Annual Review of Biophysics and Bioengineering. Vol. 2, 1973.

INTERPRETATION OF SOME MICROELECTRODE MEASUREMENTS OF ELECTRICAL PROPERTIES OF CELLS

A. PESKOFF AND R. S. EISENBERG

UCLA School of Medicine, Los Angeles, California

In recent years most measurements of the natural electrical activity of cells, such as action potentials and synaptic potentials, have been carried out using a microelectrode to record the electrical potential difference between the cell interior and a reference electrode in the surrounding saline solution. This is a sensible way to measure natural electrical activity since the potential recorded is a decent approximation to the potential across the cell membrane, and this membrane potential is usually the natural electrical activity of interest in the cell. It is well to remember that some natural electrical activity, such as that which may occur within intracellular organelles like mitochondria or sarcoplasmic reticulum, might not be simply related to the potential across the cell membrane.

The mechanism of this natural electrical activity depends on the electrical parameters of the cell, which are measured by applying current to the cell and recording and analyzing the resulting potential change. In order to perform this analysis, it is necessary to know the spatial distribution of current flow, that is to say, the magnitude and direction of current flow, especially the amount of current that crosses the membrane. While it is possible in some special cases (e.g. Hodgkin & Rushton 1) to apply current outside a cell and analyze in a reasonably unique manner where and how current is flowing across the cell membrane, it is not easy to do this in general. The problem is essentially that the membrane is a structure of very high impedance and so most of the current flows around the cell, not through it. The amount of current that crosses the membrane is only a small perturbation on the large quantity of current flowing in the extracellular solution. When current is applied inside a cell, however, all of it must flow across the cell membrane to the reference electrode outside and so the problem of analyzing the pattern of current flow across the membrane is greatly simplified. For this reason, most measurements of the electrical properties of cells use a microelectrode source of current, the current-passing microelectrode being inserted into the cell. The potential produced by this current flow is often recorded by another microelectrode inserted into the same cell, although at times a single microelectrode is used both to record potential and pass current.

The original theoretical analysis of the electrical properties of cells was carried out for other experimental arrangements (Cole & Hodgkin 2, Hodgkin & Rushton 1),

in which the electrical properties were often inferred from measurements outside the cell. Recently, a number of authors (Hellerstein 3, Rall 4, Pickard 5–7, Eisenberg & Johnson 8, Eisenberg & Engel 9, and Barcilon, Cole & Eisenberg 10) have concerned themselves with the analysis of the electric fields produced by a microelectrode source of current inserted into cells, and this paper is a review and extension of that work.

Before we introduce the physical representation of the cell used by these authors, it is important to justify the essential simplification common to all these theoretical analyses. All the results we discuss have been derived for the case where the cell membrane can be treated as a linear passive circuit consisting of a resistor in parallel with a capacitor. It may seem quite limiting to consider such restrictive conditions on the membrane but the restriction is both necessary and not so severe as it seems. The restriction is necessary because the analysis of the nonlinear time-dependent phenomena is not practical. There are mathematical difficulties in analyzing such phenomena and often the experimental information available does not completely describe the phenomena. The restrictions are not as severe as they seem since the physical processes underlying the spread of current in the linear passive situation are closely related to the physical processes underlying the spread of current in the nonlinear active case. Indeed, since the nonlinearities arise only at the membrane, the physical processes in the cell interior are identical in the two cases. We shall see that the application of a particular method of systematic approximations, singular perturbation theory (Cole 11, Lakin & Sanchez 12, Ch. 3), allows one to analyze a problem as a series of simpler problems each of which has a clear and unique meaning. The generalization of the singular perturbation analysis of a linear passive system to the nonlinear case is often straightforward, at least qualitatively, and so the analysis of the linear case may permit physical insight into the general physiological situation.

It is also important to realize that the linear passive properties of cells are of considerable interest in themselves. After all, for small enough currents the cell is linear: the linear properties describe the response of a cell to small subthreshold currents and serve as an adequate description of graded activity such as generator potentials and synaptic potentials. But there is another and more general reason for analyzing the passive linear electrical properties of cells: by using linear circuit theory one can determine the pathways for current flow across the different components of the cell, especially the membranes.

The structure of the membranes of cells is often quite complex, different parts of the membrane being specialized to perform different tasks, and this complex structure is usually intimately involved with the function of the cell. For example, the internal membrane systems of muscle fibers allow the extracellular space to invade the cell interior, thus providing a radial path by which currents associated with the action potential can depolarize membranes throughout the cell interior. This depolarization is thought to trigger the release of Ca^{++} from internal storage sites and initiate contraction. In such complex cells it is most important to determine the electrical properties of each membrane, that is, to separate the electrical properties of one part of the cell from those of another. Experimentally, a general method of measuring the electrical properties of different parts of the cell is to determine an equivalent circuit for the cell in which each circuit element in the equivalent circuit represents the

electrical property of some structural element of the cell. The most powerful method for determining the equivalent circuit relies on linear circuit theory and so is only applicable in the linear passive case. The justification for the analysis of the linear passive case is then twofold. First, the linear passive properties are of considerable physiological significance in themselves. Second, the analysis, particularly if performed using singular perturbation theory, provides physical insight into the underlying processes and so permits qualitative generalization to the nonlinear case. Furthermore, we feel that the formalism of singular perturbation theory is well suited for quantitative generalizations to more complicated problems.

REVIEW OF PREVIOUS RESULTS

The Physical and Mathematical Model of a Cell Containing a Microelectrode Source of Current

In the past, there have been many attempts to approximate the equations describing an electric field established within a cell by a microelectrode source of current. Best known are the approximation whereby a spherical cell is treated as isopotential, that is to say, where all of the interior of the spherical cell is considered to be at the same potential, and the approximation whereby the cylindrical cell is treated as a one-dimensional electrical cable. In cable theory, all the variation in potential is assumed to be in the longitudinal direction; the potential in each cross section of the cylinder is considered uniform, independent of the angular location or depth within the cylinder. Thus, the equations describing current flow include only one spatial variable and are in fact identical to the equations used to describe current flow down a transmission line. These approximate treatments have been and remain most useful since they well describe the behavior of cells under a variety of conditions. On the other hand, they suffer the grave defect of being what Van Dyke (13) calls "irrational approximations": they do not permit one even to estimate the errors in their underlying assumptions. In order to make such estimates, one must use a theory that can generate higher-order corrections. Indeed, the most important result of the theoretical analysis reviewed here is the precise specification of the conditions under which a spherical cell is reasonably isopotential and a cylindrical cell behaves like a one-dimensional electrical cable.

Experimentally, it has been clear for some time (Fatt & Katz 14, Sect. iii, p. 177) that in large cylindrical cells, such as crab muscle fibers, the usual one-dimensional cable theory does not adequately describe the spread of potential. More recently, work on the impedance of muscle fibers (Falk & Fatt 15, Eisenberg 16) and microscopic observation of muscle fibers (Adrian, Costantin & Peachey 17, Costantin 18) have shown the existence of deviations from one-dimensional cable theory. In the case of the spherical cell, experimental observations have not so clearly shown deviations from isopotentiality (however, see Kado 19), but measurements made with the single electrode bridge technique in which the same microelectrode is used to pass current and record potential, have been difficult to interpret (Schanne, Kern & Schäfer 20, Schanne et al 21, Schanne 22, Sperelakis, Hoshiko & Berne 23, Tupper, Saunders & Edwards 24, Law & Atwood 25), and it seemed likely that the reason for

this might be a local deviation from isopotentiality (Engel, Barcilon & Eisenberg 26). Therefore, several investigators have analyzed the problem of a point source of current within a spherical and cylindrical cell.

The cell is represented in these analyses as an idealized geometry, usually as a sphere or a right circular cylinder, and the membrane is treated as a very thin high-impedance structure. The appropriate equations to describe the electric field are then Maxwell's equations. Pickard (27, 28) has shown that in the biological case, at times longer than nanoseconds, these equations reduce to those of electrostatics. That is, we need not consider the delays in the change in potential caused by storage of charge within the bulk of the cell interior and exterior but only the delays caused by the storage of charge at the membrane. The potential is then described by Laplace's equation.

Inside the cell,

$$\nabla^2 V = -\frac{\delta(\mathbf{x}' - \mathbf{X}')f(t')}{\sigma_i} \qquad 1.$$

and outside the cell

$$\nabla^2 V = 0 \qquad 2.$$

where V is the change in potential at location $\mathbf{x}'$ produced by the application of a current with time dependence $f(t')$ at position $\mathbf{X}'$. The conductivity of the solution inside the cell is σ_i, the conductivity of the solution outside the cell will be called σ_0, and the delta function represents a point source.

The boundary condition, which follows from continuity of the normal component of current crossing the membrane and the electrical properties of the membrane, can be written

$$\sigma_i \frac{\partial V^-}{\partial n'} = \sigma_0 \frac{\partial V^+}{\partial n'} = G_m(V^+ - V^-) + C_m \frac{\partial}{\partial t'}(V^+ - V^-) \qquad 3.$$

where V^- is the potential immediately inside the membrane; V^+ is the potential immediately outside the membrane; t' is the time in seconds; $\partial/\partial n'$ represents the component of the gradient of potential normal to the surface membrane; G_m is the surface conductance (mho/cm^2) of the membrane; and C_m is the surface capacitance (μF cm^{-2}) of the membrane.

It is useful to rewrite Equation 2 in dimensionless form to emphasize the relative size of the different parameters of the problem:

$$\frac{\partial V^-}{\partial n} = \frac{1}{\alpha}\frac{\partial V^+}{\partial n} = \varepsilon\left[V^+ - V^- + \frac{\partial}{\partial t}(V^+ - V^-)\right] \qquad 4.$$

where $\varepsilon = aG_m/\sigma_i \sim 10^{-3}$; $\alpha = \sigma_i/\sigma_0 \sim \frac{1}{3}$; $t = t'(G_m/C_m)$; and the spatial coordinates have been normalized with respect to a, the radius or other typical cross-sectional length. Supplementary equations are necessary to describe the potential far away from the cell (i.e., the boundary condition at infinity) and the initial conditions just before current is applied to the cell.

The equation of most interest, both physiologically and mathematically, is the boundary condition. It is of great interest physiologically since it describes the membrane, which is usually the most interesting structure electrically; it is of interest mathematically because the boundary condition does not seem to have been well studied (see Garabedian 29, Sneddon 30) and because the existence of a small parameter ε in the boundary condition allows reasonably simple results to be derived (since in the limit $\varepsilon \to 0$ the boundary condition approaches the Neumann boundary condition, specifying the normal derivative at the boundary, and that has been well studied).

The parameter ε essentially measures the relative resistance of the cell membrane and cell interior. Since the purpose of the membrane seems to be to isolate the internal milieu of the cell from external disturbances, the membrane is bound to have quite small permeability to natural substances. The membrane cannot have zero permeability since it must allow the movement of essential metabolites, but its permeability must be quite low if the cell interior is to be isolated from the environment. Thus, in any description of cell properties (not just electrical), we expect there to be a parameter such as ε that measures the relative mobility of substances in the membrane and the cell interior, and that is small but not zero in magnitude. The widespread existence of such parameters in problems describing membranes leads us to name the boundary condition (Equation 4) *the membrane boundary condition.*

It is worthwhile to analyze the behavior of current flow near a boundary where Equation 4 applies. The membrane boundary condition requires that the component of current at right angles to the membrane be continuous, but places no restriction on the tangential component of current, which can in fact be discontinuous. Furthermore, the potential can be discontinuous across the membrane; the jump in potential is related to the normal component of current flow via the membrane conductance and capacitance by the boundary condition (Equation 4). The possibility of quite complicated behavior of the potential is important to keep in mind if one wishes to approximate crudely the physical problem described by Equations 1–4 as an equivalent circuit with a few circuit elements. Such an approximation must include pathways for circumferential current flow both inside and outside the cell. Circuits constructed without such pathways give incorrect results.

Representations of the Solution to Physiological Problems

The problem now facing us is to determine a formula for the potential V that satisfies the equations described above. There are several different methods for determining a formula for V and it is most important to realize that the different formulae may be very different in appearance. The uniqueness theorem for Laplace's equation guarantees that the different representations of the solution (that is, the different formulae) will have the same numerical value at any point in space and time, but different representations may have very different forms, some being closed-form solutions, others infinite series, and still others integrals. Furthermore, there are any number of approximations to the solution. Thus, the various formulae derived by different authors to describe the potential in a spherical or cylindrical cell differ in large measure because they are different representations of the same solution.

The choice of the optimum representation of a solution depends on the use to which the solution will be put. The representation that is optimum for proving mathematical theorems is rarely the one that is optimum for computation, and neither may provide much physical insight! The choice of representation is of great importance and may vary from problem to problem. In many problems it may suffice to have a computable representation since all one really wishes to know is, say, the potential everywhere in the system for a specific set of parameters. Such a representation is usually not satisfactory in biological problems where it is of the greatest importance that the representation allow physical insight into the processes underlying the problem of interest.

In the typical biological situation, one cannot measure some of the most important variables with much accuracy, or if one can measure them it is only with tremendous effort. For example, we know of no electrical measurements in which the exact position in a cell of a microelectrode tip was measured, nor do we know of any electrical measurements in which the shape of the cell was accurately measured [although the recent careful work of Hodgkin & Nakajima (31) includes estimates of cell shape]. These variables perhaps could be measured but the necessary experiments would be quite difficult and will not be undertaken unless they seem likely to yield some direct physiological insight. It seems, then, that in biological problems the coordinates of the current source and voltage recording electrode are not known with much accuracy. Thus, it does not seem that one needs to predict the potential theoretically with particular accuracy. One may even ask whether the absence of data precludes the comparison of theoretical predictions with experiments. In a quantitative sense it certainly is true that detailed comparison between theory and experiment is often not possible, but qualitatively such comparisons may be straightforward. The analysis of many biological problems, including the problems discussed here, predicts the qualitative existence of phenomena that may then be sought experimentally. Indeed, without the theoretical expectation it might be easy to misinterpret some of these experimental observations. Thus, one of the main requirements of a representation of a solution to a physiological problem is to predict the qualitative type of phenomena that should be observable experimentally.

In the case of our specific problem, this prediction of qualitative properties is of particular importance. It seems immediately obvious from a consideration of Equations 1–4 that the usual simplifying assumptions of isopotentiality or one-dimensional flow of current must fail, at least under some conditions. Since the microelectrode is described as a point source, the potential sufficiently near the microelectrode must vary as $1/r$; thus, sufficiently near the source there will be a large potential, which will surely make the cell not isopotential and make current flow in all three dimensions. Furthermore, it is clear from a consideration of the boundary condition that there must be some curvature of the current flow within the cell if the normal component of current is to be continuous across the membrane. Thus, sufficiently close to the source or to the membrane, one might expect special behavior that would violate the usual simplifying assumptions concerning the direction of current flow. This argument is weaker than it sounds, however. It is not clear what "sufficiently close" to the microelectrode means: in principle, "sufficiently

close" might be a distance less than the radius of the microelectrode! Similarly, it is not clear that the curvature of current flow that occurs near the membrane need be quantitatively important. One of the essential requirements of an analysis of Equations 1–4 is that it give answers to these questions. A useful solution will permit a qualitative statement of the conditions under which a spherical cell is reasonably isopotential and a cylindrical cell reasonably one dimensional.

Another important requirement on a representation of a formula to be used in biological problems is that it have a simple physical interpretation. This is important in order to predict the qualitative existence of phenomena as described above, but it is even more important to allow physical understanding of problems more complicated than those actually solved. For instance, few cells are actually spheres or right circular cylinders, yet most theoretical results are derived for these special cases. The generalization to irregular geometries is straightforward if the representation has simple physical significance; if the physical significance is not clear, generalization is difficult and one is always concerned that there may be different features in the problem with irregular geometry.

Eigenfunction Representations

The first published treatment of the problem, described by Equations 1–4, was by Weinberg (32, p. 112). The problem was brought to the attention of experimental electrophysiologists by Falk & Fatt (15). They (followed by Eisenberg 16, Adrian, Costantin & Peachey 17 and in part by Eisenberg & Johnson 8) determined the solution to a simplified problem by transforming the related solution of a similar heat-flow problem (Carslaw & Jaeger 33, Prob. II, Eq. 7, p. 378). The procedure for performing this transformation is described in detail in Eisenberg & Johnson (8, pp. 12–17). These solutions are in the form of an infinite sum of functions (an eigenfunction expansion), which represents the solution as an infinite sum of the natural modes (of, say, vibration in a mechanical problem) of the physical system described by the partial differential equation and boundary conditions.

The procedure of determining eigenfunction solutions from related, previously solved heat problems may save some labor but it is obviously possible to solve the electrical problem directly. Rall (4) significantly extended previous results by deriving the eigenfunction expansion for more general conditions than those considered by the above mentioned authors: Rall considered the full problem described previously (Equations 1–4), whereas the other authors considered a more limited problem in which time dependence was ignored and the extracellular region was considered isopotential. Rall's paper is particularly useful in showing how an eigenfunction expansion is derived; it is written in a clear and physically oriented manner helpful for newcomers to this field.

Although eigenfunction expansions are a mathematically correct representation of the solution, they are often not the most convenient representation for practical purposes. For instance, in the case of the infinitely long cylinder the solution involves a double infinite series, one series requiring the previous evaluation of the roots of a function of Bessel functions. The evaluation of these roots is a nontrivial matter, requiring extensive numerical analysis (Adrian, Costantin & Peachey 17) or

approximations (Eisenberg & Johnson 8, Pickard 5–7). Furthermore, all forms of the eigenfunction solution require the evaluation of many terms of the infinite series in order to approximate the infinite sum. Therefore, while one may understand the mathematical properties of each term of the infinite series (as is well described by Rall), one still has little physical understanding of the meaning of the infinite sum that represents the solution to a particular initial value problem.

These problems are vividly illustrated by considering the related problem for the spherical cell. In this case the infinite sum, derived by Hellerstein (3) and determined from the corresponding heat problem by Eisenberg & Johnson (8), is very weakly convergent and thousands of terms are necessary to approximate the sum under some conditions of interest. This very weak convergence invalidates qualitative analysis of each term of the sum and led Hellerstein to conclude incorrectly that the interior of a spherical cell was essentially isopotential.

There is another difficulty with eigenfunction solutions that should be mentioned. One cannot easily see how the solution depends on the parameter values of the problem but the solution must be computed anew for each set of parameters (cell size, membrane resistance, and so on). Since these parameters vary considerably from cell to cell, whatever values are chosen as typical will be useful in only a few cases. The application of the solution will in general require the reevaluation of the solution for each preparation and situation of interest. This problem is quite devastating since it makes generalization and physical understanding of the solutions most difficult.

Finally, these eigenfunction solutions do not allow easy comparison with the long-standing approximate treatments used in physiology. It is extremely difficult using such solutions to know when and where a spherical cell is isopotential and when and where a cylindrical cell behaves like a one-dimensional cable.

We shall see that the difficulty with these eigenfunction solutions is that they are too general, including the irrelevant case where ε is large as well as the relevant case where ε is small. The small size of ε allows one to resolve most of the difficulties just described and leads to much more useful formulae.

Approximations Based on the Eigenfunction Expansions

The original work on the problem of the point source in a cylindrical cell was motivated by the need to analyze experimental results determined from impedance measurements (Falk & Fatt 15, Eisenberg 16). The computations needed to evaluate the eigenfunction expansion were quite onerous, especially since they were done by hand. It was particularly difficult to determine the roots of the function of the Bessel functions needed to use the infinite series. Eisenberg (16) found empirically a set of rules by which these roots could be determined and it turns out that the roots were in fact quite close to well-tabulated roots of the derivative of the Bessel function $J_n(x)$, which is the $\varepsilon \to 0$ limit of the function of Bessel functions. Motivated by this result, Eisenberg & Johnson (8) carried out calculations based on an approximation to the eigenfunction solution. In the present context we would say that the eigenfunction formula had been expanded in a series arranged in increasing powers of ε. That is, Eisenberg & Johnson (8) used the fact that ε was small and so approximated

the solution by the first two terms in a Taylor series in ε. This procedure led to striking simplifications in the results. First, the results became easily computable (except for the potential close to the current electrode). Second, the comparison with the usual one-dimensional approximation became straightforward since the first term in the expansion in ε was in fact the usual one-dimensional cable expression. Third, one had some feeling for the physical significance of the correction to one-dimensional cable theory, the correction being important when ε was relatively large, that is, when the radius or internal resistivity was large or when the membrane resistance was low. Close to the microelectrode source of current (within 30 μ, say), the correction term is always significant. Finally, with this procedure one could determine one function (see Table 3 of Eisenberg & Johnson 8) that expresses the deviations from one-dimensional flow of current for all cells, of any membrane resistance or internal resistivity.

This considerable simplification and improvement in the utility of the formula led several authors to try the same procedure on related problems. Thus, Eisenberg & Johnson (8) used the same procedure to analyze the potential in a thin-plane and thick-plane cell, with some success, but the same procedure failed to provide useful formulae for the spherical cell since the formulae were weakly convergent infinite sums. Eisenberg & Engel (9) generated useful results for the spherical cell by proving in one special case an identity involving a sum of Bessel functions, and Pickard (7) determined some useful formulae for the spherical cell in a more general case. The problem of the spherical cell will be discussed again in this paper.

These analyses showed that the potential in a variety of cell geometries could be expressed as the usual approximate formula (a constant potential in the spherical case or an exponential variation with distance in the cylindrical) plus a correction term. The correction term depends on the cell parameters in a simple explicit way (it is of order ε smaller than the dominant term for the spherical cell and of order $\sqrt{\varepsilon}$ smaller for the cylindrical cell); the formula for the correction term was beautifully simple in the case of the sphere and reasonably useful in the case of the cylinder. Thus, at least part of the problem posed in Equations 1–4 was solved.

There were certain difficulties in these formulae, however. First, one had approximated the full Taylor series in ε by the first two terms and it was not clear that this procedure was always valid. Second, the physical significance of the various terms was still not clear. Third, it seemed quite inelegant to solve the full problem, for any size ε, and then treat the special case where ε was small. Might there not be some way to exploit the small size of ε from the beginning of the analysis?

Singular Perturbation Analysis

Our problem is not by any means the first in which it seemed useful to exploit the existence of a small parameter. Indeed, a whole set of techniques has been developed for writing approximate expressions for the solution of such problems, which approximations become increasingly accurate as the small parameter ε gets smaller. These techniques are brought together systematically in what is usually called *singular perturbation theory* and we shall discuss the theory since it seems particularly well suited to a variety of biological problems.

The starting point of singular perturbation theory is the attempt to develop an asymptotic series in powers of ε to approximate the solution to a problem. Rather than find the exact solution and then expand in ε, singular perturbation theory assumes (or derives in a heuristic way) an expansion in the small parameter ε and then tries to determine the coefficients of each term. Each coefficient is in fact the solution to a related simpler problem; each coefficient contains the dependence of the solution on the spatial and temporal variables. But each of these related problems is simpler than the original problem, and the dependence of the solution on the small parameter of the problem is explicit.

There are several useful references that describe various techniques in singular perturbation theory. The most important work, by Cole (11), includes a large number of examples illustrating a variety of problems and techniques to solve the problems. We have found Sections 2.1–2.2, 3.1–3.3, and 4.5.3 especially helpful. Van Dyke (13) includes considerable useful discussion of approximations that are useful in analyzing physical problems, but does not develop a unified technique as does Cole. Carrier & Pearson (34) and Lakin & Sanchez (12) include elementary chapters on the techniques.

The results of applying singular perturbation analysis to the problem posed by Equations 1–4 are given in several references. Barcilon et al (10) derive the solution to a restricted problem (a sphere at steady state with infinite external conductivity) and the solution is extensively discussed in Engel et al (26). Peskoff, Eisenberg & Cole (35) give the solution to the full time-dependent problem of a step function of current applied to a spherical cell in a bathing solution of finite conductivity. The cylindrical cell was also analyzed by Barcilon et al (10) but the physical interpretation of the results was not clear: Peskoff, Eisenberg & Cole (36) have derived simpler perturbation expansions for the cylindrical cell in the steady state bathed in a solution of infinite conductivity. To our knowledge, the time-dependent cylindrical problem including finite external conductivity has not yet been solved by perturbation techniques.

The utility of the results from singular perturbation analysis can be illustrated by the simple case of a spherical cell at steady state in a medium of infinite conductivity. The reader is sent to the above-mentioned works to find the analysis of more complicated problems. In this case the potential can be written as the sum

$$V(\mathbf{x}) = \frac{V_0(\mathbf{x})}{\varepsilon} + V_1(\mathbf{x}) + \varepsilon V_2(\mathbf{x}) + \cdots \qquad 5.$$

where V_0 is the dominant term, the solution to a problem with no source of current and no current crossing the boundary; V_0 must then be a constant potential, the potential corresponding to the usual assumption that the cell interior is isopotential.

It might be thought that V_0 would be the only important term since after all ε is a very small number. It turns out, however, that such is not the case because there are regions (near the current source) where V_1 is sufficiently large that it is important even compared with V_0/ε. V_1 is the solution to a problem with uniform current flow across the membrane and with a point source of current in the interior. This solution must, of course, have a singularity in potential and so there are regions of the cell in

which it makes an important contribution to the total potential. V_2 and higher terms have no singularities and so do not make important contributions to the total potential anywhere in the cell.

This sequence of problems corresponds exactly to the individual terms in the expansion of the eigenfunction determined by Eisenberg & Johnson (8) and Pickard (7). The difference is that now we have some physical insight into the meaning of each term. This insight is both comforting and useful, particularly when we consider generalizations to more difficult problems. A full discussion of the biological and physical significance of each of the terms can be found in Engel et al (26); a discussion of the significance of the terms in the solution to the more general problem can be found in Peskoff et al (35, 36).

Theoretical Results of Direct Experimental Significance

Most of this review has been devoted to a theoretical analysis of the potentials produced by a microelectrode source of current. It is well to end the review by discussing the theoretical results that are of immediate significance in the interpretation of published experimental work.

It is useful to summarize the theoretical results in a qualitative way: We have seen that the electric potential produced within a cell by a microelectrode source of current has two components: one, the local potential near the microelectrode (called V_1 in Equation 5 above) is quickly established (in 1 or 2 μsec) and the other (called V_0 in Equation 5 or the "far-field potential") is established according to the time constant of the cell membrane. If the cell is finite (e.g., spherical), the slow far-field potential corresponds to the usual approximation of an isopotential cell; if the cell is a long cylinder, the steady-state far-field potential corresponds to the traditional one-dimensional cable approximation. A most important result of the analysis is that these two types of potential sum—that is, they do not interact. Thus, after a few μsec, the local potential is *independent of membrane parameters* (depending only on the internal and external resistivity of the cell and the electrode location and cell size), whereas the slow far-field potential (the one that is the usual physiological approximation) is independent of the quick local potential and depends strongly on membrane parameters. The total potential is simply the sum of the slow far-field potential and the local potential, the local potential being important at short times (times less than 100 μsec in a typical spherical cell, less than 1 msec in a typical cylindrical cell), at small electrode separations (2 μ in a spherical cell, 50 μ in a cylindrical cell), or when the membrane impedance is small.[1]

With these ideas in mind, we can discuss the particular physiological cases in which the local potential, considered to be zero in the usual approximation, is important in the interpretation of experimental results.

The most common physiological situation in which consideration of local potentials is important is in the interpretation of current voltage relations (transient and

[1] These numerical estimates for a "typical" cell are meant only to convey a feeling for the orders of magnitude. Reference to the formulae presented in the original papers will permit an easy estimate of the significance of local potentials for each preparation of interest.

steady state) measured with one microelectrode (Engel, Barcilon & Eisenberg 26, Pickard 7, Sec. 3). This technique is of particular physiological importance since it is the only method available for determining the electrical properties of small inaccessible cells such as those in the central nervous system. In this technique, the microelectrode acts both as the current source and the voltage sensor and so the current and voltage electrodes overlap. It is clear, therefore, that the local potential will have important effects on the interpretation of current voltage relations. Indeed, it has been clear from strictly experimental findings that this technique can give results that differ from results determined from the same preparation with a two-microelectrode technique (Schanne et al 21, Tupper, Saunders & Edwards 24, Araki & Otani 37, Sperelakis, Hoshiko, & Berne 23, Schanne, Kern & Schäfer 20).

Theoretical analysis of this experimental technique has been performed for the case of the spherical cell (Engel, Barcilon & Eisenberg 26) and in that case at least it is possible to understand the difficulties encountered experimentally and to prescribe methods for removing most of them. The theory shows that the potential recorded in response to a step function of current applied within the cell consists of three components: a slow far-field potential, which corresponds to the potential expected if the cell were isopotential; a quickly established (μsec) local potential; and a very quickly established (nsec) potential drop within the solution filling the microelectrode. This last potential has nothing to do with the properties of the cell and so should be removed from the experimental records before the properties of the cell are analyzed. There have been several methods used to determine and remove the potential drop within the microelectrode. One method measured the potential produced by current flow when the microelectrode was located in the solution outside the cell. This potential was taken as the sum of a potential drop within the electrode and a potential drop in the "convergence resistance" of the bathing solution. These potentials were removed from the experimental records by "balancing the bridge" and the microelectrode was subsequently inserted into the cell. Analysis of this procedure (Engel et al 26) shows that the records resulting are most difficult to interpret. They contain components of the local potential as well as components due to changes in the resistance of the microelectrode upon penetration of the cell. These difficulties most likely account for the anomalous circuit parameters found by Araki & Otani (37), Schanne et al (21), Schanne, Kern & Schäfer (20), Tupper, Saunders & Edwards (24), and Sperelakis, Hoshiko & Berne (23).

Another method, more commonly used to remove the potential drop within the microelectrode, is to "balance the bridge" after the microelectrode is inserted into the cell. In that case, the response to a step function of current is observed after the current electrode is inserted into the cell and the entire quick component of potential is removed. The resulting experimental records have then been interpreted as a measure of the potential across the membrane of a cell with an isopotential interior. Analysis (Engel et al 26) shows that this is not the case. Part of the quickly established potential is not a potential drop within the microelectrode but is in fact a local potential developed within the cell. This local potential contributes to the total potential within the cell and as such cannot, in general, be ignored. There is one case, however, in which the local potential makes no difference in the interpretation of

results. If one wishes only to determine the linear circuit parameters (for example, membrane resistance and capacitance) of a cell from measurements made with the single-microelectrode technique, one can proceed as if the cell were isopotential and ignore the local potential. This surprising result occurs because the local potential is independent of the membrane parameters and adds linearly to the slow far-field component of potential, which is strongly dependent on membrane parameters.

In general, however, the existence and properties of the local potential must be kept in mind when interpreting measurements made with the single-electrode technique. Thus, the method of measuring the internal resistivity proposed by Schanne (22) and used subsequently by Law & Atwood (25) cannot generally give correct results. The difficulty is that the component of potential (the local potential), which was thought to reflect only the internal resistivity of the cell, also depends critically on the precise electrode location. Since this location is usually unknown, the local potential cannot be used to determine the resistivity of the cell interior. The measurements of internal resistivity of Carpenter, Hovey & Bak (38) and Hovey, Bak & Carpenter (39), using two different techniques, should also be analyzed taking into consideration the different size of the local potential inside a cell and in a salt solution.

The analysis of the single-electrode case also explains an occasional perplexing finding. For instance, Teräväinen & Rovainen (40) found incidentally that one of the fast motoneurons of the lamprey has an apparent threshold of only a few mV more positive than resting potential when stimulated by current applied through a single-microelectrode bridge arrangement. This phenomenon can be explained by the analysis (Engel et al 26): part of the potential change subtracted off by "balancing the bridge" in the conventional manner is not an irrelevant potential change within the microelectrode but is a true transmembrane potential and as such can exceed threshold and drive the membrane into an action potential. Thus, the proposed explanation for these strikingly small thresholds is that the membrane was in fact being depolarized by a large amount (past threshold) by the local potential, but that the usual balancing procedure removed the local potential from the experimental records of potential. This explanation is, of course, only viable if the soma of the lamprey motoneuron is excitable.

Another area of physiology where the local potential has a striking effect is in muscle physiology. Recently there has been significant progress in the analysis of the mechanism of electromechanical coupling in muscle using cinematography to analyze the radial spread of movement (and presumably tubular membrane potential). In the course of such experiments, Adrian, Costantin & Peachey (17) found large local contractions in the vicinity of the current microelectrode. They analyzed the local potential, using the full eigenfunction expansion, and showed that the local potential, computed for reasonable parameter values, was sufficient to explain their experimental results. Thus, it seems likely that the local contractions are a direct result of the local potentials we have discussed. It is not certain, however, that the important potential change is developed across the surface membrane. It might be across the tubular membrane or, less likely, across the membranes of the sarcoplasmic reticulum.

Still another area of physiology in which the local potential seems of importance is voltage clamp measurements with a microelectrode source of current. In these

experiments, it is necessary to measure the current that flows across a region of membrane, all of which has the same transmembrane potential. One of the conclusions of the theoretical analysis described above is that the local potential around the current microelectrode cannot be avoided; it is a necessary concomitant of passing current from a small source. Thus, the potential across a region of cell membrane cannot be controlled if that region contains a microelectrode source of current. It is important, then, in the design of voltage clamp experiments, to avoid measurement of potential or current flow in a region including the current microelectrode. The method of Adrian, Chandler & Hodgkin (41), in which membrane current is measured away from the microelectrode source, is not subject to these difficulties. However, in their method the spatial and temporal stability of the membrane potential should be affected by the train of action potentials initiated when the local potential near the current electrode drives the membrane past threshold.

Finally, it seems clear from measurements made by R. Valdiosera in our laboratory that local potentials have a most important effect on impedance measurements from cylindrical cells. Valdiosera has measured a large effect of electrode location, the phase angle of the impedance being significantly dependent on the angular separation of electrodes even at frequencies as low as 50 Hz. It seems clear that quantitative interpretation of impedance measurements must include consideration of the effects of the local potential.

ACKNOWLEDGMENT

It is a pleasure to thank Professor J. D. Cole for his guidance in the analysis of the spherical cell. We are grateful to Professor C. Newton, who introduced us to each other, and one of us (A.P.) to the application of mathematics to biological problems. The work was supported by NSF grant GB-24965. Dr. Peskoff was supported by NIH grant NS-05670.

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