Srivastava, ed. Red Blood Cell and Lens Metabolism

THE LENS AS A SPHERICAL SYNCYTIUM

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Many analyses of the properties of the lens have assumed, implicitly or explicitly, that the tissue is a giant single cell of roughly spherical shape. This assumption makes experiments much easier to interpret, particularly experiments analyzing the transport of solutes. Measurements of influx and efflux of solutes or tracers are then interpreted as the properties of a single homogeneous membrane with a single permeability (or conductance) coefficient to describe the movement of each solute. Properties characteristic of more complex structures are not present in single cells. Thus, if a preparation is a single cell, one can safely neglect the consequences of 1) parallel connections of membranes of different properties; 2) restricted diffusion and movement of solutes in a small extracellular space; 3) gradients of extracellular concentration or transmembrane potential; 4) steady state, i.e. standing, currents; and 5) a number of other related phenomena.

We know, of course, that the lens of the eye is not a giant single cell. The scanning electron microscope, which allows visualization of unstained membranes, has shown that all lens fibers, even those from the innermost nuclear regions of the lens, are surrounded by membranes which seem intact (Sakuragawa, et al., 1975)[1]. Images of incomplete membranes seen in the transmission electron microscope should not be taken literally since visualization of a membrane in the electron microscope requires the reaction of stain and membrane, leaving an electron dense reaction product. Failure to observe a membrane may imply the absence of a membrane, but it can just as well imply a failure of the membrane to stain. Indeed, if the inner membranes of the lens have an unusual lipid or protein composition, one might expect them not to react with the standard reagents of electron microscopy, designed as they were to stain typical biological membranes.

While we know the lens is not a single cell, we also know that many of the properties of the lens have been interpreted, without obvious failure, as the properties of a single giant cell. There are several reasons that such interpretations have been possible. First, we must remember that lens fibers are

connected by numerous cell to cell junctions, which join the cytoplasm of virtually all the cells in the preparation. These gap junctions have been demonstrated both morphologically (Benedetti, et al., 1976[2]) and physiologically (Duncan, 1973[3], Rae, 1974[4]). They evidently allow the diffusion of solutes and passage of current from any fiber in the lens to any other. This coupling between cells means that the movement of small solutes within the cytoplasm of the lens is not qualitatively different from what it would be in a giant single cell.

Another reason that the lens has been considered a single cell, without obvious failure, is that many of the gross properties of a spherical syncytium are similar to the properties of an equivalent single cell. For example, the transport properties of a syncytium--the flow of current, the movement of ions, the flow of water, and the movement of nonelectrolytes, to list just a few--are closely related to the properties of a single cell. Such properties, measured from a syncytial preparation, represent a weighted average of the properties of the different membranes in the preparation. Unfortunately, the average properties measured do not depend simply on the properties of the different membranes. For this reason, interpreting these measurements as the properties of the membrane of a spherical cell will not give specific information concerning any one membrane of a spherical syncytium. The contribution of the different membranes present depends on other factors, such as the volume and surface area of the inner and outer membranes and the branching of the extracellular space within the lens. In fact, the relative contributions of the different membranes depends as much on the anatomy of the lens as on the specific conductance of its membranes. Experimental interventions can change the relative contribution of inner and outer membranes to average properties; therefore, the meaning of the average properties depends on the unknown properties we seek to determine.

Average properties have been interpreted as the properties of a single membrane without obvious failure, despite the facts just mentioned. Perhaps some of the more complex properties which have been attributed to a single membrane really represent the contributions of several membranes, each with more simple properties. In order to demonstrate the contributions of different membrane systems, it is necessary to make measurements which depend on the local properties of different systems of membranes. It is best, perhaps even necessary, to make interventions which systematically vary the contributions of different membranes to the properties measured.

Measurements of tracer efflux from an entire lens do not isolate the contri-

butions of different membrane systems. The fact that the efflux apparently follows a simple exponential law, with a single rate constant (after the washout of an extacellular space) does not provide evidence that the preparation is a single cell. Washout from a spherical syncytium would have similar properties. The single rate constant would be from two parallel conductive paths, one across the outer membranes and the other across inner fiber membranes, through the extracellular space and then into the bathing solution. One cannot distinguish parallel conductive paths by one measurement of flux or current. For this reason, among others, washout measurements are rather insensitive to local properties of membrane systems and rarely allow systematic intervention to vary the properties or contributions of different membrane systems to the total measured efflux. The efflux (or current flow) in such a situation will certainly depend on the properties of both membranes, but neither of the membranes present need have properties which account for the total (i.e. average) efflux. The efflux might come predominantly from one membrane system under one set of conditions and mostly from another set of membranes under another set of conditions. We see then that the interpretation of flux measurements depends on the syncytial properties of the preparation, even though flux measurements are not usually sufficiently sensitive to demonstrate those syncytial properties. The simplicity of a single rate constant for efflux does not demonstrate that a preparation is simple; the apparent simplicity may mask the complexity present.

Electrical measurements, of the potential change induced by current applied to the lens, force consideration of the syncytial nature of the tissue. Such measurements can be sensitive indicators of the properties of the outer and inner membranes of the preparation, and of the intracellular and extracellular media within the lens as well. Furthermore, the contribution of the inner membranes to the properties observed depends on the independent variables of time or frequency. Measurements of the transient or frequency response will be dominated by different sets of membranes at different times or frequencies.

Electrical measurements from complex tissues are most useful when models of the preparation are available to describe the expected relation of current, voltage, and time (or frequency). Such models must be based on both the electrical and morphological properties of the preparation (Eisenberg, et al, 1977[5], Eisenberg and Mathias, 1980[6]) and must enumerate and describe all the pathways for current flow. In fact, one of the drawbacks of this approach is that the model used must describe biologically unimportant paths of current flow in as much detail as it describes functionally important pathways.

The theory describing the relationship of current, voltage, and time (or frequency) in a giant spherical cell was published some time ago (see Peskoff and Eisenberg, 1975[7], and Ramirez and Peskoff, 1975[6] and references cited there). It is of interest to see how well the theory describes the properties of the lens. If the lens were a single roughly spherical cell, and the theory were correct, one would expect the description to be quite good. If the lens were a syncytium, one would expect the theory of a spherical cell to describe only the more gross properties of the preparation. The parameters (e.g. membrane resistance and capacitance) determined using the spherical cell theory would be crude composites of the properties of the real systems and might therefore have values quite different from those of a simple spherical cell. One would expect, moreover, that the theory would fail to fit some of the more sensitive measures of the syncytial properties of the tissue.

The theory for a spherical cell predicts that step currents passed through an intracellular glass microelectrode will induce a voltage at the tip of another intracellular electrode. The induced voltage should have a fairly simple time course, consisting of two components. The fast component should be a local potential, measurable only in the immediate vicinity of the current passing microelectrode. The local potential should depend only on the cytoplasmic resistivity and the location of the electrode tips. It has no dependence on membrane properties. The second component is much slower, having an exponential time course which depends only on the parallel resistance and capacitance associated with the cell membrane. The slow component should be spatially uniform, independent of the separation of electrode tips, and it should be described by a single time constant. The slow component depends on membrane properties but not on the properties of the cytoplasm or gap junctions.

Measurements of the electrical properties of amphibian lenses (Eisenberg and Rae, 1976[9]) have been compared to the theory of a spherical cell. The potential induced by a step of current does have two components, one slow and one fast. The fast component depends as predicted on electrode separation and presumably measures the sum of the effective resistance of the cytoplasm and the effective resistance of the gap junctions. The slow component is found, as predicted, to be spatially uniform; that is to say, the slow component is found to be the same at all separations of the electrodes. The similarity of the lens to a single spherical cell stops here, however. The slow component does <u>not</u> follow the predicted time course; it cannot be described as a single exponential. Furthermore, the parameter values derived using the theory of a

single spherical cell are quite unreasonable. If the theory were inappropriate, one would expect such unreasonable parameters, because the parameters measured would actually arise in a combination of structures not included in the theory in the first place.

The single cell theory ascribes the membrane conductance and all membrane capacitance measured to a single membrane surrounding the outside of the lens. In a lens of some 4 mm equatorial diameter, the surface area of the outer membrane is about 0.5 cm^2. A lens that size is found experimentally to have a total capacitance of about 100 μF. Thus, the capacitance ascribed to the outer membrane is about 200 $\mu F/cm^2$. Biological membranes are known to have capacitances a little less than 1 $\mu F/cm^2$ and this number is quite constant since it depends mostly on the average thickness and dielectric constant of the membrane, neither of which can vary very much. The spherical cell model thus produces parameter values which cannot possibly be correct.

One can see why the spherical cell model is incorrect. The syncytial nature of the lens implies that there is much more membrane in the preparation than that on the outside. If one guessed that the inner membranes of an amphibian lens of diameter 4 mm had an effective surface area of about 100 cm^2, the total measured capacitance of some 100 μF would be produced by 100 cm^2 of membrane, implying a specific capacitance similar to that of other biological membranes. A detailed discussion of the lumped equivalent circuit of a spherical syncytium, and therefore of the meaning of the measured capacitance of such a syncytium, is given in Eisenberg, et al, 1979[10] and Mathias, et al, 1979[11].

It is useful to extend this argument to the membrane conductance measured from the lens. Using a single cell model, Eisenberg and Rae, 1976[9], found a value of 400 $\mu mho/cm^2$ for the average membrane conductance assuming that all the conductance arose in the 0.5 cm^2 of membrane on the outside of the preparation. This figure for the specific conductance is at first glance quite reasonable since most biological membranes have specific conductances of this order. Our discussion of the capacitance of the lens demonstrates, however, that current flows across some 100 cm^2 of membrane. If all that membrane contributed equally to the effective membrane conductance (as it does more or less to the effective membrane capacitance), the specific conductance of the membranes would be very low, namely some 400 ÷ 100 = 4 $\mu mho/cm^2$. The average value of membrane conductance in the lens is thus about 100 times less than in other preparations, so it is not unreasonable to expect that at least some of the membranes in the lens are quite specialized, having much less conductance (i.e. less leakage) than typical membranes.

This discussion implies that most of the membranes, inner and outer, of the lens are available for current flow. In a lens of this size some 100÷100.5 = 99.5% of the membranes are inside the preparation. Thus, if all membranes of the preparation had the same properties and if they all contributed to the overall lens properties according to their area, one would expect that some 99.5% of the transport properties of a lens would reflect the properties of the inner membranes. At first glance, this argument seems to make the treatment of the lens as a spherical cell ridiculous. It will be shown, however, that the inner membranes have quite different properties from the outer membranes, so the contribution of both membrane systems are important.

The above arguments, made in a somewhat speculative vein, can in fact be made quite specific and careful, but only with the use of rather abstract reasoning involving the definition of effective capacitance using integrals of transients (Eisenberg and Mathias, 1980[6]). It is far better--both more convincing and of more utility--to avoid the abstract argument and simply to conclude that the spherical cell model is incorrect. One must then construct and test a model which explicitly treats the lens as a spherical syncytium, containing both inner and outer membranes. That was the goal of the theoretical work reported by Eisenberg, et al, 1979[10], and the experimental work of Mathias, et al, 1979[11]. The theoretical work starts with the general description of a syncytium, proceeds to the derivation and solution of the equations for a spherical syncytium, and then interprets that solution as an equivalent circuit with effective parameters, like the effective (i.e. average) capacitance and resistance discussed previously in this paper. It is fortunate that the forbidding mathematics of syncytial cable theory can resolve itself into such simple physical models. Without such simple models it would be quite difficult to proceed with experimental analysis (for example, that described by Mathias, et al, 1979[11]) or to use syncytial theory to interpret biological properties of the tissue.

The experimental work testing the syncytial theory of the lens differs somewhat from the experimental work described above. The model which described the lens as a spherical cell was tested in Eisenberg and Rae, 1976[9] by the application of step functions of current and the recording of the induced transient potentials. It is difficult to extend these techniques to test the syncytial theory since the method does not have sufficient sensitivity to the properties of the inner membranes, nor does it allow experimental control of the relative contributions of outer and inner membranes. In particular, while it is true that the contributions of outer and inner membranes differ at

different times following the application of a step of current, it is also true that time is a difficult variable to control. Furthermore, in a syncytium the voltage response to a step function consists of sums of exponentials and it is very difficult (especially in the presence of noise and drift) to resolve uniquely the components of such transient responses. This difficulty arises from the basic properties of the transient response to a step function input. The exponential functions which describe the transient response are not orthogonal (i.e. the estimate of one exponent changes the estimate of the others); the energy of step functions is concentrated in a very narrow low frequency band (meaning that measurements of properties dominant at high frequencies are necessarily made at very small signal to noise ratio) and finally the responses are monotonic (meaning that the responses always increase or decrease with time, never having maxima or minima). Functions without maxima or minima have very little structure. Monotonic functions which are composites of other monotonic functions do not have enough richness of behavior to allow isolation of their components. At least this is what one suspects is the main reason for the notorious inability of curve fitting techniques to determine the components of transient responses (see discussion in Eisenberg and Mathias, 1980[6]).

The measurement of the response to sinusoidal currents is a much more sensitive and useful approach to the test of the syncytial theory of the lens. With sinusoidal analysis all frequencies can be weighted evenly and have equal signal to noise. The response of a linear system to a sinusoidal input (or to the sum of sinusoids) is also a sinusoid or the sum of sinusoids. These functions are orthogonal. Furthermore, many of the functions which describe responses to sinusoidal inputs have maxima or minima. The orthogonality and presence of maxima or minima in the response to sinusoidal inputs allow curve fitting routines to work quite well.

Sinusoids have another advantage. The control of the frequency of the sinusoid allows direct and convenient control of the relative contribution of outer and inner membranes to the observed response of the lens, simply by changing the frequency of the applied current. The relative contributions of inner and outer membranes can be controlled because of the fundamental difference between the two membrane systems, a difference which would be present even if the two membranes had identical properties.

The essential difference between the inner and outer membranes is that the inner membranes are in series with a substantial extracellular resistance whereas the outer membranes are not. This extracellular series resistance is simply the electrical property of the extracellular space within the lens; the

resistance is substantial because the extracellular space is narrow, long and tortuous.

> It is important to note that the extracellular resistance discussed here is analagous to the resistance R_e of Mathias, et al, 1979. It has no relation to the intracellular series resistance R_s, caused by the local potential near a microelectrode source of current, defined in Eisenberg, et al, 1979.

The extracellular series resistance ensures that the potential across the inner membranes cannot be identical to that across the outer membranes (although of course it may approximate the potential across the outer membranes in special circumstances). Any current flow across the inner membranes must flow through the extracellular space, thereby inducing a potential drop in that space. The potential across the outer membrane equals the potential across the series combination of the extracellular space and the inner membranes. When there is current flow across the inner membranes, there is a potential drop across the extracellular space. The potential across the inner membranes must then differ from that across the outer membranes. The current flow across the inner membrane and its contribution to the overall electrical properties of the lens must also differ from the current flow and contribution of the outer membranes.

The current flow across the inner membranes of the lens depends on frequency because the inner membranes have a substantial capacitance. Thus, the contribution of the inner membranes to the total properties of the lens varies with frequency. We see then that the presence of an extracellular series resistance and an inner membrane capacitance allows us to vary the contribution of the surface and inner membranes to the total properties simply by varying the frequency of applied current. The relative contributions of inner and outer membranes is a more complex issue not discussed here, because both membranes have frequency dependent behavior. It is easier to address such issues with the simple mathematics of lumped circuits rather than with verbal reasoning.

It would be misleading, but simpler, to leave the discussion of the extracellular series resistance at this stage. It would be misleading because one might suppose that there was a single value of extracellular resistance (said to be a "lumped" resistor) in series with all the inner membranes. The potential drop within the lens is, however, distributed throughout the extracellular space within the lens. Thus, the amount of potential drop in series with any particular piece of inner membrane depends on the length of the current path from that piece of membrane to the external bathing solution; that is to say,

it depends on the depth of the membrane within the lens. Membranes just under the outer surface of the lens have a short current path and therefore the current which flows to those membranes has suffered only a small potential drop. Membranes deep within the lens have a long current path within the extracellular space. Current which flows across such deep membranes has suffered a large drop in potential. The potential drop in series with any membrane, depends on the on the properties of the solution in the extracellular space as just described, but it also depends on the current flow across the inner membranes. The amount of current which can reach the extracellular space around the innermost fibers of the lens (which is a measure of the potential drop in series with the innermost fibers) depends not only on the resistance and pathlength of extracellular solution, but also on the amount of current which flows across all the inner membranes between the outer surface and the innermost fibers. If the inner membranes had a sufficiently large conductance, most of the current flowing into the extracellular space would flow across the inner membranes in the first few layers of fibers, and there would be almost no current flowing in the extracellular space deep within the lens.

The analysis above shows that the electrical properties of a syncytium depend in a complex way on the properties of both the inner membranes and the extracellular space within the syncytium. In particular, transport properties (such as the effective membrane conductance or capacitance or the rate constant for tracer efflux) depend on the properties of an extracellular space, as well as depending on the properties of membranes.

In the last few paragraphs we have qualitatively discussed the flow of current within the extracellular space inside the lens. The first paragraph described the flow in a lumped model of the lens. The second paragraph described the flow of current in a distributed model of the lens. It should be clear that verbal discussion of the latter case is awkward, imprecise, and unconvincing. Even the verbal discussion of the first case does not allow a statement of the relative importance of inner and outer membranes at high and low frequencies. That is why we must use a mathematical description of current flow in either lumped or distributed representations of syncytial tissues (Eisenberg, et al, 1979[10]).

There is one practical reason why the measurement of sinusoids might be less useful than it seems. As applied to measurements from skeletal muscle (Mathias, et al, 1977[12]), measurements of sinusoids take a long time. The protocol for a sinusoidal experiment requires the application of a sinusoidal current of one frequency, a substantial wait for the system to reach steady

state, and then a further wait of at least twenty periods (i.e. cycles) while the response is being averaged to improve signal to noise. This procedure is repeated at each frequency at which data is measured. Since the lens has so much internal membrane, and such a high specific membrane resistance, it has very slow electrical properties. To adequately investigate the low frequency behavior of the preparation, measurements to at least 0.01 Hz are needed. A measurement at 0.01 Hz, averaged for 20 cycles, takes 2000 seconds = 33 minutes. And only one frequency point has been measured. It can be seen that a determination of the entire frequency response of the lens with reasonable resolution would require an inordinate length of time. Few biological preparations, certainly none penetrated with microelectrodes, are viable--let alone stationary--that long.

Fortunately, there are other waveforms available which compress the time required for sinusoidal measurements, without running into the problems of step function responses. These are the sums of sinusoids, or waveforms which closely approximate the sums of sinusoids, with equal energy at all frequencies of interest. These signals appear stochastic to the naked eye; whether they are in fact stochastic or periodic is a matter of technical convenience and choice. For measurements involving microelectrodes, which are rarely stationary and always introduce large amounts of noise, we feel it best to use stochastic wide band signals. Such signals provide sinusoids of many frequencies at the same time. Thus, by passing broad band stochastic signals through one microelectrode while recording potential with another, it is possible to measure all frequencies in the band of interest in precisely the same time it would take to measure the sinusoid of the lowest frequency of interest. This remarkable result is true because sinusoids are orthogonal functions; thus the linear response to sinusoids of one frequency is at the same frequency and does not interact with sinusoids of other frequencies. The lowest frequency component of the stochastic signal then has the same properties as a single sinusoid of the same frequency.

The above discussion applies of course to the ideal case. The practical implementation of this technique, however, is surprisingly close to the ideal case (Mathias, et al, 1979[11]). Although such an implementation requires a certain amount of digital hardware and therefore cost, there appear to be no other drawbacks in its routine application to biological preparations.

Experiments designed to study the electrical properties of the lens with high resolution are made more precise and easier to interpret if the current passing microelectrode is placed close to the center of the lens. The results

are more precise because irrelevant (and hard to analyze) potentials in the bathing solution are circularly symmetrical if the electrode is in the center of the lens. Circularly symmetrical bath potentials can easily be removed from measurements with conventional (but high quality and wide bandwidth) differential recording techniques. The results are easier to interpret when the current electrode is in the center of the lens because the theoretical expressions simplify considerably. Then, infinite series of Legendre polynomials and spherical Bessel functions (or their sums) are no longer needed to describe the angular dependence of potential. Measurements made with the electrode in the center depend on only the radial, not the circumferential pathways for current flow in the tissue. This fact can be an advantage (since it allows experimental isolation of one of the orthogonal properties of an anisotropic preparation). It can be a disadvantage if the circumferential properties are of interest.

When a high resolution electrical analysis was performed on amphibian lenses bathed in normal (lens) Ringer solution, several important generalizations became apparent. It is important to note that these generalizations are quite independent of the details of the model. For that reason, we expect these generalizations to survive the evolution of our knowledge of the morphology and the corresponding natural selection of the fittest theoretical models of the lens.

1. The lens cannot be described as a single cell over any reasonable range of frequencies.

2. The membranes of the inner lens fibers have a specific resistance roughly three orders of magnitude larger than that of the surface cells. That is to say, the inner lens fibers are not at all leaky compared to most membranes.

3. Even though the inner membrane has a much higher specific resistance than the outer membrane, there is so much inner membrane that about one half of the total current flow or corresponding isotope flux out of the lens is across the inner membranes, at least in small frog lenses. The other half is across the outer membranes. From this fact, it is clear again that predictions made which assume solute movement across just the outer membranes are unlikely to be revealing of the properties of any one membrane system in the lens. It should be noted that both the

relative and absolute amounts of inner membranes are larger in larger lenses. Thus, analysis of small frog lenses is likely to underestimate the importance of solute movement and current flow across inner membranes in larger lenses, like many mammalian lenses.

4. The resistance of the extracellular space between lens fibers is not at all negligible. In fact, the extracellular resistance contributes noticeably to the input resistance of the preparation and to the distribution of current flow even at very low frequencies. It is this property which limits our ability to interpret measurements made with a single cell theory of the lens. The presence of significant extracellular resistance implies that the conductance measured from the lens is not a simple sum of the conductance of the two membrane systems, but rather that it involves a weighting dependent on the dimensions and resistivity of the extracellular space. The significant resistance of the extracellular space is not surprising if one considers the narrowness and tortuosity of the channels between cells. Indeed, Mathias, et al, 1979[11], have shown (see the Appendix to that paper) that a simple structural model of the lens can account for the observed extracellular resistance, using preliminary estimates of morphological parameters made by B. Eisenberg and Rae and assuming the extracellular spaces are filled with Ringer solution.

With this data in hand, it is possible to speculate concerning the functional role of ion transport in the life of the lens. One must continually remember that the main function of the lens--to refract light--places serious constraints on how it can be built and how it can function. It seems that the lens must lack both blood vessels and large extracellular spaces if it is to remain transparent. These constraints severely limit the movement of solutes into and out of the lens since they greatly decrease the efficiency of diffusion. Cell to cell coupling seems an evolutionary adaptation to these limitations since it allows diffusion in the intracellular spaces to be much what it would be in a giant spherical cell. This is certainly much worse than it would be in a spherical tissue riddled with capillaries, but the diffusion is still much better than it would be in a collection of lens fibers not coupled one to another. The requirement for transparency probably also makes oxidative metabolism difficult for the lens, since the mitochondria which perform such metabolism would almost certainly scatter light and interfere with optical

properties of the lens. The lens must then generate its ATP primarily by glycolytic metabolism, which is much less efficient than oxidative metabolism. Furthermore, one might expect that most of the glycolytic metabolism would occur near the surface of the lens, since the diffusion of substances into the depths of the tissue would severely limit the rate of ATP production.

One might expect, for all these reasons, that the lens has much less capacity to pump ions, per unit area of membrane, than other tissues. If the inner membranes were typical biological membranes, the sodium leakage, and consequent influx of water, would probably overwhelm the capabilities of the sodium pump (and cell metabolism). The fact is, however, that the inner membranes of the lens are highly atypical, having very low leakage compared to other biological membranes. One must suspect then that this special property of the inner membranes is an evolutionary adaptation to the metabolic limitations of the tissue.

Even though a given amount of the inner membranes is not at all leaky compared to the same amount of other biological membranes, the total leakage through all the inner membranes must be substantial, just because there is such a large area of inner membrane. The lens thus has an important need to pump sodium, despite the specialization of the inner membranes.

It is known that the primary sites of active ion transport in the lens are the epithelial cells which exist on the anterior side of the tissue (Kinsey and Reddy, 1965)[13]. Active ion transport by the inner membranes (the membranes of the lens fibers) has never been conclusively demonstrated, and, if it does occur, it must be considerably less important than active transport by the anterior epithelium. Presumably, the anterior epithelium controls the ion gradients for all the cells in the lens. If the lens has electrical coupling between all its fibers and its epithelium, pumping by the epithelium would be expressed throughout the tissue. For example, pumping sodium at the surface would deplete the sodium in the interior. There would be a diffusion problem through the interior of the lens, but this would be much less severe than the diffusion problem that would be faced if the pump were distributed throughout the membranes of the inner lens fibers. If the inner membranes pumped sodium, the pumping would be into a restricted extracellular space, of very small volume and considerable path length. The diffusion of sodium in that restricted extracellular volume would be much more restrictive than the diffusion of sodium in the intracellular medium. One can then rationalize the distribution of the sodium pump in the lens. Sodium pumping on the inner membranes would be

inefficient; therefore, evolution has selected adaptations which put the sodium pump in the surface epithelium. One might also speculate that the lens epithelium is located on the anterior side so that it can pump directly into the constantly flowing aqueous humor. Pumping at the posterior side would be into the more stagnant vitreous body and would probably result in substantial standing gradients of metabolites and pumped solutes near the posterior surface. Such gradients would, by the law of mass action, substantially limit the efficiency of active transport and metabolism.

The measurements of electrical properties may also be useful in the testing of the hypothesis that a key element in the formation of cataracts is the electrical uncoupling of adjacent cells in the lens. Such an uncoupling must be supposed from morphological studies of cataracts (Sakuragawa, et al, 1975[1]). These studies clearly show that isolated "bundles" of cells can undergo a local swelling and hydration while adjacent cells remain reasonably normal. This result can occur only if the adjacent non-swollen cells have electrically uncoupled from those that were swollen. Of course, it is not certain that uncoupling occurs before swelling. But if uncoupling did occur, it is certain that swelling would subsequently occur, because the uncoupled cell would no longer have access to the sodium pump of the epithelial cells to make up for the leakage of sodium across its membranes.

The value of the internal resistivity that is determined from impedance analysis should be a reasonable measure of the extent of uncoupling since this number depends on junctional resistivity as well as cytoplasmic resistivity. In fact, the internal resistivity measured is much larger than that expected for cytoplasm. One assumes, therefore, that the value of internal resistivity is a fairly direct measure of the extent of cell to cell coupling. One might then look for local changes in internal resistivity in the process of cataractogenesis. It is interesting to note that in a spherical syncytium, the voltage generated across gap junctions can increase as the cells uncouple. If the first effect of uncoupling is a decrease in the number of functional gap junctions, its first electrical effect would be an increase in the effective internal resistivity. This increase would show itself by an increase in the local potential recorded near the current electrode. Of course, eventually, when the number of functional gap junctions become essentially zero, the potential near the current electrode (in a neighboring but uncoupled fiber) would be quite small. The conventional wisdom that the potential must fall as cells uncouple does not apply to syncytia.

Although it seems clear that impedance measurements can be an important tool

in understanding transport properties of the crystalline lens, the analysis to date has several deficiences, which may prove to be severe.

1. The quantitative morphology of the lens on which impedance analysis depends is not presently known with much precision since stereological measurements have not been reported.

2. No attempt has been made to model the asymmetry in the preparation resulting from the asymmetrical distribution of the epithelial cells.

3. The analysis to date only permits two regions of lens fibers: inner and outer. It is most likely that there is a transition in membrane properties as one proceeds from surface to interior and it is clear that the outer layer must represent more than the outer membrane of the outermost layer of lens fibers and epithelia. Furthermore, differences in properties in the nucleus of the lens should be allowed.

4. Our estimate of the capacitance of the outer membranes is almost certainly too large, both because of the arbitrary definition of the outermost region of the lens and because of mathematical ambiguities in the definition of the boundary condition appropriate for syncytial tissues.

All of these deficiencies in our analysis must be addressed before ion transport and volume regulation in the lens can be considered well understood.

The recognition of two pathways for solute movement in the lens is, however, a necessary and significant advance over our previous treatment of the lens as a giant single cell. But the analysis of the problems of transport and volume regulation in the lens requires the application of syncytial theory to the electrodiffusion of ions across membranes and through extracellular spaces. One cannot hope to understand transport in the lens until one knows the dynamics of extracellular diffusion and the individual membrane conductances for each ion in each membrane system of the tissue.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Institutes of Health, EY-03282 to J.L.R., HL-20230 to R.S.E., and EY-03095 to R.T.M.

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