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Impedance Measurements as Estimators of the Properties of the Extracellular Space

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The extracellular space of a tissue is tempting to ignore; after all it is connected to the bathing solution and substances in the bath can "freely diffuse" through the extracellular space and reach the membranes of the cells. Historically, workers in many tissues have succumbed to temptation, ignored extracellular space, and eventually realized that they had ignored an important determinant of experimental results and (presumably) physiological function.

The historical path to the investigation of the extracellular space, although well trodden, has had different names in different fields, or should I say gardens. In the study of axons, the relevant extracellular space is called "the Frankenhaeuser-Hodgkin space."[1,2] In skeletal muscle it is the lumen of the tubules;[3-5] in cardiac muscle the spaces are the lumen of the tubules and the clefts between cells.[6] In epithelia it is both the lateral intercellular spaces and the "unstirred" layers of the serosal and mucosal faces of the tissue (see Clausen *et al.*[7] and references there). The multiplicity of names for the same historical path (and logical phenomena) has hidden the identity of the physical and physiological processes. This paper will try to place the study of the extracellular space of the brain in the context of earlier work.

The extracellular space of many tissues restricts movement because it is tortuous and narrow, with a long path length for diffusion and a large ratio of membrane surface to extracellular volume. In these circumstances properties of the tissue are not entirely set by their membranes, channels, and transport systems. Rather the extracellular space becomes a significant impediment to, and thus determinant of, flow. In each tissue, there are two main classes of effects of the restricted extracellular space: the changes in local concentrations (i.e., chemical potential) of ions and the change in local concentration of net electrical charge (i.e., voltage or electrical potential) caused by the resistance of the extracellular space. A similar approach has been taken in the analysis of the flow of water by Mathias, and the same theme is heard there: the extracellular space plays an important role in controlling the flow of water.

The physiological analysis of the role of the extracellular space presupposes a qualitative and quantitative knowledge of the structure of the tissue. Thus, serious physiological work in this field must be accompanied by anatomical analysis: one must know the topology, the connectivity, and the extent of the extracellular space before one can analyze it.

The techniques of electron microscopy allow a reasonably straightforward analysis of the topology of the extracellular space. Artifacts rarely change the connectivity of

the space, and when they do, they cannot escape the attention of even the most aggressive investigator. The topological analysis is often distorted, however, not by artifact but by a peculiar interaction of human motivation and technical restriction. Electron microscopy prepares much more tissue for examination than can ever be studied, let alone reported in the literature. Thus, the micrographs chosen for publication represent only a tiny fraction of those taken, and in turn those taken are a tiny fraction of the tissue. Put in this context, it is clear that extensive precautions must be taken to ensure that the tissue sample reported in the literature is representative of the structure of the tissue *responsible for the physiological process.* Unbiased random sampling is the obvious correct approach, but unfortunately this approach is in contradiction to the traditions of morphology, where the more unusual structures gain the greatest attention, if not notoriety. Since the energetic morphologist can rapidly scan huge amounts of tissue, it is quite easy for the literature to include only the most interesting and therefore *least* representative structures of the tissue of interest.

Once the qualitative structure is known, we must turn to the quantitative analysis. Here there are two fundamental problems: tissue preservation and morphometric analysis. Tissue preservation is an issue that cannot be dealt with in this short paper; suffice it to say that the art (as much as the science) of morphology concerns this issue.

Most workers, confronted for the first time with the need for morphometric information, take the same approach because they are unaware of the history (or even existence) of the science of stereology. They seek to reconstruct the entire structure of a (hopefully) representative piece of tissue and then trace that structure, using some variation of plainimetry to determine the parameters of interest. This approach is as tedious as it is unnecessary. It is tedious because of the effort involved in reconstructing a solid of many micrometers in extent from slices only nanometers thick. It is unnecessary because the information required is only a very small fraction of the total information in a micrograph and because averaging over many micrographs from many tissues is in any case a *biological necessity.* When the dominant source of variance is biological, from cell to cell, tissue to tissue, and animal to animal, as it usually is, there is no point in acquiring all the information in a given micrograph. Rather just enough information should be abstracted from the individual micrograph to ensure that the variance in that estimate is negligible compared to biological variance. With this approach, the measurement of biological structure becomes a branch of the statistical science of stereology, and the task becomes much easier, even trivial if computers are avoided (for identifying structures). The statistical sampling methods of stereology produce accurate estimates of the parameters of physiological interest without too much effort (see Weibel[8,9] and Eisenberg[10]).

Assuming now that the morphology is in hand, we turn to more physiological issues. How do we estimate the properties and effects of the extracellular space?

The answer is, of course, that we estimate it in many ways, most of which are specific to the tissue in question. But a canonical approach is also possible in which a systematic procedure is applied to any tissue. This canonical approach has the advantage of being general and not specific to a given tissue. It also has the disadvantage of being general and not specific: it cannot take advantage of special situations and properties of a tissue.

The rest of this paper describes such a canonical approach that we have called "structural analysis of electrical properties."[10,11] Structural analysis begins with the translation of the morphological structure into a mathematical model capable of predicting the outcome of experimental measurements.[12] If this model is to be useful in measuring the properties of the extracellular space, it must have as little freedom as possible; thus, it is wise and customary to model only the voltage-independent

("linear") properties of a tissue when one is seeking to measure the parameters of the extracellular space. In this way one needs to specify quite little about the channels (indeed one need only specify their aggregate conductance). Indeed, one cannot study channels very efficiently this way, and if that were the goal, another approach would be needed. (However, see the argument of Mathias[13] that a nonlinear structural analysis is both possible and physiologically useful.)

Historically, mathematical models have often been specified in the form of equivalent circuits (either lumped or in the distributed form that physiologists, following Kelvin, Rushton, and Hodgkin, call "cables," as reviewed in Jack *et al.*[14]). That approach is obviously the best when it can be done without ambiguity, but circuit models of complex tissues are "irrational approximations" in the sense that they do not allow the computation of their own error. It is hard to be sure that circuit models are correct; it is even harder to reach agreement between competitive investigators when the tissue being studied is as complex as an epithelium or a strand of cardiac muscle.

We have taken a different approach, seeking to write explicit field equations and boundary conditions to describe complex tissues.[12] This approach is hardly original, reaching back to Maxwell through many distinguished workers, most notably Carslaw and Jaeger, but it has not been as productive as it might be in physiology, probably because of the complexity of the traditional representations of the mathematical solutions to the field problems using eigenfunction expansions. These are always hard to understand and often awkward to compute even on modern mainframe computers.

Our approach has simplified these expressions by the method of systematic approximation (to the field equations and boundary conditions, not to the solution of these equations) called "singular perturbation theory" (Peskoff and Eisenberg[15] discuss the method in biological context. Kevorkian and Cole[16] and Nayfeh[17] are widely used texts, the first of which describes the biological problems discussed here). Singular perturbation theory is used because our problems have natural small parameters (namely the ratio of membrane to cytoplasmic resistance, both in comparable units, or the volume fraction of the extracellular space), yet the small parameters cannot be set to zero without the nature of the problem changing ("becoming singular"). For example, if we consider the (steady state) problem of current applied to a spherical cell from a microelectrode, we cannot allow the membrane conductance to become zero. If it did, current could not leave the cell, and the problem would have no solution, because there would be a source (physically and mathematically), yet no flow. In more familiar language, the problem would be singular because it would not allow current to flow in loops. Another interesting example is found in syncytial tissues. Here the small parameter (the volume fraction of the extracellular space) cannot be set to zero for physical/biological/common-sense reasons rather than mathematical reasons. If the volume fraction were set to zero, the problem would still have a mathematical solution, but it would not involve most of the membranes of the tissue (namely those lining the extracellular space). Thus, the biological nature of the problem would change, and in that sense setting the small parameter to zero would introduce a singularity.

Singular perturbation theory has proven quite successful in a variety of problems ranging from spherical to cylindrical cells to anisotropic syncytial tissues (see references in Eisenberg, Barcilon, and Mathias[18]). In each case we have (to our surprise) been able to derive a fairly simple solution to complex partial differential equations and boundary conditions, a solution *that can be precisely represented as an equivalent circuit,* that is to say, as an equivalent cable. In some cases the cable representation had been (or could have been) guessed correctly; in others it had not been and it seems hard to believe that it could have been. Thus, we create a circuit model of our

complex preparations by writing the appropriate differential equations and boundary conditions, solving those equations with singular perturbation theory, and recognizing the solution as the description of an anatomically meaningful equivalent circuit.

With this circuit model in hand, we can turn to the measurement of the properties of the extracellular space. A variety of methods are possible now that optical techniques allow (at least in principle) the direct measurement of the spatial variation of concentrations[19] and membrane potentials.[20] When our work was done, however, we were restricted to measurements of the potential at one location produced by current injected at another location. Given this limitation in spatial resolution, it was necessary to make measurements with the highest resolution possible and that means using measurements in the frequency domain. For reasons that are not completely understood (in the mathematical theory of inverse problems,[21,22] or the statistical theory of estimation[23]) measurements in the frequency domain (e.g., of the response of a system to sinusoids of a wide range of frequencies) determine the parameters of the system far more accurately than measurements of equivalent accuracy in the time domain (e.g., of the response to step functions). While the reasons for this result are not fully known, the result has been accepted by physical scientists since the time of Fourier. Those biologists who doubt the superior accuracy of frequency-domain measurements should try to identify the topology and measure the circuit elements of an RC circuit: first with step functions, then sinusoids. If the topology of the network is not known in advance, step functions are virtually useless. Even if the topology is known, step functions are only useful if the measurements are free of noise or systematic error and the time constants of the network are widely spread (and evenly weighted). For the class of circuits representing the effects of the extracellular space, step-function analysis is nearly useless (Eisenberg;[10] Fig. 2, p. 306).

Frequency-domain measurements have been less widely used in biology than physical sciences, probably because they require facility with the arithmetic of complex numbers. We expect that this irrational constraint on scientific technique is less of a problem now that complex arithmetic is taught in school and circuit theory in the first year of college. Another difficulty with frequency-domain analysis in the past has been that it was much slower experimentally than transient analysis. This restriction has been removed by applying input signals that have a rich harmonic content, having energy at all frequencies in the range of interest. White noise is such a signal, so the measurements we have reported are made with white-noise input; we measure the white-noise output simultaneously with measurements of the input[24] (also reviewed in Eisenberg[10]). The digital techniques of Fourier analysis (cross-power spectral estimation) are then used to compute the transfer function.

The transfer function of the electrical model is then computed and compared to the experimental measurements. The parameters of the model (e.g., the resistivity of the extracellular space) are adjusted for optimal fit.[25-27] In this manner one can use impedance measurements to determine the parameters of the extracellular space.

With these tasks behind us, impedance analysis becomes just another tool in the physiologist's armory. He or she can measure the linear parameters under a variety of conditions and seek to interpret their changes as experimental or physiological conditions change. In that manner understanding of how the tissue works may be gained.

The extracellular space is accurately described as a linear circuit parameter, namely an effective resistance consisting of a morphometric parameter representing path length and cross-sectional area for current flow and the resistivity of the bathing solution. The combination of morphometric and electrical measurements allows the separation determination of resistivity, path length, and cross-sectional area; thus, this combination of techniques, which we have called "structural analysis of electrical properties,"

provides a tool to study the role of the extracellular space in a variety of physiological phenomena.

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DISCUSSION

R. K. ORKAND (*University of Puerto Rico, Old San Juan, PR*): If I show you a picture of an intracellular action potential and an extracellular action potential, what can you tell me about the properties of the extracellular space if you know those two voltage changes?

R. S. EISENBERG (*Rush Medical College, Chicago, IL*): The essential difficulty in the tissue that I talked about is that the great majority of membrane is experimentally inaccessible. It is hidden away down a 200-angstrom space. In that kind of situation, it is not possible to do any kind of quantitative voltage analysis.

C. NICHOLSON (*New York University Medical Center, New York, NY*): When you derive an equivalent circuit, do you have to worry about uniqueness of representation: could you have other circuits?

EISENBERG: We don't know the answer to that in general. We have done this analysis for cylindrical cells, spherical cells, thin plain cells, thick plain cells, and spherical and cylindrical syncytia. In those five cases the perturbation analysis is clearly unique. In fact, we were so worried about it that we actually got the exact solution and did the expansion of the exact solution to verify that we hadn't left out any terms. In one case there is a term that isn't unique, but it is very, very small, and we actually don't understand it. The answer to the question is that we expect that it's unique, but we can't be sure. I want to emphasize, however, that the only thing the computer has when it fits the data is the actual solution that came out of the math, so the circuit diagram is really only of use for us in discussing the problem.

A. R. GARDNER-MEDWIN (*University College London, London, England*): You seem to be saying that people who work on cardiac and striatal muscle may have been led to artifactual conclusions, by not being sophisticated enough. Can you suggest specific kinds of things to do with brain function where we might have the same type of problem?

EISENBERG: I really don't want to say anything more specific than the following, which is based on a very simple case that I have thought a bit about. If I were interested in studying problems of sodium channels in squid axon, which is away from brain function, but it will illustrate my point, I would not proceed until I understood the role of the Schwann cell and could predict the linear response of the squid axon at times faster than 100 microseconds, which still hasn't been done. Now in studying brain function, it would be extremely interesting to take a tissue that one could get reproducibly and do the stereological analysis of the morphology and try to predict from that, for example, in the case of a glial preparation, the electrical

properties that one ought to get when you put in two electrodes. If this works, then you can immediately generalize to electrodiffusion and again the techniques that we heard about earlier could be used to estimate the parameters. Finally, I will mention one thing that I didn't talk about. Dr. Mathias in my department used the approach that I outlined in epithelia, and he has been able to solve some of the nonlinear electrodiffusion equations, including water flow. He finds the remarkable result that convection arises almost inevitably; it's virtually impossible to avoid convection in tissues of this complexity. This is probably the key to the understanding of transport in the lens of the eye, for example. The point here is that all the parameters in Mathias's model can be measured.