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# MEASUREMENT, MODELING, AND ANALYSIS OF THE LINEAR ELECTRICAL PROPERTIES OF CELLS\*

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Most cells and tissues use the flow of electric current to help perform their natural functions. The widespread use of electricity in the function of cells is natural from the physical point of view, since so many of the substances transported in the body are charged and since electrical signals have properties suitable for information-transduction, transfer, and processing. The widespread use of electricity is also natural from an evolutionary point of view. Cells, in most cases, must have a resting potential across their surface membrane if they are to exist at constant volume; the resting potential thus was probably present in the earliest cells in biological history. It seems that evolution is a highly conservative process; that is, if a structure or property is present, it will be modified and used in diverse ways in evolutionary development. Thus, one expects what one finds; most cells and tissues use their resting potential and associated electrical mechanisms to help perform their natural function.

It is interesting to note that electrical processes seem to be used even where they need not be used. Most systems that transport nonelectrolytes appear to involve the obligatory transport of ions as well, even though physically there is no need to transport charge when uncharged solutes are being moved across membranes. One can rationalize this fact by imagining that the active transport of ions was developed early in biological history to help maintain the volume of cells in the presence of small but persistent leakage of "impermeant" ions. Then the mechanism for nonelectrolyte transport developed as a modification of an ion-transport system rather than as an entirely new, electrically silent mechanism.

The study of the mechanisms that underlie the electrical activity of cells is of considerable biological interest. It is sometimes possible to study such mechanisms by the simple observation of natural electrical activity and by observing the change in natural electrical activity produced by ionic or experimental intervention. But it is usually necessary to study such mechanisms by direct electrical intervention; that is, by applying a perturbing current and measuring the responding voltage change. This paper is concerned with the study of the response of cells and tissues to the application of current and illustrates with two examples some of

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the information that can be determined from the measurement of the voltage response to applied current.

Our particular interest here will be the construction and test of electrical models of two preparations: the skeletal muscle fibers of frogs and the lens of the eye. These models predict the voltage response to applied current and include as the components of the model the electrical and morphological properties of the cell and its component structures and organelles. The need for an explicit electrical model may not be apparent but is, in our opinion, a general requirement for the analysis of the electrical properties of any preparation. We therefore will discuss the role of electrical models before we discuss the specific cases just mentioned.

The first role of the electrical model is to enumerate and describe the pathways for current flow. Current applied to a preparation flows in many directions. It leaves the current-passing electrode (in our cases a microelectrode inserted into the cell) and flows in the cytoplasm to a membrane, crosses the membrane into an extracellular space, and then flows in the extracellular solution to a collecting electrode, an indifferent electrode in the external bathing solution. The flow of current in the extracellular space may be rather complicated, since in many preparations some of the extracellular space is a specialized compartment within the cell. The flow of current in the intracellular saline solution is also important. In cylindrical cells like muscle fibers and nerve axons, most of the intracellular current flows longitudinally, the flow of intracellular current in other directions, both away from the microelectrode source and toward the outer membrane, producing significant effects only under extreme conditions. The longitudinal flow of current is significant because a longitudinal pathway of sufficient length (of a length constant, namely millimeters) has a resistance equal to the impedance of a similar length of outer membrane. Thus, the potential drop down the length of a cylindrical cell must be analyzed, as well as the potential drops across the outer membrane. In spherical cells there is no longitudinal pathway, and so the important intracellular potential drops occur in a different manner. As current leaves the microelectrode source in the region near the microelectrode, the great density of the lines of current flow causes significant potential drops. These three-dimensional effects, as they are called, since they involve the flow of current in all directions, are large in large spherical preparations, like the lens of the eye, for example.

Another role of an electrical model is to provide the relationship between the observed electrical properties and the properties of the individual structures and organelles that make up a cell. The measured response to applied current depends on all the pathways for current flow, many of which are not involved in the biological process of interest. Thus, a model is needed to determine the properties of the individual cell components from the observed properties of the whole preparation.

A model should include all the important characteristics of the preparation and should depend explicitly on the properties of the various structures that make up the cell (the size and shape of these structures can be measured by morphometric techniques), and it should depend on the electrical properties of individual membranes and compartments of intra- and extracellular saline solution. It is usually difficult to determine the individual electrical properties of cell components a

*priori*. For example, if one is studying muscle fibers, it is not clear how one should describe the nonlinear properties of the surface and tubular membranes, since (for the most part) measurements are available only of the nonlinear properties of the combined surface membrane and tubular system. If, however, attention is restricted to strictly *linear* properties, it is much easier to specify the electrical properties of individual membranes, since it is widely believed that the linear electrical properties of individual membranes can be represented, to a reasonable approximation, as a resistor and capacitor in parallel. The procedure we take in constructing an electrical model is then to consider only the linear electrical properties of the cell and represent each membrane as a resistor and capacitor in parallel, the unknown value of the resistor and capacitor depending on the unknown specific value of the membrane resistance and capacitance and on the known amount of membrane area. We represent the properties of the cytoplasm and extracellular solutions as resistances, with areas, volumes and shapes determined morphologically.

A quantitative description of the model—an equation—is derived with use of whatever method seems expedient. In the simplest cases a lumped equivalent circuit can be directly written; in other cases, partial differential equations that describe the continuous variation of potential are written (with boundary conditions to describe the membrane); in still other cases, difference equations are written to describe the variation in potential in a discrete number of locations; and finally, it is occasionally necessary to combine all three types of analysis. The equations are then solved; that is to say, an approximate solution is sought that has *physical meaning* and a known mathematical error. The requirement for physical meaning is critically important since the qualitative properties of the mathematical solutions are usually a particularly useful description of the physiological properties of the preparation. Furthermore, the physical meaning of the solution allows speculative extension of the mathematical approximation to cases not strictly within the domain of the analysis. For example, one often guesses that nonlinear effects will be confined to terms in the mathematical solution that depend on membrane properties and will not effect terms that depend only on intra- or extracellular resistances.

By following the procedure just described, it is possible to construct an electrical model of a cell or tissue and be in a position to test the model and interpret the electrical properties of the preparation. In this way a qualitative analysis of mechanism is possible, and indeed a reasonably precise quantitative description can be made.

#### FROG SKELETAL MUSCLE FIBERS

The electrical properties of the muscle fibers of the frog have received a great deal of attention over the years because of the intrinsic interest in skeletal muscle and the important role of electrical properties in the initiation of contraction. Interest is also aroused because skeletal muscle fibers probably represent the least

complicated structure of excitable membranes except for nerve axons.† It has been widely felt that the techniques and forms of analysis necessary to unravel the electrical properties of skeletal muscle would be a guide to the methods necessary to unravel the electrical properties of more complicated tissues of great biological and clinical importance, like smooth muscle, cardiac muscle, and the various epithelia.

The overall model of the electrical properties of frog muscle utilizes one-dimensional cable theory (see Jack *et al.*<sup>18</sup> for an excellent review) to describe the longitudinal spread of current and three-dimensional cable theory (see Peskoff *et al.*<sup>29</sup> for the most recent work and Eisenberg & Johnson<sup>10</sup> for an early review) to describe the flow of current near the microelectrode source (important at short times or high frequencies). One-dimensional cable theory includes the longitudinal and shunt (i.e., transverse) pathways for current flow. Since the longitudinal pathways are essentially resistive,<sup>22,23</sup> we confine our attention here to the shunt pathways, the pathways by which current can leave the sarcoplasm in a single sarcomere. We are mostly interested in modeling the linear properties and so will not discuss the recent work on asymmetrical capacity currents. We must emphasize, however, the importance of that work in the understanding of the nonlinear processes in skeletal muscle (Chandler *et al.*<sup>5</sup> describe and analyze the asymmetry currents and give references to other work on the subject).

There are three systems of membranes that one might suspect would contribute importantly to the electrical properties of a sarcomere of skeletal muscle: the surface membrane, the tubular system, and the sarcoplasmic reticulum. The surface membrane itself comprises a small part of the total membrane within frog muscle fibers (some 1–2% for a fiber of typical diameter) but obviously is the location of substantial current flows. The surface membrane, however, is not a simple structure, since it has infoldings called caveolae<sup>8</sup> whose function is not known. Indeed, it is not clear that all the caveolae behave electrically as part of the surface membrane. If many caveolae serve as openings of the tubular system,<sup>35</sup> they would modify the current flowing into the tubular system and so would act more as extensions of the *t*-system than as components of the surface membrane. Unfortunately, until the anatomical relationship of the opening of the tubular system and the caveolae is settled, and until the function of the caveolae is known, it will be difficult to evaluate their contribution to the overall electrical properties of the muscle fiber. At present we model the surface membrane as a simple resistance and capacitance in parallel, assuming that the only effect of the caveolae is to modify the amount of membrane that produces these electrical properties.

The electrical properties conferred on the fiber by the sarcoplasmic reticulum are customarily ignored in models, since it is widely believed that no current can flow across the tubular membrane into this compartment. Certainly there is no

†The structure of the excitable membranes of skeletal muscle is not all that simple, of course.<sup>27,21</sup> But the membranes are highly organized in a reasonably periodic arrangement with characteristics suitable for observation in the electron microscope (except at the openings of the *t*-system to the extracellular space). Thus, the complications of the structure are rather well known; indeed, the qualitative and quantitative organization of muscle membranes is known at least as well as that of the much simpler, but less studied axon membranes!

convincing evidence of substantial current flow in that pathway, and the structure of the junction between the sarcoplasmic reticulum and the tubular membrane (reviewed in Franzini-Armstrong<sup>13</sup>) is not reminiscent of junctions that do electrically couple adjoining cells. We follow the customary procedure and ignore the contribution of the sarcoplasmic reticulum to the overall electrical properties of the fiber, but we are painfully aware that some forms of coupling between the sarcoplasmic reticulum and the tubular system might influence the electrical properties of the fiber in a sufficiently subtle manner to have avoided detection but in a sufficiently substantial manner to have important effects.

The main determinant of the linear electrical properties of skeletal muscle fibers is the tubular system, since the great majority of the membrane which delimits the cell from the bathing solution is in the *t*-system. The tubular system of muscle fibers is a branching network of tiny tubules that arise as invaginations of the surface membrane and trap a component of extracellular space within the fiber. The tubules contain solution that appears to exchange readily with the extracellular bathing solution (see reviews<sup>7,24,9</sup>). Because the cross section of the tubules is small, and yet they have considerable length and membrane surface, there can be significant radial potential gradients in the lumen of the tubules. It is the description of this potential drop—and concomitantly of the entire pattern of current flow and potential distribution within the *t*-system—that we will analyze here.

The first description of the electrical properties of the *t*-system<sup>12</sup> represented the tubular network either as lumped elements—a resistance in series with the tubular membrane—or as a distributed network, a pair of discs of membrane enclosing a small extracellular space. The former description seemed sufficient to fit the experimental results, but the data did not rule out the disc or distributed representation, and a foreshortened theoretical analysis of the disc model was presented. A variety of subsequent experimental results<sup>15,3,31,16,17,4,33,34</sup> showed, however, that there were significant radial potential drops within the *t*-system and ruled out a lumped representation.

The central problem in modeling the *t*-system is then the correct representation of the branching nature of the network and radial potential drop produced by the network. This problem is sufficiently difficult that some<sup>14,26</sup> have chosen preparations with unbranched tubules, and it is probably not coincidental that the clearest results come from such preparations. Others<sup>12</sup> have ignored the branching or have analyzed the effect of branching in a few special networks with morphological properties distinct from those of frog muscle.<sup>2,31</sup> We will discuss here models that seek to include explicitly the structure of the *t*-system as reported by morphologists.<sup>27,21</sup>

Mathias<sup>19</sup> numerically constructed explicit exact solutions for two-dimensional branching networks of tubules of various structures. These exact solutions (and unpublished solutions for a hexagonal *t*-system in a hexagonal fiber; Mathias, personal communication) and the methods of deriving and computing them are keystones to future analysis, since they are the only results that one can be certain are correct, at least in a mathematical sense. All other analyses contain some form of irrational approximations: that is, approximations that do not permit complete computation of their own mathematical error. However, the exact solutions are so cumbersome as to preclude qualitative understanding. Furthermore, they would

be difficult to compute and fit to a substantial amount of experimental data. The fundamental reason that the exact solutions are cumbersome is that they use a two-dimensional description of the structure of the *t*-system; they describe each node of the tubular system with two spatial coordinates. For that reason, Mathias *et al.*<sup>20</sup> constructed a model of the *t*-system that requires only one spatial coordinate to describe each node. They classified the nodes of the *t*-system into concentric shells by a geometric construction that would tend to minimize the circumferential variation of nodal potential within any shell. Mathias *et al.* then presented a rather complex statistical derivation of a finite difference-differential equation to describe the average potential in each shell. When the shell width is small compared to the tubular length constant, the difference equation reduces to a differential equation that is of the same form as the disk model and could be easily and exactly solved. Also, it is fortunate that a quite simple and precise approximation to the solution of the difference equation for the admittance of the *t*-system could be found, this solution being valid over the entire range of length constants. Nonetheless, the complexity of their analysis is rather forbidding.

Here we shall present a physical analysis of the structural model of Mathias *et al.* We do not seek as complete an approximation as they have presented, but rather derive and solve an equation that is quite accurate within most of the physiological range, to something like 1000 Hz under normal conditions. The reader is referred to the original paper<sup>20</sup> for the more precise result and for a discussion of the applicability of the present more limited approximation.

Consider a random or deterministic network of tubules, each tubular branch being of length  $L$ , having a resistance  $R_b$  between nodes due to the lumen of one branch, having  $N_B$  branches per node (note that the number of *distinct* branches per node is  $N_B/2$ , since each branch spans two nodes), with circumferential spacing  $\delta_N$  between nodes, and with a total length of tubule per unit cross-sectional area of fiber of  $L_T/A_F$ . Such a network enclosed in a finite boundary can usually be decomposed into shells within which tubular branches can be defined as radial (that is, crossing a boundary of a shell) or circumferential (lying entirely within a shell). The spacing between *radial* branches is called  $\delta_B$ . The potential drop  $\Delta U$  in a shell of thickness  $\Delta r$  when there is radial current  $i_r$  flowing in the lumen of the tubules is

$$\Delta U = \frac{(\text{radial current}) \times (\text{resistance of one tubular branch})}{(\text{no. of radial tubular branches})} \quad (1)$$

$$\Delta U = \frac{i_r R_b}{2\pi r / \delta_B} \quad (2)$$

$$\frac{\Delta U}{\Delta r} = \frac{i_r R_b}{(2\pi r / \delta_B) \cdot \Delta r} \rightarrow \frac{dU}{dr} \text{ as } \Delta r \rightarrow 0. \quad (3)$$

The effective radial resistance is therefore  $R_b(\delta_B/\Delta r)$ , so that the factor  $\delta_B/\Delta r$  is a network parameter that depends on the manner in which the nodes of the network are connected and also on the degree of tautness in the network. For example, a network with a small spacing between radial branches will have--*all other morphometric parameters being constant*--a lower value of effective radial resis-

tance than a network with a larger spacing between radial branches, since the former network will have more parallel pathways in which radial current can flow. On the other hand, a network with a small value of  $\Delta r$  will have a larger effective radial resistance than one with a larger value—all other morphometric parameters being constant—because the total amount of tubular system in the two networks is the same, but in the former network the radial tubules that connect shells must twist and bow. Thus, the effective path length and radial resistance is greater in the network with the smaller shell thickness, all other things being equal.

In general, then, it is necessary only to determine the shell thickness from the measured morphometric parameters of the network. This can be done by equating two expressions for the total number of tubules (both radial and circumferential) in a shell

$$\begin{aligned} (\text{no. of tubules in shell}) &= (\text{no. of tubules of length } L \text{ per unit area}) \\ &\quad \times (\text{area of one shell}) \end{aligned} \quad (4)$$

$$\begin{aligned} (\text{no. of tubules in shell}) &= (\text{no. of distinct tubules per node}) \\ &\quad \times (\text{no. of nodes per shell}) \end{aligned} \quad (5)$$

$$[(L_T/A_F)(1/L)][2\pi r\Delta r] = (N_B/2)(2\pi r/\delta_N) \quad (6)$$

or

$$\Delta r = L(N_B/2)/(\delta_N L_T/A_F). \quad (7)$$

In a general expression it is preferable to write  $R_b$  in terms of resistivity of the lumen of the tubules  $R_L$  and the morphometric parameters of the network.‡ Then,

$$R_b = LR_L/A_T = LR_L \frac{L_T/A_F}{S_T/A_F} \frac{S_T}{V_F} \frac{V_F}{V_T}. \quad (8)$$

It should be noted that the expression is more useful than it is awkward, since all the morphological parameters on the right-hand side have been experimentally measured.

To complete the derivation we write Kirchoff's Current Law

$$\frac{di_r}{dr} \simeq I_w \cdot 2\pi r = U(G_w + j\omega C_w)(S_T/A_F)(2\pi r) \quad (9)$$

where  $I_w$  is the density of current crossing the tubular wall,  $G_w$  and  $C_w$  are the specific conductance and capacitance of the tubular wall, and  $S_T/A_F$  is the surface area of tubular wall per cross-sectional area of fiber.

Equations 3 and 9—using (7) and (8) if desired—can be combined to give a differential equation for the potential within the tubular system. This resulting equation is of the same form (but with different parameter values) as that used by virtually all workers in the field.<sup>2,31,16,17,25,32,6</sup>

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be difficult to compute and fit to a substantial amount of experimental data. The fundamental reason that the exact solutions are cumbersome is that they use a two-dimensional description of the structure of the *t*-system: they describe each node of the tubular system with two spatial coordinates. For that reason, Mathias *et al.*<sup>20</sup> constructed a model of the *t*-system that requires only one spatial coordinate to describe each node. They classified the nodes of the *t*-system into concentric shells by a geometric construction that would tend to minimize the circumferential variation of nodal potential within any shell. Mathias *et al.* then presented a rather complex statistical derivation of a finite difference-differential equation to describe the average potential in each shell. When the shell width is small compared to the tubular length constant, the difference equation reduces to a differential equation that is of the same form as the disk model and could be easily and exactly solved. Also, it is fortunate that a quite simple and precise approximation to the solution of the difference equation for the admittance of the *t*-system could be found, this solution being valid over the entire range of length constants. Nonetheless, the complexity of their analysis is rather forbidding.

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tance than a network with a larger spacing between radial branches, since the former network will have more parallel pathways in which radial current can flow. On the other hand, a network with a small value of  $\Delta r$  will have a larger effective radial resistance than one with a larger value—*all other morphometric parameters being constant*—because the total amount of tubular system in the two networks is the same, but in the former network the radial tubules that connect shells must twist and bow. Thus, the effective path length and radial resistance is greater in the network with the smaller shell thickness, all other things being equal.

In general, then, it is necessary only to determine the shell thickness from the measured morphometric parameters of the network. This can be done by equating two expressions for the total number of tubules (both radial and circumferential) in a shell

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$$\frac{d^2U}{dr^2} + \frac{1}{r} \frac{dU}{dr} - \Gamma_m^2 U = 0. \quad (10)$$

The propagation constant of the tubular system  $\Gamma_m$  is a generalization of the reciprocal of the usual tubular length constant, more easily applied to sinusoidal and transient problems. The propagation constant of the tubular mesh is related to the propagation constant of a single tubule  $\Gamma$

$$\Gamma_m = \left( \frac{\delta_B}{\delta_N} \frac{N_B}{2} \right)^{1/2} \Gamma(L/\Delta r) \quad (11)$$

and the tubular propagation constant can be written in terms of the specific properties of the tubular wall and lumen

$$\Gamma^2 = R_L(S_T/V_T)(G_w + j\omega C_w). \quad (12)$$

It is also useful to write (from (3) and (9)) the propagation constant of the tubular network in terms of the properties of an effective radial resistivity of the lumen and the effective wall conductivity and capacitance per unit volume of fiber:

$$\Gamma_m^2 = \left( R_b \sigma \frac{\delta_B}{\Delta r} \right) \left( \frac{S_T}{V_F} [G_w + j\omega C_w] \right)$$

$\Gamma_m^2 =$  (effective radial resistivity in units of ohm-cm)

$$\times (\text{wall admittance per unit volume of fiber in mho/cm}^3) \quad (13)$$

where  $\sigma$  is the sarcomere spacing and  $\sigma S_T/V_F = S_T/A_F$ .

The previous differential equation (10) can be trivially solved and has been applied to a wide range of situations involving the tubular system. Indeed, it serves as an adequate electrical model of the tubular system. The substantive difference between this model and earlier results is the explicit dependence of the propagation factor on the measured morphometric parameters of the tubular system. No tortuosity factors computed from deterministic models of the *t*-system appear in our result.

The question then arises, how is the model checked? What experimental interventions can be made that can be compared with the predictions of the model? First, one can determine if the model fits the impedance data measured from muscle fibers. Second, one can determine if the model describes the variation of impedance with the diameter of the fiber. Third, one can determine if the values of the parameters of the model agree with independent evidence. Fourth, one can determine whether a nonlinear extension of the model allows prediction of the shape and conduction velocity of the action potential. Fifth, one can determine whether the parameter values of the model change as they should with the change in length of the fiber. Sixth, one can determine whether the parameter values of the fiber change as they should with changes in the resistivity of the external bathing solution.

In this context it is important to point out that one essential check has already been performed. Exact solutions of networks of branching tubules similar to the *t*-system have been simulated with digital computers, and the present model has been shown to fit the properties of the exact solutions closely. The experimental

checks are not really necessary to determine whether the present model describes a branching network of tubules; rather, the experimental checks are important to see if the assumptions from which the model is constructed are reasonably correct: to see if the circuit elements in the model are reasonably apt descriptions of the structural components of muscle, to see if the division into longitudinal and shunt pathways is correct, even in the presence of a spiral *t*-system,<sup>28</sup> and, most importantly, to check if the model is reasonably complete (to check if any significant pathways of current flow have been omitted).

We shall refrain from elaborate discussion of each of the experimental checks, since the data is incomplete in most cases. Mathias *et al.*<sup>20</sup> show that the model well describes experimental data taken in a variety of conditions in which the membrane is reasonably linear. The values of the components of the model and the variation of the values, however, often do not agree with preconceptions. Some of the problem is undoubtedly caused by experimental error, particularly in the morphometric parameters which are subject to change by fixation and other preparatory procedures. However, these problems may also derive from some incompleteness of the model, the result of some structure or process not presently included. To cite a few obvious possibilities, it may be important to include explicitly the properties of the sarcoplasmic reticulum, the junction between the sarcoplasmic reticulum and the tubular system, the caveolae, the openings of the *t*-system, the asymmetrical capacity currents, and perhaps even the longitudinal components of the *t*-system.

As our knowledge of the structure of muscle increases, and as exciting new techniques such as the optical recording of membrane potentials become available, we anticipate that a much wider range of precise experimental data will become available to describe the electrical behavior and properties of muscle. We hope that theoretical models will develop hand-in-hand with the experimental developments so that the electrical behavior and properties can be properly analyzed. Perhaps in that way all the components of current flow important in the processes of excitation-contraction coupling can eventually be both measured and analyzed.

#### CRYSTALLINE LENS OF THE EYE

The lens of the eye is an interesting organ because of its clinical and biological properties: clinically it is the source of many impairments of vision, cataracts unfortunately being quite a common occurrence. Biologically, the question of how the lens is able to perform the normal metabolic maintenance functions of any tissue and still preserve its transparency is of considerable interest. The electrical properties of the lens are of interest for at least two reasons: first, the electrical coupling between cells may well be importantly involved in the processes that maintain the transparency of the lens; second, the transport processes in the surface of the lens, which clearly are important to the metabolism of the tissue, involve the flow of current. An extensive critical review of the electrical properties of the lens develops these themes in some detail (Rae<sup>30</sup>) and should be consulted.

The models of the electrical properties of the lens are much less complete than those of skeletal muscle primarily for historical reasons; the preparation has been studied by only a few workers for a rather short time. Thus, the role of the model

presented here, approximating the lens as a giant spherical cell, is analogous to the role of cable theory in the historical development of an electrical model of muscle fibers; it allows the gross analysis of the voltage response to current flow into a membrane component and an internal component. The model ignores the complex structure of the extracellular space within the lens, however, and it also ignores the specialized layer of cuboidal epithelial cells on the anterior side of the lens. Surely, the model will need to be extended, and revised, to deal with these important structural complexities. But just as in skeletal muscle, the original oversimplified model of one-dimensional cable theory has served as the foundation for later complexities, so in the lens of the eye the spherical cell model may still be of use as newer, more precise models are developed.

We crudely represent the entire lens as a spherical cell, or more precisely, as a finite cell, one with dimensions of the same approximate size in all directions. Eisenberg and Rae<sup>11</sup> have applied the three-dimensional theory of a number of authors (see Ref. 11 for references that describe the assumptions and analysis of the theory) to the lens and have shown that the overall potential recorded by a microelectrode in response to a step of current is given by

$$V = \frac{IR_m}{4\pi a^2} (1 - e^{-t/R_m C_m}) + I \cdot F(R_i, a, r_1, R_1, \theta) + I \cdot G(R_B, a) \quad (14)$$

where

$$\begin{aligned} F(\dots) = & \left( \frac{R_i}{4\pi a} \frac{r_1^2 + R_1^2}{a^2} - \frac{2r_1 R_1}{a^2} \cos \theta \right)^{-1/2} \\ & + \frac{R_i}{4\pi a} \left\{ \left( 1 + \frac{r_1^2 R_1^2}{a^4} - \frac{2r_1 R_1}{a^2} \cos \theta \right)^{-1/2} - 2 + \ln 2 \right\} \\ & - \frac{R_i}{4\pi a} \ln \left\{ 1 - \frac{r_1 R_1}{a^2} \cos \theta + \left( 1 + \frac{r_1^2 R_1^2}{a^4} - \frac{2r_1 R_1}{a^2} \cos \theta \right)^{1/2} \right\} \end{aligned} \quad (15)$$

$$G(\dots) = \frac{R_B}{4\pi a} \quad (16)$$

and where  $V$  is the potential measured,  $I$  the current applied,  $a$  the radius of the lens,  $t$  the time after onset of current,  $C_m$  (in  $F/cm^2$ ) the "specific" membrane capacitance,  $R_m$  (in  $\text{ohm}\cdot\text{cm}^2$ ) the "specific membrane" resistance,  $R_i$  ( $\text{ohm}\cdot\text{cm}$ ) the "resistivity" of the lens interior,  $R_1$  the radial location of the current electrode measured from the center of the lens (as is  $r_1$  the radial location of the voltage electrode),  $\theta$  the angular separation of the electrode tips, and  $R_B$  ( $\text{ohm}\cdot\text{cm}$ ) the resistivity of the extracellular bathing solution.

The important characteristic of this equation is the separation of the response into three components: one, which depends on membrane properties, is independent of spatial location and has a slow time course governed by the time constant  $R_m C_m$ ; the second, which depends on internal resistance, is independent of membrane properties, is highly localized, and has so fast a time course that it appears to be established instantaneously; the third is a component that arises in the extracellular bathing solution and is small and of little interest.

Experimental results show the qualitative behavior predicted by the equation: the potential can be separated into two components either by analyzing the variation of potential with position of the microelectrode or by analyzing the time course of the potential. The components then have the properties predicted by the model; namely, the spatially uniform component of potential is established slowly, while the local component of potential is established essentially instantaneously.

The structural complexity of the lens, not included in this model, precludes too much further analysis. Although the local potential can be analyzed to give a figure for the effective internal resistivity of the lens, the figure is undoubtedly a composite of the properties of the coupling between cells, the properties of the cytoplasm, and perhaps the properties of the trapped extracellular space within the lens. It is better to have such a composite figure than none at all, but it would be better yet to have a more complete theory to allow the separation of the effective internal resistance into its components. Analysis of the slow, spatially uniform component of potential is even more complicated: experimentally, it is found to have a time course quite distinct from that described by the model (Equations 14-16), and the parameter values necessary to crudely fit the model to the data are wildly different from the properties of an isolated membrane. Pursuing the analogy with frog muscle, we suspect that the cause of the discrepancy is the failure of the model to include the properties of the trapped extracellular space within the lens. The membranes lining this internal extracellular space would certainly be expected to contribute to the slow component of potential, to modify its time course, and to modify enormously the apparent parameter values. Thus, we look forward to the development of a model that will explicitly include current flow in these trapped extracellular spaces, and that will deal perhaps with the epithelial coating of cells on the anterior surface. Such a model might even help resolve the essential paradox of the electrical properties of the lens—its extremely long time constant.

It seems then that at least in the case of these two preparations—the lens of the eye and skeletal muscle fibers of the frog—the construction of linear electrical models has fulfilled many of the criteria we described earlier. The models are necessarily incomplete, both for theoretical and experimental reasons, but the general success and utility of the approach in the understanding of the electrical function of the tissues seem clear. Perhaps similar success can be expected in the application of this approach to other preparations.

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