus the density of current flowing out of the disc is uniform. The calculation consists of three steps: (*a*) the evaluation of the potential on the disc, (*b*) the averaging of the expression thus obtained over the surface of the disc, and (*c*) the conversion of the steady-state solution to the solution of the transient case, where a step function of current is applied at time zero and the membrane is represented by a resistance R_m in parallel with a capacitance C_m. The last step is not explicitly performed here since it has been described in detail (Appendix 2, Eisenberg and Engel, 1970).

Let us denote by O and O' the centers of the spherical cell and disc source, respectively, and by d the distance OO' (Fig. 5). If P and Q are two points *on* the disc source, we can denote the line segments which extend from O to these points by the vectors $\mathbf{p}$ and $\mathbf{q}$, respectively, the angle between the vectors being called θ. The lines from the center of the disc O' to the points will be called $\mathbf{p}'$ and $\mathbf{q}'$, and the length of all position vectors will be denoted by enclosing the name of the vector in vertical lines, or by writing the symbol in italics instead of boldface type. Thus, the length of the vector $\mathbf{p}$ is written $|\mathbf{p}|$ or p for short. Finally, we recall that the inner radius of the circular tip of the microelectrode (i.e., the disc source) is equal to s and that the applied current is called I.

Turning now to the actual derivation, we denote by $v(\mathbf{p}, \mathbf{q})$ the potential at P produced by a *single* point source of strength $I/\pi s^2$ located at Q. We shall approximate $v(\mathbf{p}, \mathbf{q})$ by the first two terms of the Green's function derived in Barcilon et al. (1971), i.e.

$$v(\mathbf{p}, \mathbf{q}) \simeq g(\mathbf{p}, \mathbf{q}), \qquad (A\ 1)$$

where

$$g(\mathbf{p}, \mathbf{q}) = \frac{I}{\pi s^2}\left[\frac{R_m}{4\pi a^2} + \frac{R_i}{4\pi a}\left\{g_1(\mathbf{p}, \mathbf{q}) + g_2(\mathbf{p}, \mathbf{q}) + g_3(\mathbf{p}, \mathbf{q})\right\}\right], \qquad (A\ 2)$$

and

$$g_1(\mathbf{p}, \mathbf{q}) = \frac{a}{|\mathbf{p} - \mathbf{q}|} = \frac{a}{(p^2 + q^2 - 2pq \cos \theta)^{1/2}} \quad (\text{see below}[5]), \quad (\text{A } 3)$$

$$g_2(\mathbf{p}, \mathbf{q}) = \frac{a^2}{(p^2q^2 + a^4 - 2a^2pq \cos \theta)^{1/2}}, \quad (\text{A } 4)$$

$$g_3(\mathbf{p}, \mathbf{q}) = -2 - \log \frac{1}{2} \left[1 - \frac{pq}{a^2} \cos \theta + \frac{1}{g_2(\mathbf{p}, \mathbf{q})} \right]. \quad (\text{A } 5)$$

The potential $V(\mathbf{p})$ at P due to a uniform disc source is obtained by adding the contribution of each point source Q on the disc, i.e.

$$V(\mathbf{p}) = \iint_{\text{disc}} v(\mathbf{p}, \mathbf{q}) \, dq, \quad (\text{A } 6)$$

where dq represents an element of area on the disc. This procedure can be viewed as an application of the principle of superposition to linear field problems or as an application of Green's functions to compute the solution of an inhomogeneous differential equation (see Courant and Hilbert, 1953).

If we therefore define $G(\mathbf{p})$ thus:

$$G(\mathbf{p}) = \iint_{\text{disc}} g(\mathbf{p}, \mathbf{q}) \, dq, \quad (\text{A } 7)$$

it is natural to approximate $V(\mathbf{p})$ by $G(\mathbf{p})$, viz.

$$V(\mathbf{p}) \simeq G(\mathbf{p}), \quad (\text{A } 8)$$

where

$$G(\mathbf{p}) = \frac{IR_m}{4\pi a^2} + \frac{IR_i}{4\pi^2 as^2} \iint_{\text{disc}} (g_1 + g_2 + g_3) \, dq. \quad (\text{A } 9)$$

The first integral in equation A 9 involves only the distance between P and Q and can be evaluated exactly. The calculations are greatly simplified if we introduce the coordinates χ and ϕ representing respectively the distance between $\mathbf{p}'$ and $\mathbf{q}'$, that is $|\mathbf{p}' - \mathbf{q}'|$, and the angle between $\mathbf{p}'$ and $\mathbf{p}' - \mathbf{q}'$ (Fig. 5, right). Indeed:

$$G_1(\mathbf{p}) \equiv \iint_{\text{disc}} g_1(\mathbf{p}, \mathbf{q}) \, dq = 2a \int_0^\pi d\phi \int_0^{\chi^*(\phi)} \frac{\chi \, d\chi}{\chi}, \quad (\text{A } 10)$$

where $\chi^*(\phi)$ is the distance from P to the edge of the disc along a line passing through Q, i.e.

$$\chi^*(\phi) = p' \cos \phi + (s^2 - p'^2 \sin^2 \phi)^{1/2}. \quad (\text{A } 11)$$

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[5] Note that this term represents the potential in a uniform resistive solid and thus expressions derived from this term alone will represent the properties of a microelectrode in a uniform resistive medium.

As a result

$$G_1(\mathbf{p}) = 2a \int_0^\pi \{p' \cos \phi + (s^2 - p'^2 \sin^2 \phi)^{1/2}\} \, d\phi,$$

or

$$G_1(\mathbf{p}) = 4asE\left(\frac{p'}{s}, \frac{\pi}{2}\right), \tag{A 12}$$

where $E(p'/s, \pi/2)$ is a complete elliptic integral of the second kind (Abramowitz and Stegun, 1964).

For the calculation of the second integral in equation A 9 we introduce the additional assumption that the disc is perpendicular to the line OO' which simplifies the calculations. Here again the coordinates χ and ϕ are used. To that effect, we first write p^2, q^2 and $pq \cos \theta$ in terms of χ and ϕ, viz.

$$p^2 = p'^2 + d^2,$$

$$q^2 = \chi^2 - 2p'\chi \cos \phi + d^2 + p'^2,$$

$$2qp \cos \theta = -2p'\chi \cos \phi + 2(p'^2 + d^2). \tag{A 13}$$

Substituting equation A 13 in equation A 4 we deduce that

$$G_2(p) \equiv \iint_{\text{disc}} g_2(p, q) \, dq = 2 \int_0^\pi d\phi \int_0^{\chi^*(\phi)}$$

$$\cdot \frac{a^2 \chi \, d\chi}{[(d^2 + p'^2)\chi^2 + 2p'(a^2 - p'^2 - d^2)\chi \cos \phi + (a^2 - p'^2 - d^2)^2]^{1/2}}. \tag{A 14}$$

The third integral in equation A 9 was neglected since $|g_3(\mathbf{p}, \mathbf{q})|$ never exceeds 4.3% of the value of $|g_1 + g_2|$ for electrode radii smaller than 1% of the cell radius.

The final step in our calculation consists in averaging $V(\mathbf{p})$ over all points P on the disc, i.e. in computing

$$\bar{V} = \frac{1}{\pi s^2} \iint_{\text{disc}} V(\mathbf{p}) \, d\mathbf{p}, \tag{A 15}$$

which we shall approximate by $\bar{G}$ defined thus:

$$\bar{G} = \frac{1}{\pi s^2} \iint_{\text{disc}} \left\{\frac{IR_m}{4\pi a^2} + \frac{IR_i}{4\pi^2 as^2}(G_1 + G_2)\right\} d\mathbf{p}. \tag{A 16}$$

Once again the integral of $G_1(\mathbf{p})$ can be evaluated exactly, viz.,

$$\iint_{\text{disc}} G_1(\mathbf{p}) \, d\mathbf{p} = 2\pi \int_0^s p' \, dp' \left\{4asE\left(\frac{p'}{s}, \frac{\pi}{2}\right)\right\}. \tag{A 17}$$

Using the integral form of $E(p'/s, \pi/2)$ and inverting the ϕ and p' integrations, we can rewrite equation A 17 as follows:

$$\iint_{\text{disc}} G_1(\mathbf{p}) \, d\mathbf{p} = 8\pi a \int_0^{\pi/2} d\phi \int_0^s (s^2 - p'^2 \sin^2 \phi)^{1/2} p' \, dp'.$$

With standard integration formulas (e.g. Dwight, 1961, 351.01, 432.20, and 453.32) we deduce the remarkably simple result

$$\iint_{\text{disc}} G_1(\mathbf{p}) \, d\mathbf{p} = \frac{16\pi s^3 a}{3}. \tag{A 18}$$

As a consequence

$$\bar{G} = \frac{IR_m}{4\pi a^2} + \frac{4}{3} \frac{IR_i}{\pi^2 s} + \frac{aIR_i}{\pi^2 s^4} \int_0^s p' \, dp' \int_0^\pi d\phi \int_0^{\chi^*(\phi)} \frac{\chi \, d\chi}{D^{1/2}},$$

where

$$D = (d^2 + p'^2)\chi^2 + 2p'(a^2 - p'^2 - d^2)\chi \cos\phi + (a^2 - p'^2 - d^2)^2.$$

In summary, the potential $\bar{V}$ is approximated by

$$\bar{G} = \frac{IR_m}{4\pi a^2} + \frac{4IR_i}{3\pi^2 s} + \frac{a^3 IR_i}{\pi^2 s^4} F\left(\frac{d}{a}; \frac{s}{a}\right), \tag{A 19}$$

where

$$F(\alpha; \beta) = \int_0^\beta y \, dy \int_0^\pi d\phi \int_0^{y\cos\phi + (\beta^2 - y^2 \sin^2 \phi)^{\frac{1}{2}}} $$
$$\cdot \frac{x \, dx}{[(\alpha^2 + y^2)x^2 + 2y(1 - y^2 - \alpha^2)x \cos\phi + (1 - \alpha^2 - y^2)^2]^{1/2}} \tag{A 20}$$

The first integral (over x) was performed analytically using formulas 380.001 and 380.011 of Dwight (1961). The integration over ϕ was performed numerically by a six-point Legendre-Gauss routine (Ralston, 1965) using at least 60 points. The final integration (over y) was performed by Simpson's rule over at least 31 points. Increasing the density of points used in either numerical integration made no significant difference in the results. The data are tabulated in Table I and Fig. 4 in the form of the function $\mathcal{P}(d/a; s/a)$.

$$\mathcal{P}(d/a; s/a) = \frac{3}{4} \frac{a^3}{s^3} F\left(\frac{d}{a}; \frac{s}{a}\right). \tag{A 21}$$

All the digits presented are thought to be significant.

REFERENCES

ABRAMOWITZ, M., and I. A. STEGUN. 1964. Handbook of Mathematical Functions. National Bureau of Standards, Washington, D. C.

ADRIAN, R., R. COSTANTIN, and L. D. PEACHEY. 1969. *J. Physiol. (London).* 204:231.

ARAKI, T., and T. OTANI. 1955. *J. Neurophysiol.* 18:472.

BARCILON, V., J. D. COLE, and R. S. EISENBERG. 1971. *S.I.A.M. (Soc. Ind. Appl. Math.) J. Appl. Math.* 21: 339.

COURANT, R., and D. HILBERT. 1953. Methods of Mathematical Physics. Interscience Publishers, New York. 1st English edition.

DWIGHT, H. B. 1961. Tables of Integrals and Other Mathematical Data. The Macmillan Company, New York. 4th edition.

EISENBERG, R. S., and E. ENGEL. 1970. *J. Gen. Physiol.* 55:736.

EISENBERG, R. S., and E. A. JOHNSON. 1970. *Progr. Biophys. Mol. Biol.* 20:1.

FALK, G., and P. FATT. 1964. *Proc. Roy. Soc. Ser. B. Biol. Sci.* 160:69.

FRANK, K., and M. G. F. FUORTES. 1956. *J. Physiol. (London).* 134:451.

GRAY, A., G. B. MATHEWS, and T. M. MACROBERT. 1922. A Treatise on Bessel Functions. Macmillan and Co., Ltd., London. 2nd edition. Reprinted in 1966 by Dover Publications, Inc., New York.

JOHNSON, E. A., and J. TILLE. 1960. *Aust. J. Exp. Biol. Med. Sci.* 38:509.

JOHNSON, E. A., and J. TILLE. 1961. *J. Gen. Physiol.* 44:443.

KANDEL, E. R., W. A. SPENCER, and F. J. BRINLEY, JR. 1961. *J. Neurophysiol.* 24:225.

MARTIN, A. R., and G. PILAR. 1963. *J. Physiol. (London).* 168:443.

NASTUK, W. L., and A. L. HODGKIN. 1950. *J. Cell. Comp. Physiol.* 35:39.

PICKARD, W. F. 1971. *Math. Biosci.* 10:307.

RALL, W. 1969. *Biophys. J.* 9:1509.

RALSTON, A. 1965. A First Course in Numerical Analysis. McGraw-Hill Book Company, New York. 1st edition.

RUSH, S., E. LEPESCHKIN, and H. O. BROOKS. 1968. *I.E.E.E. (Inst. Elec. Electron. Eng.) Trans. Bio-Med. Eng.* BME 15:80.

SCHANNE, O., H. KAWATA, B. SCHÄFER, and M. LAVALLÉE. 1966. *J. Gen. Physiol.* 49:897.

TUPPER, J., J. W. SAUNDERS, and C. EDWARDS. 1970. *J. Cell Biol.* 46:187.