STRUCTURAL ANALYSIS OF ELECTRICAL PROPERTIES OF CELLS AND TISSUES

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I. INTRODUCTION

The functions of electricity in living organisms are as diverse as they are important. The conduction of information in the nervous system is probably the best known function of electricity in biology; but the transduction of environmental information into nervous signals, the regulation of cellular volume, and the transport of metabolites are equally important biological functions, all intimately linked to the flow of electric current. Bioelectricity has special characteristics because biological currents are apparently always carried by ions rather than electrons. Biological potentials arise from the movement of specific ions driven by concentration gradients across membranes specialized to control the movement of those ions. Biological membranes are formed by a very thin (8 nm) high-resistance lipid matrix containing macromolecules embedded like islands in an oily sea. Conduction of ions across membranes occurs almost exclusively through these specialized macromolecules and not through the lipid phase; however, the thin lipid matrix contributes a relatively large capacitance and so it is an important factor in the time course of potential changes across membranes.

The concentration gradients which drive bioelectric currents are quite limited sources of electrochemical potential. Biological current flows themselves are also quite small, both because of the small electrochemical potentials and because of the high resistance of the protein-lipid membrane. Despite these limitations, bioelectricity allows animals many functions which would otherwise be awkward or impossible. Undoubtedly, that is why the use of electricity is so widespread among animals.

The widespread use of electricity in biological function deserves further specification, if only because that use may not be well known to physical scientists. Almost all signaling in the nervous system is electrical. The signals which move long distances in the nervous system are all-or-none action potentials, produced by the interplay of voltage-dependent membrane conductances, specific to particular ions (Hodgkin and Huxley[24]). These propagating waves would be called solitary waves or solitons in the applied mathematics literature (Whitham[51]). Local signals in the nervous system are electrical and it seems clear that information processing on a msec time scale is always electrical. Too little is known about slower processes, like memory and learning, to be sure of their electrical content. But even if these slow processes are in some sense biochemical, they must acquire and transmit data through an electrical interface.

Signals essentially similar to the action potentials of nerve fibers are also widely used in contractile tissue. The contraction of most skeletal muscle is coordinated by the rapid propagation of an action potential longitudinally down the length of a muscle

fiber and radially into the fiber through the set of tubular invaginations called the t-system. It is the propagation pattern of the cardiac action potential which allows the healthy heart to function as a pump. The failure of the propagation pattern is the immediate cause of death in many patients. Other muscles, including some amphibian skeletal muscle, smooth muscle, and many invertebrate muscles, do not need and do not use a propagating action potential to coordinate contraction. Even in these cases, however, it appears that a change in voltage across a muscle membrane is an essential step in the sequence of events which link a nerve action potential to muscular contraction.

Electricity is also used for functions other than signaling. Epithelial tissues, which function by transporting solutes from one part of the animal to another, almost always have current flow associated with their activity. Epithelia often use a potential gradient to assist in transport. Surprisingly enough, the active transport of a nonelectrolyte, such as a sugar, is usually found to be coupled to the movement of an electrolyte, even though nonelectrolytes, being uncharged, need not have any particular interaction with electrical phenomena. The membrane macromolecules which form the active transport system--called a "pump" in the biological literature—seem to link the transmembrane movement of charged and uncharged molecules.

The widespread use of electricity in biology is probably a result of the necessity of a steady resting potential across cell membranes. The resting potential found in almost all cells is some 50 to 100 mV, inside negative. In order for a cell to maintain constant volume and to trap within the cytoplasm the expensive biological macromolecules which form the metabolic apparatus and genetic code, the membrane of the cell must be impermeable to macromolecules. A necessary consequence of membrane impermeability (to charged macromolecules) is a charge imbalance in the cell interior which, although too small to be measured by chemical techniques, is large enough to produce the resting potential. Cells can be expected therefore to have had resting potentials from the earliest phases of evolution. Since evolution is the supreme pragmatist, modifying swim bladders into lungs, and fins into feet, it is not surprising that the voltage across the cell membrane is used so widely for signaling and transport. It is also not surprising that bioelectric phenomena have a central role in the function of most cells and tissues.

The analysis of electrical behavior and properties is then a central topic in biology, both the analysis of electrical function itself and the analysis of the mechanisms which produce that function. This review discusses one part of that analysis: the parsing of the overall properties of a tissue or cell into the properties of the structures which comprise it. We consider it obvious that neither function nor mechanism can be understood until the structures producing that function, and containing that mechanism, are identified and separated from structures doing other things. As obvious as this may be, the identification and isolation of the electrical properties of the components of cells and tissues has often been the rate-limiting step in the understanding of function in complex tissues. This review is devoted, therefore, to a procedure which systematically parses the electrical properties of cells and tissues into the properties of their constitutent structures. Most of this procedure is modern, but reasonably well tested. We also introduce some new ideas which, if they survive practical test, will make the procedure easier.

II. NECESSITY OF STRUCTURAL LOCALIZATION

The fundamental fact which motivates our analysis is the structural complexity usually associated with electrical function. To a physiologist, taxonomist, or evolutionary

biologist, the role of structure is always in view and should always be apparent. Each function of an animal is produced by a separate structure, an organ system, which in turn is made of cells with specialized structure, different specializations being apparent at different levels of magnification. The naked eye examines organ systems, the light microscope examines the cells and tissues of the organ, the electron microscope examines the cells and subcellular components, and X-ray diffraction can examine individual molecules. So far, as each organ is investigated, each structure is found to have a definite role in its function; and conversely, as each structure, at each level of magnification, is investigated it is found to have an identifiable function.

Structure, like all other biological phenomena, is the result of evolution and so the significance of a structure must be viewed in the context of evolutionary, not human design. Gould,[23] following in the tradition of Simpson,[44] has written eloquently, and with convincing examples, of the processes of evolution. Evolution does not proceed in what seems, to the human mind, to be a logical manner. Rather it produces well-adapted systems by selecting among the variations in natural characteristics provided by the mechanisms of heredity.* It is easier for selection to produce adaptation by making a succession of small changes in existing systems, each of which involves changes in only a few genes, than by inventing a new system which would require simultaneous and usefully correlated change in a multiplicity of genes. The range of variations provided by heredity is therefore limited and selection produces well adapted, not optimally adapted, systems: optimal adaptations would often require mechanisms beyond the possibilities provided by heredity. So we see that evolution does not build the way a human being does.

In our context then, we expect and find that evolution proceeds by adding structural complexities one on another. Each structural complexity contributes to the electrical properties one measures, and each structural complexity is expected to contribute specifically to the electrical function of the tissue.

The electrical properties of primary concern are those which the tissue or cell uses in its natural role. These are usually produced in a rather intricate manner by the different structures of the preparation. In complex tissues the analysis of structure, the analysis of function, and the analysis of mechanism all depend on the structural localization of electrical properties.

There is a separate, less profound but highly practical reason to study the role of structure in electrical function. There is usually much structure interposed between the place (or "port" in the language of electrical circuit theory) at which electrical measurements are made and the place where the electrical properties originate. Only a limited number of ports are accessible to experimentation. It is certainly true that great, perhaps the greatest, advances in physiology occur when a new port is made accessible, e.g., by penetrating cells with microelectrodes to record intracellular voltages. Each method of access to a biological tissue, however, has its own artifacts as well as advantages. Usually one measures properties which depend on all the membranes and internal structures, but with variable weighting. For example, measurements from nerve and muscle fibers rarely give the properties of the excitable membranes directly. The measurements give "input" properties, which depend on the geometry of the preparation fully as much as on the excitable membrane. Even when measurements are made directly from a membrane which has no obvious structural complexity, such as a large unmyelinated axon, there are hidden structural complications of great physiological

* It is of some importance that these variations are produced independently of the environment and of the selection processes. There is no feedback from environment to heredity. That is why evolution proceeds as described by Darwin and not as described by Lamarck: acquired characteristics are not inherited (Simpson,[44] p.270-273).

importance. The axon membrane which is producing the phenomena of interest is in series with a resistance, at the minimum the resistance of the bathing solution, and this resistance significantly alters the potential which is recorded inside the axon.

We can see from the previous discussion that structural analysis is an essential step in interpreting the electrical properties of cells and tissues and of the experiments done to measure these properties. Our procedure for structural analysis of electrical properties is essentially the procedure for creating, solving, and testing the electrical statement of the structure of the preparation. We now turn to those topics, providing an outline of the procedure and then a fuller description of each part.

III. PROCEDURE FOR STRUCTURAL LOCALIZATION

1. Structural description
   - Qualitative: a diagram of the topology
   - Quantitative: the morphometric parameters
2. Theory: the electrical structure
   - Physical laws and structural description
   - Partial differential equations boundary conditions
3. Theoretical predictions
   - Solution of equations, with known error
   - Physical meaning and circuit representation of solution
   - Linear properties, then nonlinear properties
4. Electrical measurements
   - Curve fitting to transients or frequency response
   - Determination of circuit parameters
5. Integrals of transients
   - An alternative, promising, but untested method
   - Lumped circuits with the minimal number of elements
   - Redundant lumped circuits
   - Distributed circuits
   - Experimental utility and verification
6. Experimental verification
   - Interventions to modify parameters predictably
   - Measurement of morphological and electrical parameters
   - Comparision of predictions and measurements

A. Structural Description: Qualitative

The first step in a morphological analysis of electrical properties is clearly the analysis of structure. This analysis starts with a qualitative understanding of the tissue, the overall organization of the cells, membranes, and organelles. A morphologist expresses such with a diagram, a mathematician would call the diagram a topological description, since it is designed to illustrate the connections of the various structural components but not their amounts. While this kind of qualitative analysis might be thought to be simple, or already accomplished, that is rarely the case. In the nervous system, e.g., most of the current work of both electrophysiologists and anatomists is devoted

to determining the connections between cells. Even in less complicated and better studied tissues, there is much "knowledge" that is still changing.

For example, the pattern of striations in vertebrate skeletal muscle has been examined for hundreds of years, but only recently has that pattern, and therefore the pattern of the internal membrane systems and contractile filaments, been understood (Peachey and Eisenberg[18]). It is not uncommon for profound changes to occur in the perception of a structure as morphological techniques, particularly tissue preservation, improve. The analyst is then confronted with the frustrating necessity to reanalyze a tissue, just when the first analysis is complete. At times a structure is successfully analyzed, by theoreticians, just when it is revised by the morphologists. Indeed, that happened recently with the t-system of frog skeletal muscle. The week that Mathias et al.[14] first derived their description of the expected properties of a branching planar network of t-tubules was the same week that Peachey and Eisenberg[18] showed the t-system to be helicoidal, not planar. Fortunately, in this case the consequences of the third dimension were not profound.[12]

The structural description of a tissue requires then the identification of the membrane systems and compartments which make up the tissue. Some of the aspects of the necessary morphology are not so straightforward, however. It is often necessary to use stains, with ill-defined chemical properties, to identify subcellular structures. Electron dense extracellular markers are usually needed to identify components of extracellular space not obviously connected to the exterior of the cell. Such components often are tubules which pass out of the thin sections of tissue used in electron microscopy. Identification of the connection between different compartments of tissue is also difficult with purely structural techniques, since the specialized junctions which connect these components may not always be apparent. Finally, as good as present techniques of tissue preservation are, morphologists, like the rest of experimental scientists, work up to and just beyond the limit of resolution of their methods. It is characteristic of the most significant, but unresolved questions concerning small structures that quite different images are seen when the tissue containing the structures is prepared in different ways.

The topological description of a tissue, as important as it is, is still only the first step in the analysis of structure in electrical tissues. The morphology of a tissue identifies the possible paths for current flow, but it does not tell us how much current is likely to flow into each structure. Morphometrics are needed fully as much as morphology to answer this question.

B. Structural Analysis: Quantitative

We now consider methods of measuring the amounts of the various structures described in the qualitative diagram of the tissue or cell. The first difficulties in measuring the amounts of various structures in a tissue arise from two incompatible requirements: on the one hand, the components of the tissue must be manipulated to be observable in the light or electron microscope; on the other hand, the tissue must be preserved as close to the natural state as possible. It is beyond the scope of this review, and probably of current knowledge, to discuss this subject definitively. A great deal of work is underway to understand and improve currently used techniques, and some workers are enthusiastic that rapid freezing may make tissue preservation and detailed tissue observation compatible. Suffice it to say here that the folklore of morphology provides a variety of poorly understood techniques which preserve structure surprisingly well, at least in those cases where independent measurements of structure are available. We proceed now to the discussion of morphometric analysis, leaving the subject of tissue preservation to other workers.

The first method of morphometric analysis is akin to planimetry. Fundamentally the method is to digitize the observed structure and ask the computer to perform the required measurements. As straightforward as is the idea, so is the complexity of the

implementation. At the moment the human visual and nervous systems are required to recognize and identify structures having any degree of ambiguity or complexity. Computer algorithms have serious difficulty recognizing even contrasty objects of simple structure on noiseless backgrounds. The presence of noise, low-contrast images, irregular, even broken, structures tend to confuse the computer and reduce the utility of this approach.

Curve tracing is a compromise system for planimetry which avoids the digitization of the entire micrograph. Here a cursor is traced by human hand over the structure of interest, various technologies being used to continually record the location of the device. The digital record of the cursor location is a sequence of numbers which define the boundary of the structure of interest. From this sequence, the area enclosed, the perimeter, and other parameters of interest (shape factors, center of "mass", and so on) can be determined. In the case of two-dimensional objects these parameters can completely specify the structure. Of course, most biological objects exist in three dimensions and so cannot be reconstructed from a single two-dimensional image. A deterministic analysis of the full three-dimensional structure requires serial sectioning, a technique which is as impractical (because of crumbling of material at the face of the sections) as it is tedious (because of the difficulties of cutting, handling, and observing so many sections without losing even one). A sampling of structures in different orientations, however, may be used to reconstruct the three-dimensional structure. If normal morphological procedures provide a fair representation of all the different aspects of the tissue (or in statistical language, if they provide an unbiased uniformly distributed random sample of the structure), then random sections can be used to measure structural parameters. Such a statistical approach to the reconstruction of the third dimension suggests the possibility of using a statistical approach in the analysis of even two-dimensional images. This statistical approach is called stereology.

The method of stereology, as it is usually if somewhat imprecisely called, allows the efficient measurement of many micrographs by giving up the attempt to determine all the information in a single micrograph. No attempt is made to measure the area or perimeter of the piece of a structure seen in a single picture. Rather stereological procedures measure an unbiased sample of each micrograph. The average of many samples from many micrographs can then be considered to be a good representation of the mean structure. In other words, stereology reconstructs a two-dimensional image, and then a three-dimensional structure, statistically, using unbiased samples of the structure. The mean of the samples becomes the representation of the mean structure. The methods used in stereology are beyond the scope of this review (see Weibel,[49] for a pleasant and useful introduction; see Eisenberg, et al.[14] for an application to a tissue of complex structure).

The statistical aspects of stereology are in their infancy. Little work has been done to determine the best estimators of the biological parameters of interest. The word "best" implies most practical as well as the usual statistical properties of most efficient, unbiased (or with known bias) and so on. And there are undoubtedly parameters of interest for which no estimators are known at all. The reader may wish to consult Solomon[45] for an admirable discussion of the statistical properties of a classical stereological problem: the determination of the number π using a statistical process introduced by Buffon.[8] Unfortunately, Solomon does not discuss the stereological techniques commonly used in the laboratory. One can only guess that these might be significantly improved by trivial changes in the experimental process, just as the estimates of π can be so improved (Solomon,[45] p. 11). The paper of Franklin[22] may also be consulted for what appears to be a practical estimator of the number of objects per unit volume, a parameter that had previously been difficult to measure in many cir-

cumstances. Finally it should be added that little is known about the application of stereological methods to partially or fully oriented structures, particularly the anisotropic structures so characteristic of the complex tissues we are interested in here. It seems likely that the anisotropic electrical parameters necessary to describe these tissues (Eisenberg et al.[16]) must be accompanied by estimates of anisotropic morphometric parameters. These in turn probably must be determined from measurements of specifically oriented sections (see Appendix of Mathias et al.[35]). On the other hand, the average properties of such preparations may well require measurements from unoriented, random sections. It seems unlikely that a general prescription can be constructed.

In our opinion, the methods of stereology are the methods of choice for the measurement of biological structures as much because of their accuracy as their efficiency. But the method of curve tracing is so appealing that we feel it important to justify our opinion. Human errors are more commonplace in curve tracing than might be realized. Operators will tend to measure the outside or the inside of curves; often they will miss the curve altogether; operators tend to miss corners; they will follow dangerous procedures when the curve being traced is difficult to follow, as is so often the case when the curve is a membrane caught in a grazing section. These errors are both random and systematic. The former produce much unnecessary variance; the latter can produce errors.

The tedium involved in curve tracing, and its effects, should not be minimized. Although the human operator can trace curves of structures the computer cannot identify, he cannot trace them without a great deal of effort. Tedium produces mistakes, discourages massive investigations, and encourages dangerous short cuts. These embarrassingly practical matters often turn out to determine the course of scientific research and so deserve mention here.

Another set of difficulties concerning the method of curve tracing arises from a combination of the cost (i.e., human time and effort) of the technique and certain statistical realities. The piece of a tissue seen in most micrographs, certainly in most electron micrographs, is an exceedingly small sample of a rather variable structure. Variation is present at many different levels. The micrograph is usually a small sample of a single cell, and there is considerable variation to be expected from place to place within the cell. The cell itself is a small sample of the tissue; the tissue is a small sample of the preparation; and the preparation itself has usually been insulted by an experimental procedure which is not entirely reproducible. For all these reasons, the measurement of many micrographs, from many cells, from many tissues, of many preparations, is required to produce reliable results. The expense of the curve tracing method makes it impractical to acquire so much data; and the use of small amounts of data may produce serious errors. Stereological procedures are much less expensive and require large amounts of data. It is therefore much easier to make stereological measurements independent of sampling errors.

We conclude that stereological methods are preferable where possible but, as is usually the case in an experimental science, one must tailor the methods to the questions and use statistical sampling where appropriate, oriented sectioning where appropriate, and resort to curve tracing if appropriate statistical estimators are not known.

C. Theory: The Electrical Structure

We turn now to theory to convert the structural information just described into predictions of the electrical properties of the preparation. It is easy to interpret electrical measurements with arbitrary theories, in the guise of canonical but anatomically naive equivalent circuits. But that method of interpretation cannot determine the electrical properties of structural components, since elements of the canonical circuit will

not usually correspond to individual structural components. The elements of a canonical circuit are composites of the properties of many structural components, and so the canonical elements will vary in a complex manner with experimental conditions even if the structural components vary in a simple manner. A circuit model which corresponds directly to the structure of the preparation would not have this property; its parameters are the properties of individual structural components. We feel that the theory or circuit used to interpret experimental data must be based directly and convincingly on the morphology of the preparation, with each component of the circuit corresponding to a structure. Otherwise the resulting estimates of electrical parameters are as arbitrary as the canonical circuits themselves.

The theoretical approach we advocate starts with as fundamental a set of physical laws as possible. The partial differential equations and boundary conditions of electrostatics are the correct starting point if they can in fact be solved. These equations specify the microscopic electrical potential at every point within the preparation. If the preparation consists of cells of simple geometry, the equations can be easily solved. Descriptions of the derivation and solution of one dimensional problems can be found in Jack et al.;[27] three-dimensional problems are described in Eisenberg and Johnson;[17] subsequent work is reviewed in Peskoff and Eisenberg,[40] and Eisenberg et al.[18] In the case of invaginated cells or syncytial tissues, made of many electrically connected cells, the application of the physical laws specifying the microscopic potential yields unwieldy expressions which appear awkward to solve in the general case. Barcilon et al.[7] have coped with these microscopic expressions in an interesting special case and derived much simpler but approximate equations for an average or macroscopic potential. Their derivation is for the particular situation of straight unbranched tubules with random orientation. The appropriate description of other more complicated situations, the more common situations biologically, remains an open question mathematically until a derivation is performed which includes branched and wiggling tubules and clefts between cells.

Fortunately, a heuristic description of such preparations is available (see Eisenberg et al.,[16] which includes references and discussion of other papers on this subject). Equations are derived there for the average potential in a small but macroscopic piece of tissue, a piece large enough to contain a representative sample of the entire tissue, but small enough to be meaningfully described by a single potential, or pair of potentials if an intracellular and extracellular medium are present. These equations can be derived for anisotropic situations and appear to have some validity, judging from the microscopic analysis of Barcilon et al.[7] The boundary condition used by Eisenberg et al.[16] appears to be in error, however. Or perhaps, to put it both more kindly and more precisely, it appears to be a special case of the boundary condition appropriate for complex tissues. The appropriate boundary condition for tissues containing an extracellular space other than straight unbranched tubules is not known. One can anticipate, however, that it is likely to be of the general form derived by Barcilon et al.[7] with a different relationship between the measurable effective electrical parameters and the specific electrical and morphometric parameters of the components of the tissue. It is even possible that the original membrane boundary condition, as used by Eisenberg et al.,[16] may apply to some tissues with branched and wiggling extracellular spaces, if the definition of the effective parameters is modified.

The theory just described uses partial differential equations to specify the electric field within the tissue or cell and uses boundary conditions to specify the flow of current at the edge of the preparation. The theory requires, however, an electrical description of the fundamental structural elements of a tissue, just as the analysis of an electric circuit requires a description of the electrical properties of a resistor, capacitor, or inductor. Compartments filled for the most part with saline solution, like intra- and

extra-cellular spaces, can be described as resistive. While this description must be (and fortunately can be) tested experimentally, it is not the most likely source of error. In the linear case, one can begin in the same spirit by describing a small patch of membrane as a resistor in parallel with a capacitor. The resistor describes the macromolecules which span the membrane and allow ionic current flow, perhaps through an aqueous channel in the center of a protein. The capacitor represents the dielectric behavior of the oily lipid matrix which forms the bulk of the membrane. Note that by restricting ourselves to the strictly linear situation, we mean to exclude the nonlinear ionic conductances as described in, e.g., Hodgkin and Huxley.[24] It is certainly reasonable, and probably correct, to describe membrane permeability as a fixed conductance, although errors may be introduced in a secondary manner by nonlinear phenomena inside or outside of membranes, e.g., accumulation and/or depletion of ions in small regions of extra- or intracellular space, near membranes. It is also reasonable, but not so certainly correct, to describe displacement current in the membrane as that through a fixed—voltage and time independent—capacitance. That is certainly a good description of artificial membranes made of lipids, but perhaps is not such a good description of biomembranes.

The structural analysis of nonlinear properties is of greater biological significance than analysis of the linear properties we have been discussing, for the simple reason that most physiological functions are highly nonlinear. The current voltage relationship for even the simplest ionic channel through a membrane will not be a straight line and only by keeping the voltage excursions small can one justify the assumption of linearity. Indeed the voltage dependence of many ionic channels is very steep and usually involves time dependence as well. The sodium selective channel which initiates the nerve action potential is one well-known example of a voltage and time-dependent system, as quantitatively described in Hodgkin and Huxley.[26] The analytical description of nonlinear, time-varying conductances has usually been a kinetic scheme where the rate of a chemical reaction (e.g., the opening of a gate in a channel) depends on transmembrane potential. In the case of the sodium channel, it is widely thought that three such reactions must occur in succession before the channel can conduct, since the conductance depends on a probability function cubed. Other ionic channels have different kinetic behavior and in fact it may not be possible to represent some channels by a chemical kinetic scheme.

The detailed description of an ionic channel is relevant to our goal of structural analysis, since the equations describing a tissue will depend on the properties of its membranes. If the nonlinear case is to be treated, a nonlinear representation of the membranes must be used. Since a general description of a nonlinear ionic channel is not available, and may not be possible, one cannot write equations that are appropriate for the general nonlinear situation. A further complication is that nonlinear time-varying conductances often give rise to current flows which can mimic delayed (i.e., reactive) currents produced by structural complexities.*

For these reasons, it is necessary, in our opinion, to perform a structural analysis of linear electrical properties before one can hope to determine the nonlinear properties of the individual components of the cell. The linear properties are themselves of considerable interest, so it is not necessary to apologize for their study. Nonetheless, the

* The reactive currents arising from the nonlinear properties of ionic channels are a nuisance if one is only interested in the capacitance of the lipid matrix of membranes or the resistance of extracellular compartments. On the other hand, it may be possible to localize reactive currents arising from ionic channels just as one can localize reactive currents arising from the capacitance of the lipid matrix of membranes. In this way the techniques developed here for the linear case might provide a short-cut to the goal of the structural analysis of nonlinear properties.

essential justification for the study of the linear properties of tissues which function nonlinearly is that we must study the more definable linear case first, if we are to proceed without piling ambiguity upon assumption.

D. Theoretical Predictions

The differential equations and boundary conditions describing the electrical properties of tissues and cells range in difficulty from trivial to intractable. The equations describing the steady-state potential in a small spherical cell need not be even differential equations. The equations describing current flow in a thin axon are relatively simple: one-dimensional current flow in such a preparation is described by the "telegrapher's equation" of 19th century fame. The equations describing current flow in multidimensional tissues or near the tip of a microelectrode are more difficult; finally, the equations describing nonlinear phenomena are essentially intractable, requiring numerical analysis on a digital computer.

The fundamental goals of analysis of biological preparations are rather different from those in analagous physical situations, and this difference colors the entire approach to the problem. Often the biologist is more interested in qualitative, parametric results than in quantitative predictions of the field in space and time. Thus the precise distribution of potential in a cell is rarely important. Much more significant is the variation of the properties of the cell or tissue with changes in the properties of its components, with changes in size, shape, membrane resistance, and so on. Peskoff and Eisenberg[40] argue this case in some detail and conclude that the techniques of singular perturbation theory are particularly well suited to biological needs.

In a perturbation analysis, the solution of a differential equation is represented by an asymptotic series in powers of some small parameter. The differential equation is then broken into a series of problems, the solution of each problem giving a coefficient of the power series expansion. Note that each coefficient is in fact a function, describing the spatial and temporal distribution of a component of the solution. The properties of singular perturbation theory which are most useful are quite specific: first, the breaking of a problem into subproblems, each of some mathematical complexity but each with a simple physical interpretation; and second, the isolation and relative sizing of the important dimensionless parameters of a problem. Other methods may be used to give similar results, but it should be emphasized that the limiting factor in the validity of perturbation results lies in the properties of the resulting expansion, no matter how that expansion is generated. These expansions are usually sufficiently complex, involving a significant number of physically (but not mathematically) interrelated parameters, that a discussion of uniformity is rarely undertaken. This is an important enough point to warrant further discussion.

Approximate solutions of field problems are usually leading terms in an expansion of the solution of that problem. Since the solutions and their expansions involve many dimensionless parameters, the expansion cannot be expected to be valid for all possible values of the parameters. Consider an expansion of the form $V(\vec{r},\varepsilon) = V_0(\vec{r}) + \varepsilon V_1(\vec{r}) + \varepsilon^2 V_2(\vec{r}) + \ldots + \varepsilon^n V_n(\vec{r})$. The coefficients $V_n(\vec{r})$, as well as ε, will usually depend on the morphometric properties of the tissue. We assume $\varepsilon << 1$, but the functions $V_n(\vec{r})$ may contain a morphometric parameter β such that $V_n(\vec{r}) \propto \beta^n$. The expansion introduced may thus contain terms of the form $(\beta\varepsilon)^n$. Since we must expect that under some conditions $\beta\varepsilon \geq 1$, our solution will diverge under those conditions and may not be a useful approximation. The use of the leading terms of the expansion as an approximate solution requires the additional assumption that the parameter β must be about equal to one. In more formal language, the expansion is nonuniform in the parameter β, and is valid only if β is order one.

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D. Theoretical Predictions

The differential equations and boundary conditions describing the electrical properties of tissues and cells range in difficulty from trivial to intractable. The equations describing the steady-state potential in a small spherical cell need not be even differential equations. The equations describing current flow in a thin axon are relatively simple: one-dimensional current flow in such a preparation is described by the "telegrapher's equation" of 19th century fame. The equations describing current flow in multidimensional tissues or near the tip of a microelectrode are more difficult; finally, the equations describing nonlinear phenomena are essentially intractable, requiring numerical analysis on a digital computer.

The fundamental goals of analysis of biological preparations are rather different from those in analagous physical situations, and this difference colors the entire approach to the problem. Often the biologist is more interested in qualitative, parametric results than in quantitative predictions of the field in space and time. Thus the precise distribution of potential in a cell is rarely important. Much more significant is the variation of the properties of the cell or tissue with changes in the properties of its components, with changes in size, shape, membrane resistance, and so on. Peskoff and Eisenberg[40] argue this case in some detail and conclude that the techniques of singular perturbation theory are particularly well suited to biological needs.

In a perturbation analysis, the solution of a differential equation is represented by an asymptotic series in powers of some small parameter. The differential equation is then broken into a series of problems, the solution of each problem giving a coefficient of the power series expansion. Note that each coefficient is in fact a function, describing the spatial and temporal distribution of a component of the solution. The properties of singular perturbation theory which are most useful are quite specific: first, the breaking of a problem into subproblems, each of some mathematical complexity but each with a simple physical interpretation; and second, the isolation and relative sizing of the important dimensionless parameters of a problem. Other methods may be used to give similar results, but it should be emphasized that the limiting factor in the validity of perturbation results lies in the properties of the resulting expansion, no matter how that expansion is generated. These expansions are usually sufficiently complex, involving a significant number of physically (but not mathematically) interrelated parameters, that a discussion of uniformity is rarely undertaken. This is an important enough point to warrant further discussion.

Approximate solutions of field problems are usually leading terms in an expansion of the solution of that problem. Since the solutions and their expansions involve many dimensionless parameters, the expansion cannot be expected to be valid for all possible values of the parameters. Consider an expansion of the form $V(\vec{r},\varepsilon) = V_0(\vec{r}) + \varepsilon V_1(\vec{r}) + \varepsilon^2 V_2(\vec{r}) + \ldots + \varepsilon^n V_n(\vec{r})$. The coefficients $V_n(\vec{r})$, as well as ε, will usually depend on the morphometric properties of the tissue. We assume $\varepsilon << 1$, but the functions $V_n(\vec{r})$ may contain a morphometric parameter β such that $V_n(\vec{r}) \propto \beta^n$. The expansion introduced may thus contain terms of the form $(\beta\varepsilon)^n$. Since we must expect that under some conditions $\beta\varepsilon \geq 1$, our solution will diverge under those conditions and may not be a useful approximation. The use of the leading terms of the expansion as an approximate solution requires the additional assumption that the parameter β must be about equal to one. In more formal language, the expansion is nonuniform in the parameter β, and is valid only if β is order one.

The form of the expansion will depend on the relative size of the different parameters in the problem. Since the form of the expansion determines the form of an equivalent circuit, different relative sizes of parameters can produce quite distinct images of the tissue. These different images correspond to different physical and physiological situations, which often have not been recognized. In this manner the mathematical investigation of nonuniformity can produce important physiological insights.

Perturbation analysis has been widely used in problems specifying the electrical properties of cells and tissues of complex structure. The solutions generated by perturbation methods have so far always reduced to simple circuits with known error terms. These simple equivalent circuits are useful because they summarize a wide range of properties in a neat form understood by most electrophysiologists. They also can be studied with the methods of circuit theory, which are often more easy to apply than the equivalent techniques of applied mathematics. Finally, these circuits have in themselves an obvious relation to the structure of the preparation: individual circuit components represent the effective properties of individual structural systems, whether membranes or compartments of intra- or extracellular space. The equivalent circuit therefore has a life of its own and can be used to explain phenomena that are beyond the conditions under which the original partial differential equations were derived and solved.

Equivalent circuits can be, and often have been, introduced into the scientific literature without the use of field theory because they are simply a listing of the significant pathways for current flow in a preparation. Such pathways can often be determined without much formal theory. And so the reader may wonder why the complexity of field theory is necessary. The mathematical analysis is obviously necessary to provide estimates of error, and so to avoid controversy concerning the appropriateness and range of validity of the equivalent circuits. Furthermore mathematical analysis is needed to determine the relationship between the measurable effective parameters and the underlying specific parameters which describe the properties of the cellular components. Finally, it is not always possible to identify *a priori* the significant pathways for current flow—this was certainly the case in the analysis of syncytial tissues (Eisenberg et al.[16])—and then the analytical approach is essential.

The theoretical analysis of complex structures must be done in a certain manner if it is to serve its proper role as a tool in the measurement of the electrical properties of biological structures. The theory should be

1. Reductionist and rigorous, reducing physiological phenomena to fundamental physical laws with as few interposed approximations as possible
2. Realistic, involving as precise a description of the tissue as possible
3. Accurate, giving error bounds on all approximate expressions
4. Usable, giving expressions which can be directly compared to commonly used heuristic results and to experimental data

Theoretical analyses which satisfy many of these criteria are now available for a number of preparations as summarized in the reviews already cited. But the reader must not be lulled into thinking this a closed field. The following is an incomplete list of significant open problems, all apparently solvable with known perturbation methods, all awaiting solution:

1. The electric field expected in an anisotropic cylindrical, thin plane, or thick plane syncytial tissue
2. The frequency and/or time dependence of the potential near a point source in a single cell or syncytial tissue, the solution being written in a form directly comparable to experimental data

3. The electric field expected outside a cylindrical preparation
4. The electrical interaction expected between neighboring cells, with the common biological shapes, assuming no specialized connections between cells
5. The meaning of "tortuosity" for branched and wiggling clefts or tubules in preparations of several different geometries
6. The formulation, solution, and testing of the appropriate differential equations to describe the accumulation and depletion of ions in small extracellular compartments within cells and tissues
7. The formulation, solution, and testing of the approriate differential equations to describe the spread of potential in dendritic trees, recognizing the analogy with syncytial tissues and satisfying the criteria just described

E. Electrical Measurements

The practical use of electrical measurements to specify properties of the components of complex tissues has been the subject of many papers (Valdiosera et al.,[46,47] Mathias et al.,[34] Eisenberg et al.,[16-18] Schneider and Chandler,[43] Chandler and Schneider,[10] Schoenberg et al.,[41] Schoenberg and Fozzard,[42] see the many earlier papers cited in those articles) and the recent paper of Mathias et al.,[35] presents a review of current knowledge. For that reason another long discussion is neither needed nor appropriate. Here we will concentrate on a few general issues and develop a new method which may make the structural interpretation of electrical measurements easier.

The assignment of particular electrical properties to the individual structures of a cell or tissue requires the comparison of at least one experimental response to a theoretical prediction. It is better, of course, to compare many predictions and responses. It is better yet to compare a number of predictions and responses measured and computed under different conditons, with different patterns of current flow. Living tissue

The assignment of particular electrical properties to the individual structures of a cell or tissue requires the comparison of at least one experimental response to a theoretical prediction. It is better, of course, to compare many predictions and responses. It is better yet to compare a number of predictions and responses measured and computed under different conditons, with different patterns of current flow. Living tissue is too delicate to allow multiple experimental manipulations which change the pattern of current flow. For example, pharmacological agents and physiological interventions will change the conductive properties of membranes, but most such experiments are prolonged and difficult to perform without damage to the tissue. However, the pattern of current can be changed without detrimental manipulations because of the capacitive properties of membranes. The capacitive properties of a biomembrane guarantee that different frequencies of applied current will induce different patterns of induced current flow (and of induced potential). It is possible then to compare experimental results and theoretical predictions under many conditions of current flow simply by comparing the temporal variation of potential with that predicted by theory.

F. Analysis in the Frequency Domain

The confrontation between theory and experiment can be made either in the frequency domain or the time domain. The phrase "in the frequency domain" originally meant that the input signal and resulting output were sinusoids. Here "frequency domain" refers to the sinusoidal components of a wide class of inputs and outputs, as determined from their Fourier transforms.

There are several different ways to perform a frequency domain analysis:

1. The system can be perturbed with a sinusoidal signal of just one frequency, which

ensures, for a linear time invariant system, that the response will also be a sinusoid at just that one frequency, but perhaps with a different amplitude and phase.

2. The system may be perturbed by the sum of sinusoids of different frequencies with prescribed energy at each frequency. A stochastic version of such a signal, with equal energy (on the average) at each frequency, is called "white noise". A deterministic approximation to such a signal can be produced by periodic repetition at the rate F (in Hz) of a waveform that appears to be (but is not) random. Such a periodic waveform is easy to make by analog filtering of the binary output of a shift register oscillator. The resulting waveform is often called pseudo-random noise, but it should be clearly realized that the waveform is in fact a strictly deterministic periodic signal, containing a rich spectrum of those sinusoids with frequencies greater than F.
3. The system might be perturbed by a typical transient excitation, a step function, or an impulse, and then appropriate mathematical steps taken to numerically convert the waveform, and the response to that waveform, into the frequency domain.

In an ideal system any of these methods might be expected to work reasonably well, but in the real world the third approach, using step functions or impulses, is quite difficult. For example, if a step function input were used, one would in effect be concentrating all the energy of the input at frequencies close to zero, since the energy content of a step is proportional to the reciprocal of frequency. The advantages of frequency domain analysis would then be lost, since the range of frequencies examined, and therefore the distributions of potential, are limited. Furthermore if the amplifiers and electrodes add contaminating noise at many frequencies, the signal-to-noise ratio would be very high at zero frequency but very low at higher frequencies. Measurements of circuit parameters which depend only on low-frequency behavior would be possible, but measurement of parameters which depend on high-frequency behavior would be difficult. Even prolonged signal averaging does not help very much since, in the real world, the low-frequency signals, containing so much energy, limit the usable dynamic range. The analysis of a response to a step function must be expected to give less information than the analysis of a broad band signal.

The use of a signal with equal energies at all frequencies would seem to offer a way out of these difficulties and signals approximating white noise are sometimes used for this very reason. Those are the pseudo-random, periodic signals we have just discussed. The only nonperiodic transient signal with a flat frequency spectrum is, however, particularly impractical to use. That signal is a so-called delta function (better "delta functional"): a very large, supposedly infinite spike, containing unity area. Even if such a signal is approximated as a triangle or rectangle of short duration and large height, it is most difficult to use. The signal is so large that it tends to excite confusing nonlinearities in the biological preparation or irrelevant nonlinearities in the recording apparatus. For these reasons, frequency domain analysis cannot easily be done with transient waveforms. Direct analysis of the time domain response to step functions can provide a great deal of useful information concerning the electrical parameters of a preparation. The analysis, however, must use a fundamentally different approach than analysis in the frequency domain.

Analysis in the frequency domain follows the procedure, detailed in many papers—particularly, Valdiosera et al.[46,47] and Mathias et al.[35]—of applying a sinusoidal or noise input, measuring the output, converting those measurements to estimates of the input/output relation of the system, fitting the theoretically expected input/output relation to the one experimentally observed, and thereby determining the elec-

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trical parameters of the cell or tissue. When the data are in the frequency domain, this procedure is found to work—both in principle and in practice.

G. Analysis in the Time Domain

It is only natural to expect that curve fitting to time domain data (e.g., the response to a step function) would be as successful as curve fitting in the frequency domain. Unfortunately, this expectation is not fulfilled: despite many attempts to use them, curve fitting procedures do not work well in the time domain.

Transient measurements have been widely used in physiology (and are extensively reviewed by Jack et al.,[27]) to measure the electrical parameters of preparations. But careful reading of the literature will show that such investigations have been successful in essentially two situations: one, when the system is highly nonlinear and a frequency domain analysis produces a multitude of confusions; two, when a particularly simple electrical structure is assumed for a preparation.

In the first situation, when the tissue is highly nonlinear, transient measurements are entirely appropriate and necessary. However, it has not yet been possible to determine the nonlinear electrical structure of complex tissues. That is to say, it has not been possible to assign the different nonlinear properties of a preparation to the cellular structures which produce them. Such a nonlinear structural analysis clearly requires a previous linear structural analysis.

Curve fitting to transient meaurements have been used in one other situation: when the electrical structure is assumed to be quite simple. In this case the entire preparation has been assumed to be a single cell, either a spherical cell (a resistor and capacitor in parallel) or a cylindrical cell (where the resistor and capacitor are distributed along the resistance of the cell interior). This assumption is usually in conflict with the known anatomy, so transient measurements are then used to determine the "effective" or "total" capacitance and conductance of the preparation, meaning the sum of all the membrane capacitors or conductors. But, as Adrian and Almers[3,4] point out, even the determination of "total" capacitance or conductance is correct only if the preparation really is a single cell as assumed; the determination is incorrect if the electrical structure of the preparation is complex (Vaughan et al.[48]).

The use of transient measurements to determine circuit parameters of more complex circuits is usually unsuccessful and has in fact rarely been used. The mathematical properties of a linear system guarantee that the response to a transient input is a sum of exponentials. Thus fitting experimental results with a theoretical model in the time domain means in practice the fitting of noisy data with a sum of exponentials. The purpose of the fitting is to determine the amplitudes, time constants (i.e., the exponents) and the number of exponential terms. The amplitudes and time constants in turn determine the parameters of the equivalent circuit, which are the electrical properties of individual structures of the preparation.

It is common knowledge that the transient response of circuits does not well determine the form of the circuit or the values of the parameters of that circuit. Estimates of parameters made from curve fitting to transient responses are found to be sensitive to noise and small errors. Or to put things the other way around, different forms of circuits or different values of the parameters produce responses which are practically indistinguishable. As Lanczos[28] (pp. 274-275) concludes from an extensive analysis of the problem, there is an "... extraordinary sensitivity of the exponents and amplitudes [and thus the circuit parameters] to very small changes of the data, which no amount of least square or other statistics could remedy. The only remedy would be an increase of accuracy to limits which are far beyond the possibilities of our present measuring devices." Acton[1] (pp. 252-253), includes "An Interlude-What Not To Compute" in

his textbook *"On Numerical Methods that [Usually] Work,"* where he warns of such problems in vivid terms: "One of the perennial problems that plagues [the numerical analyst]...is the fitting of data by a series of exponential functions.... The answer to this problem lies in the...laboratory [doing a different kind of experiment] ...and the sooner the hopeful innocent can be sent there and away from the computer room, the better everyone will be. For it is well known that an exponential equation of this type...is extremely ill-conditioned. That is, there are many combinations of [parameters]...that will fit the most exact data quite well indeed (will you believe four significant figures?) and when experimental noise is thrown into the pot, the entire operation becomes hopeless...." These authors conclude, as may we, that curve fitting to transient data contaminated with noise is an undesirable way to determine electrical parameters. Indeed, it is this very fact which motivated the first workers (Falk and Fatt[20] and Fatt[21]) to use measurements in the frequency domain to determine the properties of individual cell structures.

H. Integrals of Transients

One might conclude for these reasons that transient analysis is of little use in structural analysis, but Adrian et al.[5] have introduced a quite different method of treating transient data. Their method provides good estimates of the effective (but not always the total) capacitance of a preparation. Their approach avoids curve fitting altogether; rather it is based on the numerical evaluation of the integral of the transient current following a step change in voltage. This integral directly gives an estimate of the effective capacitance, since it measures the charge movement due to the voltage change. We will spend some time deriving and extending this method and will point out several applications which are not discussed in the literature to our knowledge (although related results have been independently derived and kindly communicated to us by Dr. Roger Tsien and Drs. Vaughan and Loo).

The fundamental idea introduced by Adrian et al., which we feel deserves the name breakthrough, is the computation of an integral of the transient response of a preparation and the evaluation of that integral in terms of properties of theoretical models of the preparation, usually an equivalent circuit with a structural interpretation.

We consider a class of integrals of the following type

$$I_n = \int_0^\infty (-t)^n \{g(t,y;x) \quad H(0,y;x) f(t,x)\} dt \qquad (1)$$

where n = 0,1,2, ...; t is the time after the stimulus; the input f(t,x) is applied at the spatial location x. The response to the input is g(t,y; x) measured at a different spatial location y, still within the tissue. The spatial coordinates (x,y) may in general be vectors representing the locations of, e.g., a current passing microelectrode and a voltage recording microelectrode within a three-dimensional cell or tissue.

The Laplace transforms of time signals are defined in the usual manner (Churchill,[11] Widder[52])

$$G(s,y;x) \triangleq \mathcal{L}\{g(t,y;x)\} \triangleq \int_0^\infty g(t,y;x) e^{-st} dt \qquad (2)$$

where the lower limit of the integral in Equation 2 and in all subsequent integrals of this type is taken to be 0^-; that is to say, the lower limit of the integral is just before any discontinuity which occurs at time zero.

The input/output relationship for the network (i.e., tissue) is defined as

$$H(s,y;x) \triangleq \frac{G(s,y;x)}{F(s,x)} \qquad (3)$$

The quantity H(0,y,x), used in the above integral, defines the steady-state (time invariant) response of the network. We consider biological tissues, working in their linear regions, without inductive elements. The function H(0,y; x) can then be determined from the resistive elements of the network with the capacitive elements treated as open circuits. The function H(0, y; x) can be considered either an input resistance or an input conductance, depending on whether the input f(·) and output g(·) are current and voltage, or vice versa.

We now evaluate the integrals in terms of the input/output function H(s,y; x). We later will show that, in many cases, all the circuit parameters of the preparation can be determined from the input/output function and thus from the experimentally determined integrals. Previous work has used only one integral to determine just one parameter, the effective capacitance.

Several properties of the Laplace transform are used which are derived and described in most texts concerning Laplace transforms (Churchill,[11] Widder,[52,53] give the domains of validity of the following expressions):

1. The steady value of a function is given, if it reaches a steady value, by

$$\lim_{t\to\infty} g(t) = \lim_{s\to 0} sG(s) \tag{4}$$

2. The steady value of an integral of a function is given, if it reaches a steady value, by

$$\lim_{t\to\infty} \int_0^t g(t)\,dt = G(0) \tag{5}$$

3. A class of integrals of a function g(t) can be written in terms of the derivatives $G^{(n)}(0)$ of the Laplace transform of the function: In particular, the first and all higher order derivatives can be determined from integrals:

$$\int_0^\infty (-t)^n g(t)\,dt = \lim_{s\to 0} \frac{d^n G(s)}{ds^n} \triangleq G^{(n)}(0) \tag{6}$$

Use of these properties allows the integral I_n in Equation 1 to be written as

$$I_n = \lim_{s\to 0} \frac{d^n}{ds^n}\left(\frac{H(s) - H(0)}{s} \cdot sF(s)\right) \tag{7}$$

The rule for repeated differentiation of a product gives

$$I_n = \lim_{s\to 0} \sum_{k=0}^{n} \binom{n}{k} \frac{d^{n-k}}{ds^{n-k}}\left[\frac{H(s) - H(0)}{s}\right] \cdot \frac{d^k}{ds^k}\,[sF(s)] \tag{8}$$

where

$$\binom{n}{k} = \frac{n!}{k!(n-k)!} \tag{9}$$

We expand H(s) about s = 0; apply the definition of the derivative:

$$H^{(1)}(0) = \lim_{s\to 0} \frac{H(s) - H(0)}{s}$$

set the limit of the product equal to the product of the limits; and use the property that, for the class of inputs where $f(0^-) = 0$, $sF(s) = \mathcal{L}[\frac{df}{dt}]$. Then we obtain an expression for a sum of the coefficients of the expansion of H(s)

$$I_n = \sum_{k=0}^{n} \left\{ \binom{n}{k} \frac{H^{(n-k+1)}(0)}{n-k+1} \int_0^\infty (\ t)^k \frac{df}{dt}\ dt \right\} \qquad (10)$$

This general formula does not look too elegant, let alone useful, but hidden in the complexities is a most useful result. The integrals on the right-hand side of the expression can all be directly determined from a pair of experimental records, namely the input function f(t) and the response function g(t). Since the coefficients of the Taylor expansion of H(s) determine all the properties of an equivalent circuit, this set of integrals allows the circuit parameters of an equivalent circuit, or tissue, to be determined from a transient measurement without curve fitting. At least that is what the formula implies.

Two special cases reduce the complexity of Equation 10 and have been of greatest practical use in experimental work. First, the integral computed with n = 0, namely I_0, gives $H^{(1)}(0)$ for a general input f(t,x)

$$H^{(1)}(0) = \frac{1}{f(\infty,x)} \int_0^\infty [g(t,y;x) - f(t,x)H(0,y;x)]\,dt \qquad (11)$$

Note that in this special case the sum given in Equation 10 reduces to just one term and so the expression (11) has been widely used to determine the "effective" capacitance of preparations (Adrian and Almers,[4] Chandler, et al.[9])

The second special case of the general expression (10) also reduces the sum to one term. In this case the input (current or voltage) is taken to be a step function with final value $f(\infty,x)$. Then, one has a simple result:

$$H^{(n+1)}(0) = \frac{n+1}{f(\infty,x)} \int_0^\infty (-t)^n [g(t,y;x) - g(\infty,y;x)]\,dt \qquad (12)$$

This result permits complete identification of a circuit from either current clamp or voltage clamp experiments, i.e., from experiments in which either steps of current or voltage are applied to the preparation and the resulting transient response is observed. One of the experimentally useful, and theoretically intriguing, features of Equation 12 is that it directly determines the values of the circuit parameters of a network without curve fitting, and yet still uses all the data points in the determination of each parameter.

I. Integrals of Transients: Lumped Circuits

It is now possible to use the integrals of transients to determine, without curve fitting and its attendant complexities and ambiguities, the parameters of many circuits which describe the electrical structure of biological cells and tissues. We consider circuits made of resistors and capacitors, since the linear electrical properties of many cells and tissues arise from the resistive properties of solutions and the capacitive properties of the lipid matrix of membranes. In order to minimize the algebraic complexity of our expressions, we first deal with circuits with a finite number of elements (called lumped circuits). The main points of the analysis are best made by dealing with two specific cases of physiological interest.

Consider a lumped circuit approximation (Figure 1A) to a spherical cell or tissue* (or to a small piece of a cylindrical muscle fiber); consider also a lumped circuit approximation to a tight epithelium (Figure 1B), consisting of a single functional layer of cells. The spherical tissue might be a spherical aggregate of cardiac muscle (De Haan and Fozzard,[13]) or the lens of the eye (Eisenberg and Rae;[19] Mathias et al.[35]), both containing an extensive system of inner membranes. The epithelium might be the rabbit urinary bladder whose properties can be explained by a series combination of apical and basolateral membranes (Lewis et al.[29-31]).

Physiologists will immediately note two biologically unrealistic features of the circuits shown. First, they do not contain all the resistive paths likely to be in the preparations. Second, they assume that all the inner membranes, or all the lateral membranes, have the same potential across them. The latter assumption is made to avoid the complexity of a distributed system. But as we shall see later, the analysis of a distributed system is only more complex in the algebraic, not the intellectual sense.

The absence of extra resistive paths across the inner membranes of the lens circuit and the absence of a shunt path in parallel with the epithelium is deliberate and important. Such paths require quite separate analysis, since they introduce redundancy into the equivalent circuit. That is to say, the structure of the preparation introduces a surplus of circuit elements; there are more circuit elements in the anatomically defined circuit than are needed to produce electrical properties observed from the outer terminals, at least under one set of conditions. The surplus elements cannot, of course, be identified by a single set of measurements from a preparation for the same reason that a measurement of the resistance R of a black box cannot tell whether that black box contains one resistor R, two parallel resistors of value 2R, two series resistors of value R/2, or so on. The surplus elements can only be determined by making experimental interventions to change the circuit parameters or by making measurements from different terminals. For example, experiments can be designed in which the preparation is modified by selective physiological or pharmacological interventions. Comparison of the properties of such modified preparations with those expected can often allow all circuit elements to be measured.

We will examine these redundant circuits later. Now we develop methods to identify the components of nonredundant circuits, circuits with the minimal number of elements necessary to specify their electrical behavior.

Consider a lumped minimal approximation to the admittance of a small piece of skeletal muscle, which is also an approximation to the membrane and extracellular properties of a spherical preparation like the lens:

$$Y_1 = \frac{1}{R_S} + sC_S + \frac{1}{R_e + \frac{1}{sC_W}} \tag{13}$$

Consider a lumped minimal approximation to the impedance of a tight epithelium:

$$Z_2 = \frac{\frac{1}{C_a}}{s + \frac{1}{C_a R_a}} + \frac{\frac{1}{C_{b1}}}{s + \frac{1}{C_{b1} R_{b1}}} \tag{14}$$

* The circuit shown is a crude approximation to a spherical cell or syncytium since it omits, for the sake of simplicity, the series resistance produced by point source effects (see Eisenberg and Johnson [17] and Eisenberg et al.[16]), which resistance is rarely negligible. Inclusion of a series resistance term is straightforward since it simply adds an IR drop which can be readily analyzed.

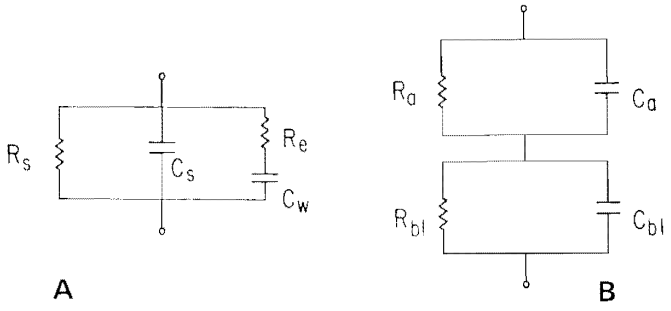

FIGURE 1. Lumped circuit approximations. (A) The lumped circuit approximation to a spherical cell or tissue. (B) The lumped circuit approximation to a tight epithelium consisting of a single functional layer of cells.

We write the Taylor expansion of the impedance functions just given to relate the circuit elements to integrals of transients. The coefficients of the Taylor series are the same derivatives, evaluated at $s = 0$, given by the integrals of transients in Equations 10 to 12. The Taylor series of lumped circuits, about $s = 0$, is usually easier to determine by long division than differentiation, and gives for our lumped circuits

$$Y_1 = Y(0) + sY^{(1)}(0) + \frac{s^2}{2!} Y^{(2)}(0) + \ldots \frac{s^n}{n!} Y^{(n)}(0) + \ldots \tag{15}$$

where

$$Y(0) = \frac{1}{R_s}$$

$$Y^{(1)}(0) = C_s + C_w$$

$$Y^{(2)}(0) = 2C_w{}^2R_e$$

$$Y^{(n)}(0) = n!\,(-1)^{n-1}R_e{}^{n-1}C_w{}^n; \quad n > 1 \tag{16}$$

For epithelia

$$Z_2 = Z(0) + sZ^{(1)}(0) + \frac{s^2}{2!} Z^{(2)}(0) + \ldots + \frac{s^n}{n!} Z^{(n)}(0) + \ldots \tag{17}$$

where

$$Z(0) = R_a + R_{b1}$$

$$Z^{(1)}(0) = [C_aR_a{}^2 + C_{b1}R_{b1}{}^2]$$

$$Z^{(n)}(0) = n!\,(-1)^n(C_a{}^nR_a{}^{n+1} + C_{b1}{}^nR_{b1}{}^{n+1})$$

$$n \geq 1 \tag{18}$$

Since each of the derivatives $Z^{(n)}(0)$ and $Y^{(n)}(0)$ can be determined by an experimentally determined integral of a transient (using Equations 10 to 12), each combination of circuit elements can be measured directly from a single experimental record. Note that, at least in principle, an infinite set of combinations of circuit elements can be determined from a single experimental record. Since there are only a finite number of elements in the circuits analyzed—indeed, only four—these combinations provide redundant estimates of the same parameters. It seems unlikely that averaging of redundant estimates will be useful, because they are based on the same experimental data, but there are situations where averaging of redundant estimates made from overlapping data sets is of great benefit (Welch[50]).

In the case of some circuits, e.g., the circuit for Y_1, it is possible to write explicit formulae for the circuit parameters in terms of the derivatives or, equivalently, in terms of experimentally determined integrals.

For the lumped lens circuit, we have

$$C_w = -\frac{3}{2}\frac{[Y^{(2)}(0)]^2}{Y^{(3)}(0)} \qquad C_s = Y^{(1)}(0) - C_w$$

$$R_s = \frac{1}{Y(0)} \qquad R_e = -\frac{2}{9}\frac{[Y^{(3)}(0)]^2}{[Y^{(2)}(0)]^2} \tag{19}$$

Here the total capacitance equals the effective capacitance and both are given by a single derivative, $Y^{(1)}(0)$, which is determined from a single integral I_1, given by Equations 11 and 12.

It seems too much to expect that the relations analagous to Equation 19 can be solved for a general circuit, since usually the relations will include high-order polynomials for which explicit solutions do not exist. In the case of lumped circuits there is a tantalizing predictability to the higher order coefficients, and it is possible that one might prove some useful theorems concerning component identification and Taylor series coefficients. We have not analytically solved the relations for the epithelial circuit; nonetheless, the circuit of the epithelial circuit can be determined from the Equations 17 and 18 using straightforward numerical methods.

It is instructive to consider the meaning of effective capacitance for the epithelial equivalent circuit. In this case the "first" integral—the integral with n = 1—will determine $Z^{(1)}(0)$ and the effective input capacitance is given by $Y^{(1)}(0) = -Z^{(1)}(0)/[Z(0)]^2$, or more explicitly,

$$C_{eff} = \left[\frac{R_a}{R_a + R_b}\right]^2 C_a + \left[\frac{R_b}{R_a + R_b}\right]^2 C_b \tag{20}$$

Thus the effective capacitance for an epithelial equivalent circuit is not given by the sum of the capacitors in the circuit. Rather the effective capacitance depends on the resistors in the circuit fully as much as on the capacitors; the effective capacitance is the sum of the capacitors scaled by an attenuation factor, the resistive voltage divider ratios, squared.

It is tempting to try to construct integrals to give each circuit component directly, thus avoiding the solution of simultaneous nonlinear equations. We do not know yet whether these integrals can be constructed.

The procedures just outlined permit the identification of circuit parameters of nonredundant lumped circuits from integrals of transients easily measured from biological

preparations. Circuits that correspond to the structure of a tissue, however, will usually be redundant. For example, the circuit shown in Figure 1A does not correspond very well to the properties measured from the frog lens (Mathias et al.[35]), since even the lumped approximation to the real lens has a substantial conductance in parallel with the capacitance C_m, corresponding to the total or effective membrane conductance of all the inner membranes. On the other hand, the circuit shown in Figure 1A does correspond quite well to the properties of a small piece of frog skeletal muscle in conditions in which the conductance of the tubular membranes has been reduced (Adrian and Almers[3]). Similarly the epithelial circuit shown in Figure 1B well represents the properties of a tight epithelium, mounted between two chambers without substantial artifactual leakage around its edge, but it does not represent even the lumped approximation to leaky epithelia nor does it well represent epithelia mounted with a definite amount of artifactual leakage. In both those cases, a parallel resistor must be included if the circuit is to have anatomical and physiological reality.

J. Integrals of Transients: Redundant Lumped Circuits

We are forced then by experimental and biological reality to deal with redundant circuits. The analysis of such circuits is not done here in a general way, because the experimental maneuvers available to determine the parameters of redundant circuits are different for each preparation and so do not seem amenable to generalization. Rather, we discuss a particular important case.

The general property of all redundant lumped circuits is that identification of their components requires more experimental information than is available by measurements from a single pair of terminals under one set of conditions. Separate experimental information, often with additional assumptions, is needed to determine the value of the redundant resistors, shown in Figure 2. For example, circuit elements which represent the capacitance of membranes are usually assumed to be independent of ionic conditions, at least if the resting potential of the preparation is reasonably constant. This assumption can be exploited by studying a preparation in different conditions, assuming that the membrane capacitance is the same in those different conditions. Then there may be sufficient information to identify all circuit elements.

There are a number of other experimental interventions, practical in many preparations, which can also help determine redundant circuit parameters.

1. Variation of the conductivity of the bathing solution, which sometimes can be done without modifying membrane properties.
2. Change in the size or shape of the preparation, either by experimental manipulation or by natural variation (see Hodgkin and Nakajima[25,26] for the classical application of the latter approach). The results of these interventions are interpreted with the assumption that all specific morphometric and electrical variables are independent of size and shape.
3. Change in volume induced by solutions of varying tonicity and/or osmolality. This is often a dangerous technique, since changes in cell volume tend to sufficiently disrupt the homeostasis of the cell that nothing can be assumed to remain constant.
4. Changes in the properties of specific membranes induced by drugs, natural pharmacological agents, or ionic interventions.

It should be obvious that the analysis of each of the interventions requires measurement of both electrical and morphometric parameters. Unfortunately, as obvious as that might seem, there are almost no interventions for which both sets of data have been measured.

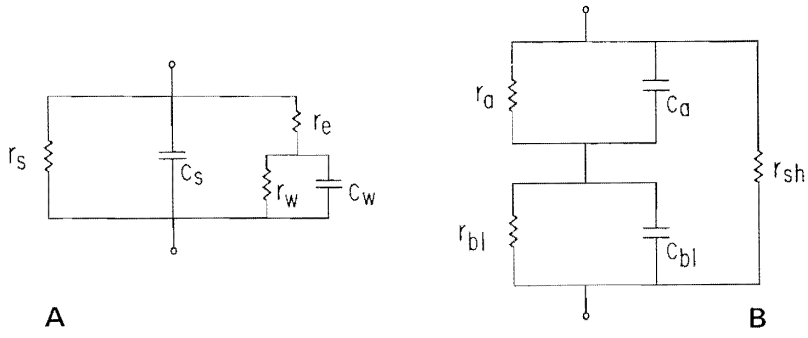

FIGURE 2. Lump circuits containing redundant resistors. (A) A lumped approximation to the transverse path for current flow in a skeletal muscle fiber or the radial path for current flow in the lens. (B) A lumped approximation to a leaky epithelium consisting of a single layer of functional cells.

We now turn to the particular redundant circuit which approximately describes the transverse path for current flow in a long skeletal muscle fiber or the radial path for current flow in the lens, again neglecting point source effects for simplicity (Figure 2A). We will analyze the circuit to show the information available from integrals of transients and then briefly discuss the experimental approach to identification of the circuit elements.

The admittance of the circuit in Figure 2A is

$$y_1 = \frac{1}{r_s} + sC_s + \frac{1}{r_e + \dfrac{1}{\dfrac{1}{r_w} + sC_w}} \tag{21}$$

which can be expressed in precisely the same form as the admittance of the nonredundant circuit shown in Figure 1A.

$$y_1 = \frac{1}{r_s} + \frac{1}{r_w + r_e} + sC_s + \frac{1}{(r_e/r_w)(r_w + r_e) + \dfrac{1}{sC_w[r_w/(r_w + r_e)]^2}} \tag{22}$$

Thus the redundant and nonredundant circuits have identical properties if the relationship between their circuit elements is

$$\frac{1}{R_s} = \frac{1}{r_s} + \frac{1}{r_w + r_e}$$

$$R_e = \frac{r_e}{r_w}[r_w + r_e]$$

$$C_s = c_s$$

$$C_w = c_w \left(\frac{r_w}{r_w + r_e}\right)^2 \tag{23}$$

The coefficients of the Taylor expansion of Equation 22 are given by

$$y(0) = \frac{1}{r_s} + \frac{1}{r_w + r_e} \tag{24}$$

$$y^{(1)}(0) = c_s + c_w [r_w/(r_w + r_e)]^2 \tag{25}$$

$$y^{(2)}(0) = -2c_w{}^2 r_e (r_w/(r_w + r_e))^3 \tag{26}$$

and so on. Each of these coefficients can be measured by the integrals defined in Equations 11 or 12.

If the first integral of the transient is measured or interpreted with the redundant circuit, one does not obtain the sum of the capacitors in the circuit, but, as with the nonredundant epithelial circuit already discussed, the capacitance of the inner membranes is attenuated by a factor squared. The squared attenuation factor probably occurs because the voltage across the capacitor is attenuated once by the resistor divider ratio and the charge measured at the outer terminals is attenuated a second time by the same factor. It is clear that the effective capacitance does not necessarily equal the total capacitance, either for redundant or nonredundant circuits. The effective capacitance measured by the integral of a transient can indeed change even if the total capacitance is fixed, since the effective capacitance depends as much on membrane and solution resistances as it does on membrane capacitances. Thus changes in effective capacitance cannot be directly intepreted as changes in membrane capacitance. Adrian and Almers[3] have in fact measured the resistance of membranes by measuring effective capacitance.

K. Integrals of Transients: Distributed Circuits

The circuits considered up to now have included only a finite number of circuit elements. In fact, they describe the electrical properties of biological preparations only if each system of membranes within the preparation has one transmembrane potential, whereas the typical preparation has significant variation of potential along a single membrane system. Most of the circuits which describe biological preparations must describe the continuous variation of potential along one or more spatial coordinates, and so must include distributed elements. The simplest example of such a circuit is the telegrapher's equation widely used to describe a cylindrical unmyelinated axon. (This equation, under the name "one dimensional cable theory", is discussed in detail in Jack et al.[27]) Current flows predominantly in two pathways in such a preparation; at least this is true if one avoids regions close to a point source, and if the diameter of the preparation is small compared to the distance current spreads. Current flows longitudinally down the length of the axon and current is shunted radially across the membrane which forms the axon. The membrane has a quite high specific resistance R_m (say, 3 kohm^2) and an exceedingly high resistivity (say, 3×10^9 ohm-cm) so the little current which leaks across a small length of membrane produces a large change in transmembrane potential. The interior of the axon is filled with salt solution with a resistivity R_i of only some hundreds of ohm-cm. The axon has a small cross section and so a sufficient length of the interior has a total resistance to longitudinal current flow (measured in ohms) comparable to the resistance of the same length of membrane to transverse current flow (also measured in ohms). If one defines a as the radius of the axon, and λ as the length of axon in which the transverse resistance equals the longitudinal resistance, then

$$\lambda \frac{R_i}{\pi a^2} = \frac{1}{\lambda} \frac{R_m}{2\pi a} \tag{27}$$

which shows that

$$\lambda = [aR_m/2R_i]^{1/2} \tag{28}$$

We recognize the definition of the d.c. length constant λ widely used in physiology.

The spread of d.c. potential in a long thin cylinder is described by

$$v(\infty, x) = \frac{i_0}{2} \lambda \frac{R_i}{\pi a^2} e^{-x/\lambda} \tag{29}$$

where i_0 is the constant current applied at $x = 0$, and $v(\infty,x)$ is the steady potential produced in response to that current at distance x from the current source.

We turn now to the analysis of a specific pair of distributed circuits, those of a cylindrical axon and then of a cylindrical muscle fiber. The methods developed can be used, together with perturbation theory, to analyze a wide variety of biological preparations.

The first circuit is a generalization to the time-dependent or frequency-dependent case of the steady state cable equation previously presented. The input/output relation Z(s,x), in ohms, connects the observed response V(s,x), in volts, to the applied current $I_0(s)$, in amps. The following equation gives the input impedance Z(s,x) as a function of the shunt admittance y(s) (mho/cm) to radial current flow and the impedance $z_i(s)$ (ohm/cm) to longitudinal current flow.

$$Z(s,x) \triangleq \frac{V(s,x)}{I_0} = \frac{1}{2}[z_i/y]^{1/2} e^{-x(z_i y)^{1/2}} \tag{30}$$

In axons (Cole and Hodgkin[12]) and skeletal muscle fibers (Mobley et al.[36,37] the longitudinal pathway is essentially resistive, so z_i is written as r_i. In axons the admittance of the shunt pathway is produced by the surface membrane only. Since all of that membrane at a given longitudinal distance from the source is at the same potential, the radial or shunt admittance of an axon can be written as a lumped circuit, namely

$$y = g_m + sc_m = 2\pi a (G_m + sC_m) \tag{31}$$

where the upper case variables are the specific membrane conductance and capacitance in units of mhos/cm^2 and μF/cm^2, respectively.

The thrust of our analysis will be to first determine the coefficients $Z^{(n)}(s=0,x)$ of the Taylor series for Z(s,x) by integrals of transients. Then we relate the coefficients $y^{(n)}(s=0)$ of the Taylor series for the shunt element y (s) to the coefficients $Z^{(n)}$ (0,x). Since the circuit components g_m and c_m of the shunt element can be determined from $y^{(n)}(0)$, we can determine the shunt circuit elements from integrals of transients. The derivatives of the input/output relation of the cylindrical cell can be determined just as before, giving

$$Z^{(n)}(0,x) = \frac{2n}{I_0} \int_0^\infty (-t)^{n-1} [v(t,x) - v(\infty,x)]\, dt$$

$$n \geq 1 \tag{32}$$

where

$$v(\infty, x) = \frac{1}{2} I_0 Z(0, x) \tag{33}$$

We define the input admittance Y(s,x) as the reciprocal of the input impedance Z(s,x). The derivatives, $Y^{(n)}(s)$, of the input admittance are then given by

$$\frac{Y^{(1)}}{Y} = -\frac{Z^{(1)}}{Z} \tag{34}$$

$$\frac{Y^{(2)}}{Y} = -\frac{Z^{(2)}}{Z} + 2\frac{Z^{(1)}}{Z} \tag{35}$$

$$\frac{Y^{(3)}}{Y} = -\frac{Z^{(3)}}{Z} + 6\frac{Z^{(2)}}{Z}\frac{Z^{(1)}}{Z} - 6\left[\frac{Z^{(1)}}{Z}\right]^3 \tag{36}$$

and so on. The shunt admittance and the derivatives $y^{(n)}(0)$ of the shunt admittance can now be written in terms of the input admittance and the derivatives $Y^{(n)}(0,x)$ of the input admittance. Note that in the following formulae several arguments of the admittances and their derivatives have been omitted to simplify the notation: the admittances, and derivatives all are evaluated at s = 0; they also all depend on x.

$$y = Y\frac{e^{-x/\lambda}}{\lambda} \tag{37}$$

$$\frac{y^{(1)}}{y} = \frac{2}{1 + x/\lambda}\frac{Y^{(1)}}{Y} \tag{38}$$

$$\frac{y^{(2)}}{y} = \frac{2}{1 + x/\lambda}\frac{Y^{(2)}}{Y} + 2\left[\frac{Y^{(1)}}{Y}\right]^2 \frac{1 - \frac{x}{\lambda} - \frac{x^2}{\lambda^2}}{(1 + x/\lambda)^3} \tag{39}$$

$$\frac{y^{(3)}}{y} = \frac{2}{1 + x/\lambda}\frac{Y^{(3)}}{Y} + 6\frac{Y^{(2)}}{Y}\frac{Y^{(1)}}{Y}\frac{1 - \frac{x}{\lambda} - \frac{x^2}{\lambda^2}}{(1 + x/\lambda)^3}$$

$$+ 2\frac{x}{\lambda}\left[\frac{Y^{(1)}}{Y}\right]\frac{3 - 6 + 5\frac{x^2}{\lambda^2} + 2\frac{x^3}{\lambda^3}}{(1 + x/\lambda)^5} \tag{40}$$

These formulae look awkward, but promise to be very useful nonetheless. They permit the measurement of the properties of the radial (that is, shunt) component of admittance from measurements of the transient response to an applied current, even with the electrodes separated.

The procedure for measuring the electrical properties of components of an axon from the response to applied steps of current can then be summarized:

1. Insert two microelectrodes at several different separations to measure the d.c. length constant, as described in Jack et al.[27]
2. At one or more separations, measure the response v(t,x) to the step of current and determine from it the integrals of transients.

3. From the integrals of transients, determine the coefficients $Y^{(n)}(0,x)$ of the Taylor expansion for the input admittance, using equations 34 to 36.
4. From the coefficients $Y^{(n)}(0,x)$ determine the coefficients $y^{(n)}(0)$ of the Taylor expansion of the shunt admittance, using Equations 37 to 40.
5. If the shunt admittance y is simply a parallel conductance g_w and capacitance c_w, the problem is solved: $g_w = y(0)$, $c_w = y^{(1)}(0)$.

The extension of the current pulse method to more complex preparations is straightforward, if tedious. Consider as an example frog skeletal muscle fibers. In that case, the shunt admittance is not the property of a single membrane system, but of two membrane systems and a compartment of infiltrating extracellular space. These two pathways are approximated by the circuit shown in Figure 2A and so one must perform an additional step to determine the structural parameters from the coefficients $y^{(n)}(0)$. If a skeletal muscle fiber had no distributed properties in the radial direction, the analysis presented in Equations 24 to 26 and 41, combined with independent measurements of the attenuation ratio, would permit identification of the circuit elements from integrals of transients.*

In reality, there is a still further degree of complexity, since the inner membranes will generally not have the same transmembrane potential. For example, skeletal muscle fibers contain a system of tubules, in which the predominant variation of potential is radial, not longitudinal (Mobley et al.[32,36,37]). The description of such a situation must include the distribution of potential in at least two directions, radial and longitudinal. Similarly, cylindrical syncytial preparations, e.g., cardiac muscle, include inner membranes across which potential must be expected to vary in many directions (Schoenberg et al.,[41] Schoenberg and Fozzard[42]). Even spherical preparations, like the lens of the eye (Mathias et al.[35]) and aggregates of cardiac muscle (de Haan and Fozzard[13]) require complex analysis, since the inner membranes of spherical syncytia must also be expected to have potential variation in many directions.

The complete description of the variation of potential in such complex tissues, including infolded membranes and point sources requires field theory (Barcilon et al.,[7] Eisenberg et al.,[16] Peskoff[39]). The exact solutions of the resulting partial differential equations are difficult to interpret mathematically, let alone physically. Fortunately, the results can be simplified using singular perturbation theory (Eisenberg et al.[16]). In the past, perturbation expansions have shown that the dominant (order zero) potential is a simple function, with an obvious interpretation in terms of the membranes of the tissue, while higher-order corrections describe the three-dimensional flow of current around the microelectrode. In this manner it has been possible to analyze rather complex tissues as a collection of distributed circuit elements. Each of the distributed elements can in turn be analyzed just as we have analyzed a cylindrical axon. In the case of a muscle fiber with a radial distribution of potential across the inner membranes, the analysis of the shunt admittance y would begin with its Taylor series. One would then write the formulae for the coefficients $y^{(n)}(0)$ in that series from the distributed representation of y_w. The procedure for introducing the radial distribution of potential into the Taylor series of the shunt admittance y is entirely analagous to the procedure

* The reader interested in applying the above analysis to a lumped representation of skeletal muscle should be warned that morphometric parameters have been omitted from Figure 2A for the sake of simplicity. The full equations are presented in Mathias et al.[34] There is, however, a misprint in Figure 5 of that paper; the correct expression for the effective (i.e., lumped) resistance of the tubular lumen is $R_L/[(8\pi\tau)(V_T/V_F)]$, the symbols being defined in that paper.

used earlier (Equations 30 to 40) to introduce a longitudinal distribution of potential into the Taylor series for the input impedance Z. In the radial case, the expressions for the distributed admittance y_w (see Equations 43, 44, and 47 of Mathias et al.[34]) are sufficiently awkward, and the computations of the coefficients of the Taylor series sufficiently complex, that we do not present the results here. It should be clear to the reader, however, that in principle a complete identification—involving several interlocking distributed admittances—can be made from measurements of the integrals of transient potentials induced by steps of applied current.

The question of redundant versus nonredundant circuits has not appeared explicitly in the discussion of distributed circuits, because the presence of an infinite number of pathways for current flow from terminal to terminal of a distributed circuit does not necessarily imply that the circuit contains surplus parameters. The distributed conductance of the inner membranes in skeletal muscle (Adrian and Almers[3]) and of the lens of the eye (Mathias et al.[35]) has in fact been measured with techniques similar to those discussed here. Of course, in the case that the radial length constant is quite large compared to the radius of the preparation, the distributed circuit is well approximated by the redundant lumped circuit shown in Figure 2A and the ambiguities previously discussed reappear.

L. Integrals of Transients: Experimental Verification

The power of the technique just presented is really quite considerable, since it promises a complete analysis of a complex tissue using time domain techniques. Until now, the applicability of integrals of transients to these cases, involving steps of current applied to tissues with distributed admittances, has not been clear. Rather it seemed that these integrals could be used in only some rather special, albeit important, cases, where the potential in the preparation could be controlled using specialized voltage clamp techniques. Most of the complex tissues in which structural localization is important are difficult to voltage clamp, so the integrals of transients have not been used for structural analysis. Now it seems that a complete structural analysis of electrical properties may be possible from the relatively simple measurement of the transient response to a step of current, measured with the electrodes apart.

Despite the promise of the last paragraph, the reader should be warned that these formulae have not yet been used to measure circuit parameters in either models or real systems. Until that is done successfully, one must suspect that problems will arise which are not apparent in the mathematical analysis presented here.

M. Experimental Verification of Structural Analysis

We have presented a general procedure for determining the structural location of the electrical properties of complicated tissues and cells. The procedure began with an analysis of structure, continued with the conversion of that structure into predictions of electrical properties, went on to the measurement and fitting of electrical data with the structural theory, and finally introduced a new method which hopefully can bypass some of the difficulties of collecting and fitting frequency domain data. This procedure, whether based on the well-tried methods of curve fitting, or on the direct computation of integrals of transients, permits the reconstruction of the properties of the entire tissue from those of its components. In this manner the function of the tissue can be analyzed into the contributions of its parts. But the validity of the entire approach to structural localization cannot be determined by pure thought; rather, like most other scientific procedures and results, it must be verified by direct experimentation, including the measurement of both electrical and morphometric properties under a variety of physiological conditions.

We are unaware of a preparation which has been subject to complete analysis; frog muscle has been studied under the widest variety of conditions, but even there morphometric measurements are available only for muscle in normal conditions. We look forward to the measurement of sufficient data from a variety of preparations to test the validity of our approach.

The ultimate justification of our analysis is, however, its utility in

1. Understanding the natural behavior of tissues and the way that behavior is produced by the components of the tissues
2. Isolating the individual molecular mechanisms which produce the electrical properties of structural components; thereby making possible the study of molecular mechanism, even in tissues with complex structure

Perhaps our readers will find this article helpful in applying, extending, and revising the techniques of structural analysis.

REFERENCES

1. **Acton, F. S.,** *Numerical Methods That Work,* Harper & Row, New York, 1970.
2. **Adrian, R. H. and Almers, W.,** Measurement of membrane capacity in skeletal muscle, *Nature (London) New Biol.,* 242, 62, 1973.
3. **Adrian, R. H. and Almers, W.,** Membrane capacity measurements on frog skeletal muscle in media of low ion content, *J. Physiol.,* 237, 573, 1974.
4. **Adrian, R. H. and Almers, W.,** The voltage dependence of membrane capacity, *J. Physiol.,* 254, 317, 1976.
5. **Adrian, R. H., Almers, W. and Chandler, W. K.,** Appendix: muscle membrane capacity and ionic strength, *J. Physiol.,* 237, 601, 1974.
6. **Barcilon, V., Cole, J. D. and Eisenberg, R. S.,** A singular perturbation analysis of induced electrical fields in nerve cells, *SIAM. Appl. Math.,* 21, 339, 1971.
7. **Barcilon, V., Eisenberg, R. S. and Mathias, R. T.,** Microscopic and Macroscopic Description of the Electrical Properties of Syncytial Tissues, in press.
8. **Buffon, G.,** Essai d'Arithmetiqe Morale, Supplement a l'Histoire Naturelle, 4, 1777.
9. **Chandler, W K., Rakowski, R. F., and Schneider, M. F.,** Effects of glycerol treatment and maintained depolarization on charge movement in skeletal muscle, *J. Physiol.,* 254, 285, 1976.
10. **Chandler, W. K. and Schneider, M. F.,** Time-Course of potential spread along a skeletal muscle fiber under voltage clamp, *J. Gen. Physiol.,* 67, 165, 1976.
11. **Churchill, Ruel V.,** *Operational Mathematics,* McGraw-Hill, New York, 1958.
12. **Cole, K. S. and Hodgkin, A. L.,** Membrane and protoplasm resistance in the squid giant axon, *J. Gen. Physiol.,* 22, 671, 1939.
13. **DeHaan, R. L. and Fozzard, H. A.,** Membrane response to current pulses in spherioidal aggregates of embroyic heart cells, *J. Physiol.,* 65, 207, 1975.
14. **Eisenberg, B. R., Kuda, A. M., and Peter, J. B.,** Stereological analysis of mammalian skeletal muscle. 1. Soleus muscle of the adult guinea pig, *J. Cell Biol.,* 60, 732, 1974.
15. **Eisenberg, B. R., Mathias, R. T., and Gilai, A.,** The intracellular localization of markers within injedcted or cut frog muscle fibers, *Am. J. Physiol.,* 6, 50, 1979.
16. **Eisenberg, R. S., Barcilon, V., and Mathias, R. T.,** Electrical properties of a spherical syncytium, *Biophys. J.,* 25, 151, 1979.
17. **Eisenberg, R. S. and Johnson, E. A.,** Three-dimensional electrical field problems in physiology, *Prog. Biophys. Mol. Biol.,* 20, 1, 1970.
18. **Eisenberg, R. S., Mathias, R. T., and Rae, J. L.,** Measurement, modelling and analysis of the linear electrical properties of cells, *Ann. N.Y. Acad. Sci.,* 303, 342, 1977.
19. **Eisenberg, R. S. and Rae, J. L.,** Current-voltage relationships in the crystalline lens, *J. Physiol.,* 262, 285, 1976.
20. **Falk, G. and Fatt, P.,** Linear electrical properties of striated muscle fibers observed with intracellular electrodes, *Proc. R. Soc. London Ser. B.* 160, 69, 1964.

21. **Fatt, P.**, An analysis of the transverse electrical impedance of striated muscle, *Proc. Soc. London Ser. B.*, 159, 606, 1964.
22. **Franklin, J. N.**, Some stereological principles in morphometric cytology, *SIAM J. Appl. Math.*, 33, 2, 246, 1977.
23. **Gould, J.**, *Ever Since Darwin, Reflections in Natural History*, W. W. Norton and Company, New York, 1978.
24. **Hodgkin, A. L. and Huxley, A. F.**, A quantitative description of membrane current and its application to conduction and excitation in nerve, *J. Physiol.*, 117, 500, 1952.
25. **Hodgkin, A. L. and Nakajima, S.**, The effect of diameter on the electrical constants of frog skeletal muscle fibers, *J. Physiol.*, 221, 105, 1972.
26. **Hodgkin, A. L. and Nakajima, S.**, Analysis of the membrane in frog muscle, *J. Physiol. (London)*, 221, 121, 1972.
27. **Jack, J. J. B., Noble, D., and Tsien, R. W.**, *Electric Current Flow in Excitable Cells*, Clarendon Press, Oxford, 1975.
28. **Lanczos, C.**, *Applied Analysis*, Pitman Medical Publishing, London, 1957.
29. **Lewis, S. A., Diamond, J. M., and Eaton, D. C.**, The mechanisms of Na transport by rabbit urinary bladder, *J. Membr. Biol.*, 28, 41, 1976.
30. **Lewis, S. A., Eaton, D. C., Clausen, C., and Diamond, J. M.**, Nystatin as a probe for investigating the electrical properties of a tight epithelium, *J. Gen. Physiol.*, 70, 427, 1977.
31. **Lewis, S. A., Wills, N. K., and Eaton, D. C.**, Basolateral membrane potentials of a tight epithelium ionic diffusion and electrogenic pumps, *J. Membr. Biol.*, 41, 117, 1978.
32. **Mathias, R. T.**, An analysis of the electrical properties of a skeletal muscle fiber containing a helicoidal T-system, *Biophys. J.*, 23, 277, 1978.
33. **Mathias, R. T.**, An analysis of the consequences of electrical continuity between the T-system and sarcoplasmic reticulum, an Appendix to Neville, M. C. The extracellular compartments of frog skeletal muscle, *J. Physiol.*, 1979.
34. **Mathias, R. T., Eisenberg, R. S., and Valdiosera, R.**, Electrical properties of frog skeletal muscle fibers interpreted with a mesh model of the tubular system, *Biophys. J.*, 17, 1, 1977.
35. **Mathias, R. T., Rae, J. L., and Eisenberg, R. S.**, Electrical properties of structural components of the crystalline lens, *Biophys. J.*, 25, 181, 1979.
36. **Mobley, B. A., Leung, J., and Eisenberg, R. S.**, Longitudinal impedance of skinned frog muscle fibers, *J. Gen. Physiol.*, 63, 625, 1974.
37. **Mobley, B. A., Leung, J., and Eisenberg, R. S.**, Longitudinal impedance of single frog muscle fibers, *J. Gen. Physiol.*, 65, 97, 1975.
38. **Peachey, L. D., and Eisenberg, B. R.**, Helicoids in the T-system and striations of frog skeletal muscle fibers seen by high voltage electron microscopy, *Biophys. J.*, 22, 145, 1978.
39. **Peskoff, A.**, Electrical potential in three-dimensional electrically syncytial tissues, *Bull. Math. Biol.*, 41, 163, 1979.
40. **Peskoff, A. and Eisenberg, R. S.**, Interpretation of some microlectrode measurements of electrical properties of cells, *Annu. Rev. Biophys. Bioeng.*, 2, 65, 1973.
41. **Schoenberg, M., Dominguez, G., and Fozzard H. A.**, Effect of diameter on membrane capacity and conductance of sheep cardiac Purkinje fibers, *J. Gen. Physiol.*, 65, 441, 1975.
42. **Schoenberg, M. and Fozzard, H. A.**, The influence of intercellular clefts on the electrical properties of sheep cardiac Purkinje fibers, *Biophys. J.*, 25, 1, 217, 1979.
43. **Schneider, M. F. and Chandler, W. K.**, Effects of membrane potential on the capacitance of skeletal muscle fibers, *J. Gen. Physiol.*, 67, 125, 1976.
44. **Simpson, G. G.**, *The Meaning of Evolution*, Yale University Press, New Haven, 1950.
45. **Solomon, H.**, Geometric Probability, Society for Industrial and Applied Mathematics, Philadelphia, 1978.
46. **Valdiosera, R., Clausen, C., and Eisenberg, R. S.**, Measurement of the impedance of frog skeletal fibers, *Biophys. J.*, 14, 295, 1974.
47. **Valdiosera, R., Clausen, C., and Eisenberg, R. S.**, Impedance of frog skeletal muscle fibers in various solutions, *J. Gen. Physiol.*, 63, 460, 1974.
48. **Vaughan, P., Howell, J., and Eisenberg, R. S.**, The capacitance of skeletal muscle fibers in solutions of low ionic strength, *J. Gen. Physiol.*, 59, 347, 1972.
49. **Weibel, E. R.**, *Stereological Techniques for Electron Microscopic Morphometry Principles and Techniques of Electron Microscopy*, Hayat, M. A., Ed., Van Nostrand Reinhold Company, New York, 1973, 6.
50. **Welch, P. D.**, The Use of Fast Fourier Transform for the Estimation of Power Spectra: A Method Based on Time Averaging Over Short, Modified Periodograms, *IEEE Trans. Audio Electroacoust.*, 335, 1967.

51. **Whitham, G. B.,** *Linear and Nonlinear Waves*, John Wiley & Sons, New York, 1974.
52. **Widder, D. V.,** *The Laplace Transform*, Princeton Mathematical Series, 6, Princeton, N.J., 1946, 391.
53. **Widder, D. V.,** *An Introduction to Transform Theory*, Academic Press, 42, Department of Mathematics, Harvard University, Cambridge, 1971, 242.