EFFECT OF TORTUOUS EXTRACELLULAR PATHWAYS ON RESISTANCE MEASUREMENTS

RICHARD T. MATHIAS
Department of Physiology, Rush Medical College, Chicago, Illinois 60612

ABSTRACT There are many instances in which we are limited to measuring macroscopic quantities such as a bulk flow or an average field. In biology, we are frequently interested in using such macroscopic measurements, for example, the total current from a tissue, to determine the microscopic properties of the cells or tubules of the tissue. The microstructure of the tissue will generally increase the resistance to flow over what would be measured in an unstructured medium. This paper derives a fairly general expression for the relationship between effective resistance to macroscopic flow and the specific resistance of the medium conducting the microscopic flow. This expression, called a tortuosity factor, is defined entirely in terms of measurable morphometric and geometric parameters of the tissue.

INTRODUCTION

Tortuosity factors arise when a flow is channeled by the microstructure of the medium through which it occurs. The channeling may be through clefts or through a lattice or by pores. In the physiological context, tortuosity factors have been applied to the flow of solutes in three instances: (*a*) ionic current flow through the T-tubules of a skeletal muscle fiber (Adrian et al., 1969; Schneider, 1970; Mathias et al., 1977); (*b*) ionic current flow through the intercellular clefts of electrically syncytical tissues such as cardiac muscle or the crystalline lens (Eisenberg et al., 1979; Mathias et al., 1979, 1981); and (*c*) solute diffusion through the extracellular spaces of the brain or cardiac tissue (Nicholson and Phillips, 1981; Lammel, 1981; R. A. Levis, et al., 1983). These are but a few examples taken from a limited context.

Tortuosity factors are generally recognized in branches of applied mathematics that involve flows (see Babuska [1976] for numerous references), and they often are implicity included in the description of resistive properties of a medium (e.g., Maxwell, 1891). The approach taken by applied mathematicians (called homogenization [Babuska, 1976]) is essentially to first develop microscopic field equations and then volume average the microscopic field in order to define a macroscopic field. The approach taken here is more intuitive, in that an average structure, called the unit cell, must be defined, and it is not clear exactly what is being averaged. Nonetheless, if one can define a unit cell, then the macroscopic field can be explicitly related to the structural morphometric parameters of the medium, and such an explicit relationship is not provided by homogenization.

Because the need for defining a tortuosity factor will surely recur again and again, whenever one considers ionic current flow, diffusion of solutes, or water flow in the tubes, or clefts of biological tissues, it seems worthwhile to have a fairly general derivation. This paper begins such a derivation based on the assumption that one can define a unit cell of the structure conducting the flow.

Unit Cell

The unit cell is the smallest piece of a medium that possesses all of the morphometric properties of the gross structure. In the biological context we are fortunate because all living tissue is comprised of cells. Thus, if we want to determine a tortuosity factor for the extracellular clefts between the cells of a syncytial tissue, we can assume the unit cell of the tissue is a typical biological cell from which the tissue is constructed.

If, instead of a tissue, we consider subcellular flow through branching tubes, such as a dendritic tree or the T-system of a skeletal muscle fiber, then one may identify a unit cell by the node-to-node spacing of the network. Because the network can be built from small volume elements, each of which encloses one node, the typical such volume element will be considered the unit cell.

The definition of the word typical in the two above mentioned situations is more intuitive than rigorous. In the case of a syncytial tissue, we fundamentally wish to analyze a small cuboid of tissue which has the average volume of a cell, the average surface area of membrane surrounding a cell, and the average amount of wiggling in the clefts between cells. For convenience, we would like the unit cell to have as much symmetry as possible, hence we place the node of intersection of clefts at the center. However, because the average biological cell is rarely a cube, we must allow the size in each spatial coordinate to differ. Moreover, in order to mathematically analyze a structure, one must select a coordinate system, and this typical cell must be fit into the appropriate volume element

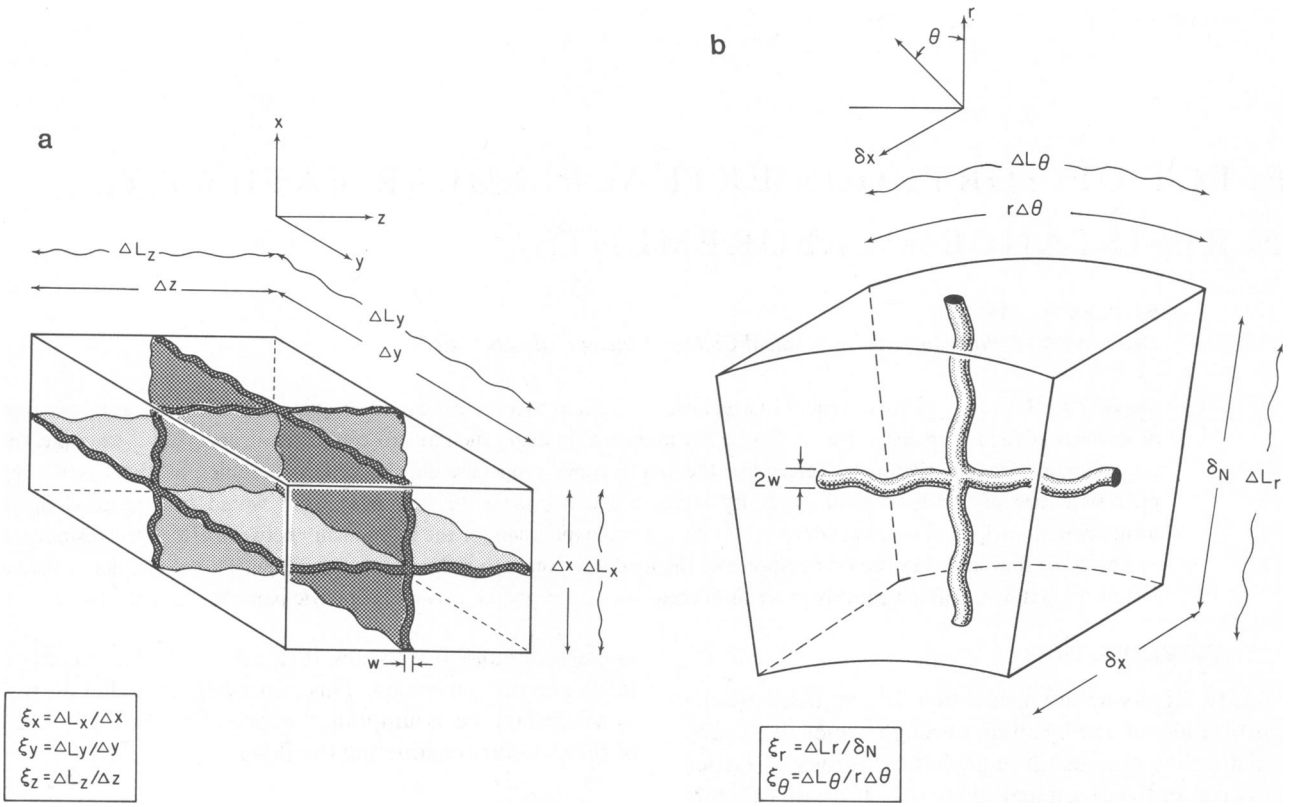

FIGURE 1 Two fanciful sketches of unit cells. (*a*) A unit cell with the dimensions of a typical biological cell from the tissue, but constructed so the intersection of four biological cells falls at its center. The extracellular clefts between cells are stippled. (*b*) A unit cell constructed about one node of a branching, two-dimensional network of tubules. In this instance, the node-to-node spacing of the network defines the dimensions of the unit cell.

for the coordinate system of choice. For example, in Fig. 1 *a* a Cartesian coordinate system is chosen, so the appropriate volume element is rectangular.

If we consider subcellular flow through the branching tubules of a cardiac or skeletal muscle fiber, then the unit cell is an imaginary subcellular cuboid that encloses one node of the branching network of tubules. The dimensions of the unit cell are equal to the average node-to-node spacing of the tubular network, and the unit cell encloses a typical node. A typical node is again one whose properties are determined by the average properties of all the nodes within the fiber. Thus, the node should have the average number of branches and the average amount of wiggling in each branch. Moreover, the cuboid should contain the average surface of membrane in a unit volume of fiber and the average volume of tubular lumen in a unit volume of fiber. The unit cell pictured in Fig. 1 *b* was presumed to come from a cylindrical fiber, so it has the appropriate shape to pack into a cylinder.

Although there are probably some classes of networks that cannot be packed into a unit cell and analyzed by this method, we are aware of no rigorous mathematical work on this question. Mathias et al. (1977) analyze several deterministic networks, clearly of the class that allows the analysis presented here. Because there are infinite possibilities, we simply leave the construction of the unit cell as a plausible precondition for the analysis that follows.

Once the unit cell is constructed, one must quantitatively describe the nodes defined by the intersection of tubes, or the lines defined by the intersection of clefts. An hexagonal array will have three branches from each intersection; in a rectangular array there will be four such branches. In general a tissue contains a mixture of intersections, some with three branches, some with four or more. Thus, the average or typical intersection will have some noninteger number of branches and therefore cannot be visualized or sketched on a piece of paper. The sketches in Fig. 1 show four branches at each intersection, but this is an idealization. Each illustrated branch exists with a probability, given by $N_B/4$, when branches are randomly and uniformly oriented, where N_B is computed from the entire tissue and represents the average number of branches at an intersection. In the computation of extracellular volume or membrane surface area, the properties of each branch will be multiplied by the probability of the branch (usually $N_B/4$), so that morphometric parameters for the illustrated unit cell will match those parameters for the tissue from which it came.

DERIVATION

Many problems in classical physics involve the relationship between a potential energy field and the flow produced by such a field. The general form of such a relationship is

$$\mathbf{J} = -\sigma \nabla \Psi, \tag{1}$$

where the vector $\mathbf{J}$ is the flux density of the flow, Ψ is the potential energy function, ∇ is the gradient operator (Jackson, 1975), and σ is the proportionality constant often referred to as a conductivity. If the flow, $\mathbf{J}$, is channeled by the microstructure of the medium, the value of the proportionality constant, σ, must be modified to include geometric as well as specific properties. We assume the specific properties are known from bulk measurements in an unstructured medium, whereas the effect of structure requires a theory.

Consider the potential energy drop along the intercellular clefts, shown in Fig. 1 *a*, oriented in the x direction. If σ is the specific conductivity, then we can write an approximate relationship between the potential at the nodes of two adjacent cells, $\Psi[x + (\Delta x/2)]$, $\Psi[x - (\Delta x/2)]$ and the flow along the cleft $J_x(x)$, which holds for small Δx, namely:

$$\left[\Psi\left(x - \frac{\Delta x}{2}\right) - \Psi\left(x + \frac{\Delta x}{2}\right)\right]\frac{S_c}{S_x}\frac{\sigma}{\Delta L_x} \approx J_x(x).$$

The dimensions of the unit cell are assumed to be sufficiently small so the potential function, Ψ, varies approximately as a linear function of position over changes in position of the size Δx, Δy, or Δz. Furthermore, the distance, W, illustrated in Figs. 1 *a* and *b* is assumed to be small compared with the dimension of the unit cell, so that flow in clefts is approximately two-dimensional, or flow along tubules is approximately one-dimensional.

Then for clefts

$$J_x \approx -\frac{\partial \Psi}{\partial x}\frac{S_c}{S_x}\frac{\sigma}{\xi_x}, \tag{2}$$

where S_c is the surface area of extracellular clefts cut by the x-face of the unit cell, S_x is the total surface area ($\Delta y\,\Delta z$), and ξ_x is the wiggle factor that allows the x-oriented length of cleft ΔL_x to be longer than the x-oriented unit cell dimension Δx (see Mobley and Page, 1972, or Hellam and Studt, 1974 for typical values of ξ_x in heart muscle). Note that the units of J_x are flux/(cm^2 of total surface area) so we have converted a microscopic tortuous flow into a macroscopic tissue flux density.

Eq. 2 includes both the specific and geometric properties of the new effective proportionality constant for x-directed flow;

$$\sigma_x \equiv \frac{S_c}{S_x}\frac{\sigma}{\xi_x}. \tag{3}$$

However, the term S_c/S_x should not be mistakenly construed as a real morphometric parameter. It exists only in the abstract sense the unit cell exists, and it must therefore be defined in terms of measureable morphometric parameters. Stereology will provide estimates of S_m/V_T (in cm^{-1}), the surface area of membrane in a unit volume of tissue, and V_e/V_T, the fraction of extracellular or cleft volume in a unit volume of tissue (Weibel, 1972; Mobley and Eisenberg, 1975; Eisenberg and Cohen, 1983). If the dimensions of the trapped extracellular compartment are uniform, one expects the effective conductivity will be reduced from the actual conductivity by a factor at least as small as the volume fraction through which the flow occurs. Thus, we wish to derive an alternate expression for Eq. 3, one which is closely related to the experimentally measurable parameter V_e/V_T, namely

$$\frac{1}{\xi_x}\frac{S_c}{S_x} = \tau_x \frac{V_e}{V_T} \tag{4}$$

and Eq. 4 defines the tortuosity factor for x-directed flow, τ_x. From consideration of Fig. 1 *a*, if we assume uniformly distributed branches (i.e., an equal number intersect each face of the cuboid), we may write

$$\frac{S_c}{S_x} = \frac{W\Delta L_y\, {}^1\!/_4\, N_B + W\Delta L_z\, {}^1\!/_4\, N_B}{\Delta y\,\Delta z}, \tag{5}$$

where W is the width of a cleft. Similarly,

$$\frac{V_e}{V_T} = (W\Delta L_y\,\Delta L_x\, {}^1\!/_4\, N_B + W\Delta L_z\,\Delta L_y\, {}^1\!/_4\, N_B + W\Delta L_y\,\Delta L_z\, {}^1\!/_4\, N_B)/\Delta x\,\Delta y\,\Delta z. \tag{6}$$

Substituting Eqs. 5 and 6 into Eq. 4 gives the expression for τ_x

$$\tau_x = \frac{(\xi_y\,\Delta y + \xi_z\,\Delta z)\,\Delta x}{(\xi_x\,\xi_y\,\Delta x\,\Delta y + \xi_x\,\xi_z\,\Delta x\,\Delta z + \xi_y\,\xi_z\,\Delta y\,\Delta z)\,\xi_x}. \tag{7}$$

If the tissue is isotropic so that $\xi_x = \xi_y = \xi_z = \xi_c$ and $\Delta x = \Delta y = \Delta z$, then Eq. 7 reduces to a simple result

$$\tau_{ic} = \frac{2}{3}\frac{1}{\xi_c^2} \quad \text{(isotropic clefts)}. \tag{8}$$

The appearance of the factor $^2\!/_3$ can be physically interpreted if Eq. 7 is written in a slightly different form

$$\tau_x = \frac{S_c\,\Delta L_x}{V_e}\frac{1}{\xi_x^2}.$$

The numerator in the above equation is the volume of extracellular cleft for x-oriented flow, which includes just two of the clefts pictured in Fig. 1 *a*, whereas V_e is the total volume of extracellular cleft, which includes all three of the clefts pictured. If the clefts are isotropic, then the ratio $S_c\Delta L_x/V_e$ reduces to the geometrically determined value of $^2\!/_3$. τ_{ic} may therefore be separated into a geometric factor divided by a wiggle factor squared.

Consider next the two-dimensional network (two-dimensional in the sense W is small and flow in any one tubule is one-dimensional) of tubules embedded in the unit cell of Fig. 1 *b*. The surface of tubule lumen cut by the r face of the unit cell is

$$\frac{S_c}{S_r} = \frac{\pi W^2 \tfrac{1}{4} N_B}{\delta_x r \Delta\theta} \tag{9}$$

and

$$\frac{V_c}{V_F} = \frac{\tfrac{1}{4} N_B \pi W^2 (\Delta L_r + \Delta L_\theta)}{r \Delta\theta \delta_x \delta_n}, \tag{10}$$

where V_c/V_F is the volume of tubules contained within a unit volume of fiber. In this instance, we have assumed the probability of an x-directed branch is zero and that r and θ branches are equally probable. By analogy with Eq. 4 we can write

$$\tau_r = \frac{\delta_n}{(\delta_n \xi_r + r \Delta\theta \xi_\theta) \xi_r} \quad \text{(two-dimensional tubules)}, \tag{11}$$

and for isotropic tubules in two dimensions

$$\tau_{2T} = 1/(2 \xi_T^2) \quad \text{(two-dimensional isotropic tubules)}. \tag{12}$$

The geometric factor is now ½ because each tubule directs the flow into one dimension, whereas the total flow is assumed to be two-dimensional. If we allow the tubules to branch in the δ_x direction as well as r and θ directions, then the number of possible directions for flow becomes three, and for an isotropic three-dimensional network of tubules only one-third of the tubules conduct current in any one direction, thus

$$\tau_{3T} = 1/(3 \xi_T^2) \quad \text{(three-dimensional isotropic tubules)}. \tag{13}$$

Another tissue geometry of general physiological interest is elongated cells in a synctical tissue. This situation is representative of the fiberlike cells of the crystalline lens or the elongated cardiac cells in a Purkinje fiber. Elongated cells may be approximately modeled by assigning the y-coordinate as the axis of the fiber and then allowing $\Delta y \rightarrow \infty$ in Eq. 7. However, this assumption implies the unit cell is not differentially small. Alternatively, one can assign the probability of an x-z branch to be zero, whereupon there is no structural determinant of Δy and it can remain a small, arbitrary differential distance for the derivation of differential equations. Either formulation yields Eq. 14,

$$\tau_c = \frac{\Delta x}{(\xi_x \Delta x + \xi_z \Delta z) \xi_x} \quad \text{(elongated cell clefts)}. \tag{14}$$

If the cells are isotropic in the x- and z-coordinates (equivalent to r- and θ-coordinates in a cylinder), then Eq. 14 reduces to $1/(2\xi_c^2)$.

One final geometry of interest is a single layer of cells that have x-y and x-z clefts but no y-z cleft. Such a geometry is representative of many epithelial tissues. The appropriate tortuosity factor may also be computed by assigning zero probability to a y-z cleft.

$$\tau_c = 1/\xi_c^2 \quad \text{(clefts, epithelial)} \tag{15}$$

In summary, a tortuosity factor depends on two factors (*a*) a geometric factor that for isotropic networks may take on values (1, 2/3, 1/2, 1/3); which value depends on the number of clefts or tubes channeling the flow in a particular direction divided by the total number of clefts or tubes; (*b*) a wiggle factor, ξ, that does not depend on branching but measures the added length of extracellular flow must follow as it moves a given length through the tissue.

DISCUSSION

Comparison with Previous Results

Adrian et al. (1969) introduced the idea of a tortuosity factor for computing the effective luminal conductivity of the T-system in skeletal muscle fibers. They found the value to equal ½ for several two-dimensional networks of one-dimensional tubules, but did not consider wiggling of the tubules. Thus, their result is consistent with Eq. 12, $\xi_T = 1$.

Mathias et al. (1977) derived a more general expression for the tortuosity factor of the T-system based on the solution of difference equations that were derived by breaking the T-system into circular shells. Our 1977 expression for the tortuosity factor (Fig. 5 of that paper) appears to differ from the present expression, Eq. 12, because it was written in terms of the branch-to-branch spacing, δ_B, as well as the nodal spacing, δ_N. Moreover, the parameter L_T/A_F, which represents the length of tubule in a unit area of z-disk, appears in the 1977 expression. The parameter L_T/A_F can be derived from Fig. 1 *b* and is $N_B \xi_T / 2\delta_N$. Comparison of our earlier result with Eq. 12 indicates the two expressions are identical if $(\delta_N/\delta_B) \doteq \tfrac{1}{4} N_B$. If we consider k unit cells side by side, then the distance in the θ direction will be $k\delta_N$ or $j\delta_B$, where j is the number of branches in the r direction. However, the probability of a branch in the r direction, in an isotropic network, is $N_B/4$, thus $j = kN_B/4$ and indeed $(\delta_N/\delta_B) = N_B/4$.

Eisenberg et al. (1979) introduce the concept of a tortuosity factor for the flow of ionic current through the intercellular clefts of a syncytial tissue but they do not evaluate the tortuosity factor in terms of morphology. Although the output equations from that analysis are correct insofar as where the tortuosity factor appears, the intermediate steps include a misleading definition of tortuosity. Eq. 5 of Eisenberg et al. (1979) implies the tortuosity factor is the same as $1/\xi_x$ described in Eqs. 2 or 4 of this paper. However, in the companion paper, Mathias et al., 1979, Eq. A-1, the tortuosity factor is defined as in Eq. 3 of the present derivation.

Form of the Tortuosity Factor

We have chosen to present the tortuosity factor as the product of what we have called a geometric factor and a wiggle factor. This choice of presentation is intuitively appealing but it is clearly not unique; moreover, it may not be the most practical form in terms of what one can measure. Another choice of presentation is to use Eqs. 5 or 9 to define S_e/S_x. This choice obviates the need to introduce either a volume fraction or a tortuosity factor. Finally, one might choose to keep the idea of tortuosity, but to eliminate the wiggle factor, ξ, in favor of more readily measureable parameters. For example, Eqs. 6 or 10 define isotropic wiggle factors in terms of V_e/V_F, N_B, W, and the dimensions of the unit cell. Another definition in terms of S_m/V_F is possible by simply noting $V_e/V_F = (1/2)\ W\ S_m/V_F$. These equivalent relationships can be trivially derived from the unit cells pictured in Fig. 1. Clearly, the unit cell approach allows one a great deal of flexibility in relating many parameters that have been reported in different literature references.

Further Investigation

This paper provides a reasonably general expression for a tortuosity factor in a number of different geometries. The most heuristic parts of the analysis are the assumptions (*a*) one can uniquely define a unit cell in a random structure, and (*b*) one can pack that cell into the appropriate cuboid for mathematical analysis. These assumptions can, at present, be considered no more than a plausible hypothesis for biological tissues. The field of geometric probability is a relatively small area of mathematics (e.g., Solomon, 1978) and to my knowledge, no work has been done on this sort of problem. The existence and rigorous definition of a unit cell is therefore an open question for future mathematical investigation.

Furthermore, this derivation assumes that the dimensions and wiggling of the extracellular space are uniform, but this is not always the situation in biological tissues. Mathias et al. (1981) reported nonuniformities in the dimensions of extracellular clefts in spherical clusters of tissue cultured chick heart cells, and they were able to estimate the effect on measured values of the effective resistivity of the clefts. Situations where the extracellular dimensions are nonuniform have to be individually investigated and, depending on the nonuniformity, a specific evaluation of the effective resistivity might be possible. Such nonuniformities of dimension were also observed by B. R. Eisenberg and I. S. Cohen (1983) in dog Purkinje strands, so investigation of the effective resistivity of nonuniform, tortuous structures needs further investigation.

I first beame interested in the problem of tortuosity because of discussions with Bob Eisenberg. He has subsequently provided many useful comments as well as continuing enthusiasm and support.

The work was supported by the National Institutes of Health (grants EY03095, HL29205) and The American Heart Association (grant 79-851).

Received for publication 22 July 1982 and in final form 1 December 1982.

REFERENCES

Adrian, R. H., W. K. Chandler, and A. L. Hodgkin. 1969. The kinetics of mechanical activation in frog muscle. *J. Physiol. (Lond.).* 204:207–230.

Babuska, I. 1976. Homogenization and its application to mathematical and computational problems. *In* Proceedings of Numerical Solutions of Partial Differential Equations -3. B. Hubbard, editor. Academic Press, Inc., New York. 89–116.

Eisenberg, B. R., and I. S. Cohen. 1983. The ultrastructure of the cardiac Purkinje strand in the dog. A morphometric analysis. *Proc. R. Soc. Lond. B. Biol. Sci.* In press.

Eisenberg, R. S., V. Barcilon, and R. T. Mathias. 1979. Electrical properties of spherical syncytia. *Biophys. J.* 25:151–180.

Hellman, D. C., and J. W. Studt. 1974. A core-conductor model of the cardiac Purkinje fibre based on structural analysis. *J. Physiol. (Lond.).* 243:637–660.

Jackson, J.D. 1975. Classical Electrodynamics. John Wiley and Sons, Inc., New York.

Lammel, E. 1981. A theoretical study on the sucrose gap technique as applied to multicellular muscle preparations. I. Saline-sucrose interdiffusion. *Biophys. J.* 36:533–553.

Levis, R. A., R. T. Mathias, and R. S. Eisenberg. 1983. Electrical properties of sheep Purkinje strand. Electrical and chemical potentials in the clefts. *Biophys. J.* In press.

Mathias, R. T., R. S. Eisenberg, and R. Valdiosera. 1977. Electrical properties of frog skeletal muscle fibers interpreted with a mesh model of the tubular system. *Biophys. J.* 17:57–93.

Mathias, R. T., J. L. Rae, and R. S. Eisenberg. 1979. Electrical properties of structural components of the crystalline lens. *Biophys. J.* 25:181–201.

Mathias, R. T., L. Ebihara, M. Lieberman, and E. A. Johnson. 1981. Linear electrical properties of passive and active currents in spherical heart cell clusters. *Biophys. J.* 36:221–242.

Maxwell, J. C. 1891. A Treatise on Electricity and Magnetism. Dover Publications, Inc., New York. 1:363.

Mobley, B. A., and B. R. Eisenberg. 1975. Size of components in frog skeletal muscle measured by methods of stereology. *J. Gen. Physiol.* 66:31–45.

Mobley, B. A., and E. Page. 1972. The surface area of sheep cardiac Purkinje fibers. *J. Physiol. (Lond.).* 220:547–563.

Nicholson, C., and J. M. Phillips. 1981. Ion diffusion modified by tortuosity and volume fraction in the extracellular microenvironment of the rat cerebellum. *J. Physiol. (Lond.).* 321:225–257.

Schneider, M. F. 1970. Linear electrical properties of the transverse tubules and surface membrane of skeletal muscle fibers. *J. Gen. Physiol.* 56:640–671.

Soloman, H. 1978. Geometric Probability. The Society for Industrial and Applied Mathematics. J. W. Arrowsmith, Ltd. England.

Weibel, E. R. 1972. A stereological method for estimating volume and surface of sarcoplasmic reticulum. *J. Microsc. (Oxf.).* 95:229–242.