Exp. Eye Res. (1982) **35**, 471–489

Physiological Role of the Membranes and Extracellular Space within the Ocular Lens

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(*Received 4 November 1981 and accepted 14 April 1982, New York*)

Most physiological properties of the lens have been interpreted without explicit reference to the properties of the inner membranes or extracellular space within the tissue. Rather, the lens has been analyzed as if it were a giant spherical cell, ignoring the internal structural complexity known to be present in the tissue. We present measurements which show that the capacitance and conductance of inner membranes and the resistance of the extracellular space are important determinants of the properties of the lens.

Measurements were made of the slow response of the lens using either steps of voltage or stochastic currents. The response to stochastic currents was analyzed in bathing solutions of differing potassium concentration to estimate the specific capacitance of the membranes of lens fibers. The specific capacitance varied insignificantly as potassium was varied from 2·5 to 30 mM. This result is expected if the slow response of the lens arises from the dielectric properties of the membranes of the lens fibers; it is not expected if the slow response is produced by changes in potassium concentration in the extracellular space. The response to stochastic currents in bathing solutions of varying conductivity was also measured and analyzed to estimate the distributed resistance in series with the membranes of the lens fibers. The distributed series resistance was found to double when the extracellular conductivity was decreased by a factor of 1·9, suggesting that the distributed series resistance is in fact produced by the resistance of the solution in the extracellular space within the lens. The transient current flowing in response to a step voltage was measured in two solutions of different conductivity. The time course of the current was substantially different in the two solutions, showing that the conductivity of the extracellular solution between lens fibers has a dramatic effect on the electrical properties of the lens.

These results support the previous conclusions that inner membranes and extracellular space between lens fibers are both important determinants of the overall properties of the lens. We suggest that physiological measurements of the overall properties of a lens – whether of isotopic flux, ionic current, or voltage – cannot be interpreted as the properties of a single membrane. Rather, they must be interpreted as the response of a syncytial tissue, with important contributions from inner and outer membranes and extracellular space.

Key words: extracellular space; ion accumulation; lens; conductivity; impedance; capacitance; syncytial theory; electrophysiology.

1. Introduction

The ocular lens consists of many layers of lens fibers, coupled together at specialized sites by gap junctions, with a small but definite extracellular space between fibers (Wanko and Gavin, 1959; Cohen, 1965; Rafferty and Esson, 1974). Scanning electron microscopy (SEM) has shown that cellular structure extends nearly to the center of the tissue (Kuwabara, 1975; Dickson and Crock, 1972; Kuszak, Alcalá and Maisel, 1980): each lens fiber appears bounded by a cell membrane. The extracellular space within the lens is lined by fiber membranes across which solute and water can flow. These 'inner membranes' are distinguished from the membranes of outer lens fibers, and epithelial cells, exposed to the bathing solution outside the tissue. The amount of membrane lining the extracellular clefts within the lens is very large, amounting

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0014-4835/82/110471+19 $02.00/0

to more than 99% of all the membranes of the lens. Thus, physiologically important properties of the lens like current flow, flux of water or flux of solute must depend on the properties of the inner membranes.

The extracellular space within the lens is narrow and tortuous, typically 10–20 nm wide as it twists and winds around the numerous interdigitations between neighboring fibers (Kuwabara, 1975; Kuszak et al., 1980). A narrow and tortuous extracellular space should significantly impede movement of substances to the inner membranes. We expect then that physiological properties of the lens will be strongly affected by the properties of the extracellular space, as well as membrane properties.

Most physiological properties of the lens have been interpreted without explicit reference to the properties of inner membranes or extracellular space. Rather, the lens has been considered a giant spherical cell, ignoring the internal structural complexity known to be present in the tissue (Thoft and Kinoshita, 1965; Kinsey and Reddy, 1965; Epstein and Kinoshita, 1970; Patmore and Duncan, 1980; Paterson, 1980; Delamere, Duncan and Paterson, 1980a; Hightower and Reddy, 1981). This paper tries to show that the extracellular space and inner membranes are important determinants of the properties of the lens. Detailed analyses of the electrical properties of the lens (Eisenberg and Rae, 1976; Rae, 1978; Mathias, Rae and Eisenberg, 1979, 1981) have already reached this conclusion, but the methods used are somewhat inaccessible. Here we use simple general arguments and results to show the importance of the extracellular space and membranes within the lens. (1) We show that the slow time course of the response to applied current reflects the capacitance of the inner membranes. It is not caused by accumulation or depletion of ions. (2) We show that the resistance of the bathing solution influences the electrical properties of the lens. These results show that current flow through the extracellular space and across membranes within the lens contributes significantly to the physiological properties of the tissue.

We conclude that physiological properties of the lens cannot be understood unless the properties of the membranes and extracellular space within the tissue are explicitly analyzed.

2. Methods

Lenses were prepared by removing the eye of a frog *Rana pipiens* and excising the posterior part of the globe, the cornea, and the iris. Four equally spaced slits were cut in the posterior part of the remaining globe down to the pars plana, producing four flaps which could be pinned to the bottom of a Sylgard lined chamber. Most experiments were done with the posterior side up, since the thinner posterior capsule was more easily penetrated by microelectrodes.

Glass micropipettes were made with a Brown–Flaming gas jet puller (Sutter Instruments). Pipettes used to measure voltage were filled with 3 M-KCl and had resistances of 3–7 megohms. Pipettes used to pass current were pulled from thin walled Pyrex tubing and filled with 3 M-potassium acetate to give resistances of 1–2 megohms. Impedance measurements were made with the techniques and circuitry of Mathias et al. (1981). Sucrose Ringer and KCl Ringer were made by substituting sucrose or KCl for some of the NaCl in the normal Ringer described by Mathias et al. (1979), keeping osmolarity constant.

Voltage clamp measurements were made using the circuit shown in Fig. 1. This circuit establishes the desired voltage at the tip of the voltage microelectrode in less than 100 μsec. Sampling of the current transient was done at 1 msec intervals by a 12 bit analog to digital converter or connected to a Digital Equipment DECLAB/11 computer.

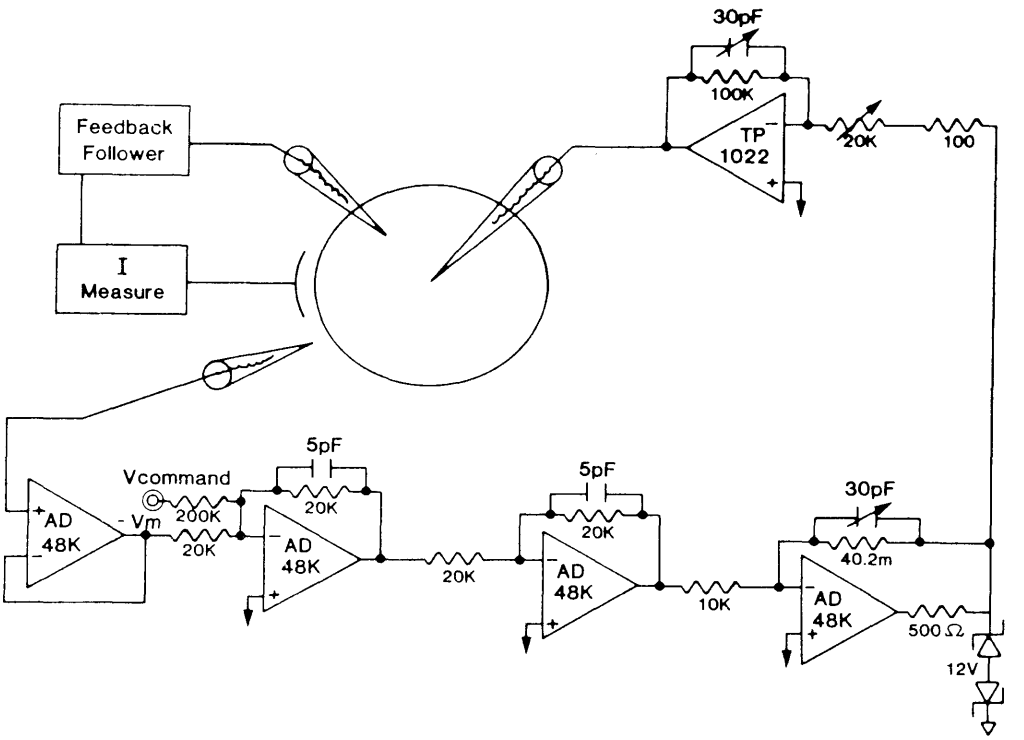

FIG. 1. Voltage clamp circuit for the lens. All resistors are 1% metal film. Teledyne-Philbrick, TP; Analog Devices, AD. Feedback follower and current (I) measuring circuit are described in Mathias et al. (1981).

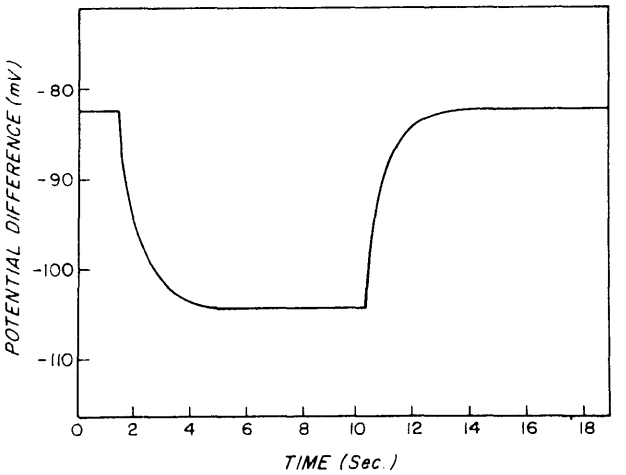

FIG. 2. The voltage response of a typical lens to a step current of 4 μA. In this experiment the current electrode was in the center of a lens of equatorial 'diameter' 0·32 cm. The voltage electrode was inserted 200 μm below the posterior surface.

3. Results

The slow voltage response of the lens

The voltage response of the lens to an applied step of intracellular current is a slowly rising voltage with a characteristic time τ of about 1 sec for a lens of 0·16 cm radius (Fig. 2). The voltage response does not resemble the non-linear responses of excitable membranes produced by changes in the conductance to specific ions; neither does the current response to a step voltage. Rather, both resemble the response of excitable membranes to small applied currents or voltages, responses which represent the

charging of a membrane capacitance in parallel with a weakly non-linear resistance. Specifically, the response of the lens to 'excitation' (i.e. either applied current or applied voltage) of one direction is always in one direction; if the direction is reversed, the direction of the response reverses; the time course of the transient response following the turn on of the excitation is close to the time course of the transient following the turn off; there is no sign of non-monotonic (i.e. biphasic) behavior; finally, the effect of the starting potential on subsequent currents does not resemble the effect on channel currents.

If we crudely model the voltage response of the lens as the exponential response of a parallel RC circuit (Eisenberg and Rae, 1976), we can estimate the effective capacitance of the lens as $C_{\text{eff}} = \tau G_{\text{eff}} \simeq 120\ \mu\text{F}$, where G_{eff} is the conductance determined from the steady voltage and current. The outer membrane of this lens has about 0·1 cm^2 area; thus, the capacitance referred to the surface area of outer membrane, ignoring the presence of inner membranes, would be $\sim 1200\ \mu\text{F/cm}^2$, far exceeding the 1 $\mu\text{F/cm}^2$ typical of biological membranes (Cole, 1972). This argument is qualitatively useful but has its weaknesses. The voltage response is not well described by a single exponential and a time constant is only a crude measure of the transient response.

The mechanism of the slow transient response

Confronted with surprisingly slow transient responses to applied current, electrophysiologists usually find one of two explanations: either they find that membrane area is wildly under-estimated (for example, in skeletal muscle, see Jack, Noble and Tsien, 1975; Eisenberg, 1983); or they find that the response does not reflect the capacitive charging of membranes. In the latter case, the slow change in voltage is often found to reflect a slow change in the equilibrium potential for potassium, as potassium accumulates or depletes in a small space around the membrane. (Orkand, 1979, is a symposium containing articles and references on accumulation and depletion phenomena in nerve, glia, skeletal and cardiac muscle. Barry, 1977, contains references to the literature on epithelia and plant cells, as well as skeletal muscle; Neumcke, 1971, contains references to the literature on bilayers. Andersen, 1978, and Andreoli and Schafer, 1978, discuss the closely related problem of unstirred layers in bilayers and epithelia.)

Eisenberg and Rae (1976) measured the transient response of the lens and suggested that the slow response of the lens reflects the capacitive charging of inner membranes. Further tests of this idea required a structural analysis of the electrical properties of the lens (Eisenberg and Mathias, 1980), involving impedance measurements interpreted with a detailed model of lens structure. Measurements of impedance are used because they have much greater resolution than measurements of transients: they display the response of the system to sinusoidal inputs of many frequencies. Mathias et al. (1979) interpreted such impedance measurements with a model (Eisenberg, Barcilon and Mathias, 1979) in which the lens is described as a spherical syncytium of fibers, electrically connected one to another, with inner and outer membranes and extracellular space within the preparation.

A typical impedance measurement is shown in Fig. 5, curve R. Current is applied by a glass microelectrode located in the center of the preparation and voltage is recorded by a second microelectrode inserted about 200 μm below the surface. The slow properties of the lens are reflected in this frequence domain data by the low frequencies at which the current and voltage are displaced in time: significant phase

shift is apparent at frequencies well below 1 Hz. If one assumes that the inner membranes have a typical specific capacitance, the surface/volume ratio (i.e. surface of inner membranes/volume of lens) needed to fit the data is some 5000–6000 cm^2/cm^3. This figure is close to the amount of inner membrane present in an idealized model of the lens (Rae, pers. comm.) in which the lens is composed of concentric spherical shells. Each shell is taken as a layer of hexagonal lens fibers with the dimensions given in Mathias et al. 1979 (fig. 8, p. 199). The total membrane surface area in the model is the sum of the membrane surface in each shell and the total lens volume is that of a sphere having the equatorial diameter of the lens.

The agreement of these estimates, and the a priori likelihood that inner membranes contribute significantly to electrical properties of the lens has not been apparent to all workers, however, and so further experiments are needed. The other possibility, already mentioned, is that the slow time constant of the lens reflects accumulation of potassium ions in a small space, just as the slow properties of skeletal muscle are thought to arise in changes of potassium concentration in the lumen of the tubular system.

The accumulation of potassium would occur if more potassium entered a region of extracellular space than could diffuse away, for example, if more potassium crossed the inner membranes of the lens than could diffuse away through the extracellular space between lens fibers. In that case, the concentration of potassium would vary with time; then the equilibrium potential for potassium would vary with time; and the resting potential would also vary with time. Quantitative analysis (see Appendix 1) shows that in the simplest case the variation of potential with time would resemble the slow charging of a large capacitor, forming a 'polarization capacitance' instead of the dielectric capacitance of a pure lipid bilayer.[1]

Slow potentials, produced by changes of ion concentration, and crudely modeled by a 'polarization capacitance', depend steeply on the resting concentration of the ion flowing. For example, accumulation of potassium is much more important than accumulation of sodium or chloride, because the resting concentration of potassium is much less, roughly 40 × less, than the resting concentration of the other ions. An accumulation of 3 mM-potassium in the extracellular space would roughly double the potassium concentration, whereas an accumulation of 3 mM-sodium would have less than a 3 % effect on sodium concentration. Hence, an equal flow of potassium, sodium, and chloride would produce a much larger change in the equilibrium potential of potassium than in the equilibrium potential of other ions.

Models in which changes of ion concentration are responsible for slow changes in potential can be tested by varying the resting concentration of permeant ions, for example, potassium. If the potassium concentration is low, at its plasma concentration, a given flux of potassium would produce a large relative change in potassium concentration and a large change in the equilibrium potential for potassium. If the resting potassium concentration is raised, then, all other things being equal, the same flux of potassium would produce a much smaller fractional change in potassium concentration, and therefore a smaller change in the equilibrium potential and membrane potential. If the resting extracellular potassium were some 3 mM, an accumulation of 3 mM would be a 100 % effect. But if the resting extracellular

[1] Phenomena of ths sort have been extensively studied in electrochemistry (Vetter, 1967; Breyer and Bauer, 1963). In general, potentials and currents limited by diffusion cannot be modelled as a combination of resistors and capacitors; rather the currents and voltages are non-linearly related.

potassium were adjusted to 30 mM, an accumulation of 3 mM-potassium would only be a 10 % effect. The resulting slow potential change would be reduced and the value of the polarization capacitance would also be smaller at the larger potassium concentration.

We then have a simple method to distinguish dielectric capacitance from polarization capacitance. The dielectric capacitance of a single membrane should be independent of the concentration of permeant ions; polarization capacitance, associated with changes in ion concentration, should vary with concentration.

The effect of ionic concentration is easy to study in simple preparations. Artificial lipid bilayers, small spherical cells, and space clamped axons can be described by a membrane conductance in parallel with a membrane capacitance. The capacitance of such preparations should not vary with potassium concentration, if the membrane capacitance is a dielectric phenomenon. Of course, even if the capacitance is constant, the voltage response would vary with potassium. Changing potassium will change the conductance of the membrane G_{eff}, thus changing the steady value of the voltage response. Changing potassium would also change the time course of the voltage response, because the time constant depends on the conductance as well as capacitance, $\tau = C_{\text{eff}}/G_{\text{eff}}$.

On the other hand, if the slow change in voltage is the result of changes in the concentration of potassium, the capacitance C_{eff} will be a polarization capacitance and it, along with G_{eff} and τ, will vary markedly with potassium concentration.

Capacitance measurements in simple preparations were reported early in the history of electrophysiology (reviewed in Cole, 1972). These show little or no dependence on ionic concentration and so have been taken as evidence that the capacitance of biological membranes is a dielectric phenomenon.

The lens, however, is a much more complex tissue than the simple systems just described. It is a syncytial tissue with distributed properties; that is to say, the voltage across a single membrane is not uniform, as it is in the artificial lipid bilayer, small spherical cell, or space clamped axon.

Most cells, as well as all syncytial tissues, are complex enough to require distributed models. Distributed models are needed to describe unmyelinated axons: Cole and Hodgkin (1939) and Hodgkin and Rushton (1946) described axons as transmission lines. Muscle fibers were first crudely described as transmission lines by Fatt and Katz (1951); now they are more accurately described as transmission lines containing distributed networks, see Jack et al. (1975) and Eisenberg (1983). Epithelia include lateral intercellular spaces analogous to the extracellular space within the lens. These spaces are described by distributed networks, see Clausen, Lewis and Diamond (1979).

Preparations like the lens, with significant distributed properties, cannot be well described by a parallel combination of G_{eff} and C_{eff}. If one attempts such a description, the effective capacitance will depend on membrane conductance and intra- or extracellular resistance, as well as on the membrane capacitance. For this reason, measurements of *effective* capacitance from distributed preparations may vary with potassium concentration, even if the *membrane* capacitance does not change with potassium. Such difficulties in the interpretation of effective capacitance of distributed preparations are well known, and have been evident to workers in excitable tissues for many years (see references in Adrian and Almers, 1974). Indeed such difficulties were one of the main motivations for the development of cable theory of nerve cells and muscle fibers (see Jack et al., 1975, for a review and references) and its extension to syncytial tissues (see Eisenberg et al., 1979, for historical references).

Cable theory allows estimates of membrane capacitance, as opposed to effective capacitance; if the theory is a proper description of the tissue, such estimates will be independent of the membrane conductance, intra- and extracellular conductances, and geometry of the preparation. In fact, estimates of the capacitance of the inner membranes made with a correct theory should give the same value as measurements of a small piece of excised inner membrane (Horn and Patlak, 1981; Hamill, Marty, Neher, Sakmann and Sigworth, 1982).

We measured the impedance of the lens at different concentrations of potassium and used the syncytial model of the lens to estimate membrane capacitance. If the slow properties of the lens were a result of ion accumulation, the capacitance determined this way should vary drastically with ion concentration. On the other hand, if the slow properties of the lens entirely represented capacitive charging of membranes, the measured membrane capacitance should be independent of ion concentration.

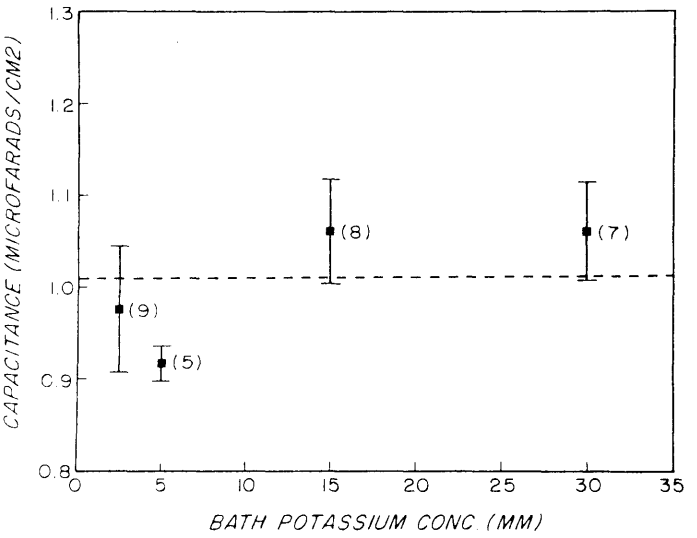

FIG. 3. Plot of the capacitance C_m of the inner membranes vs. the potassium concentration in the bath. The capacitance was determined by fitting the syncytial theory of Eisenberg et al. (1979), to the lens impedance data. Values are the mean ± S.D. with the number of lenses measured at each concentration given in parentheses. The mean capacitance determined from all measurements is shown (----).

Figure 3 shows that the membrane capacitance of the inner membranes is quite independent of the potassium concentration of the bathing solution.

The interpretation of this result depends, of course, on the syncytial theory used to analyze the data. If the theory were oversimplified, or incorrect in other ways, the parameter we call 'membrane capacitance' would not be the capacitance of a small piece of inner membrane; rather, it would be a composite parameter, depending on the conductance of membranes and the conductivity of the extracellular space. In that case changing the bath potassium would be expected to change the estimate of capacitance, even if the true membrane capacitance arose in a dielectric independent of ion concentration. An estimate made with an incorrect theory would depend on parameters that vary with potassium and so the estimate would vary with potassium.

The finding that the capacitance of the inner membranes does not vary significantly with the potassium concentration of the bathing solution thus indicates (1) that syncytial cable theory correctly describes the contributions of membranes to the measured response and (2) that accumulation of potassium plays only a minor part in the slow voltage response of the lens.

Role of extracellular resistance

If the slow response of the lens is interpreted as capacitive charging of membranes, it must arise from current flow across the inner membranes; no other membrane system has enough area. The inner membranes are separated from the bath by the long narrow tortuous extracellular space within the preparation. This space is likely to provide a significant barrier to ionic diffusion: changes in concentration in the bathing solution are likely to spread very slowly into extracellular space within the lens. The voltage response to a quick change in potassium concentration should then have a complex time course. One expects a rapid change in voltage, as potassium changes its equilibrium potential (and thus the trans-membrane potential) across the outer membranes; the rapid change should be followed by a slow change in potential as potassium penetrates the clefts between fibers, changes the equilibrium potential across inner membranes, and changes the potential recorded by the voltage microelectrode. To check these predictions, we quickly changed the potassium concentration around the lens. The concentration had to be changed in less than 1 sec to give unambiguous results and had to be maintained for 30–40 min to allow adequate time for diffusion (see Appendix 2). Figure 4 shows a typical result. Note the rapid change in potential,

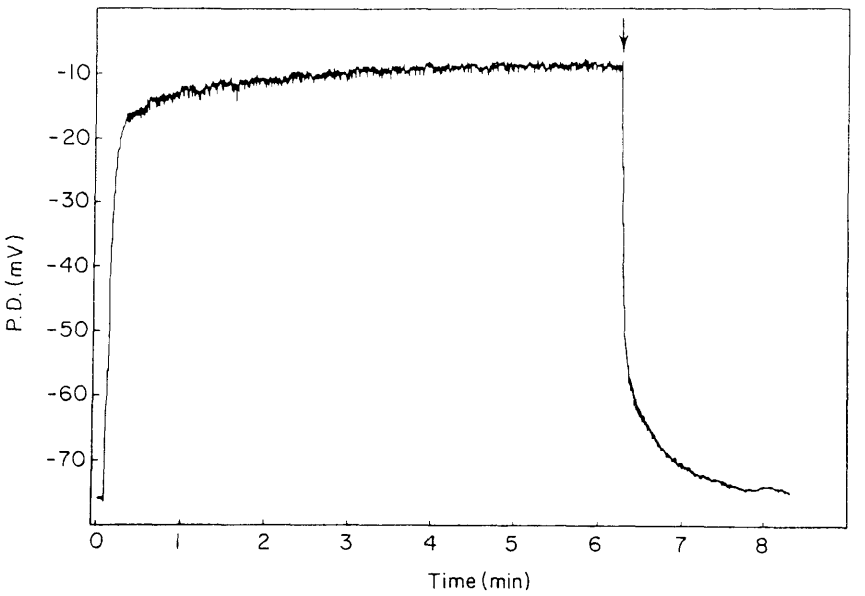

FIG. 4. The voltage change following a rapid change in bath potassium concentration, from 2·5 to 90 mM. The voltage measuring microelectrode was inserted 200 μm below the posterior surface in a lens of equatorial 'diameter' 0·33 cm. The potassium change was started at the time indicated (→) and was essentially complete in less than 1 sec.

followed by a slow creep. These records are difficult to analyze quantitatively, because they involve several physical processes, including diffusion, membrane permeation, water and current flow, as well as the non-linear dependence of potential on concentration. But the qualitative meaning is clear: two processes are involved in the response to potassium, one slow, one fast, just as one would expect from a system consisting of inner and outer membranes.

We turn now to the contribution of extracellular resistance to the overall properties of the lens. We have done three types of experiment to show that the resistance of the extracellular solution is important in determining overall lens properties, properties which have often been interpreted as if they were entirely produced by one membrane.

The first set of these experiments are extensions of the impedance analysis of Mathias et al. (1979), using the syncytial theory of Eisenberg et al. (1979). Curve fits of these data can give values of extracellular resistance and the resistance of the entire set of inner membranes (r_e and $1/g_m$, units ohms, defined in fig. 7 of Mathias et al., 1979). The series combination of these two resistances determine the total d.c. current flow through the inner membranes. If the extracellular resistance is equal to the membrane resistance, then the extracellular space is as important as the inner membranes in determining current flow though the inner membranes, because all current across the inner membranes must flow through the extracellular space. In fact, measurements show that r_e is some 5 kohm and $1/g_m$ is some 20 kohm. Quantitative analysis suggests then that the resistance of the extracellular space has a significant, but not dominant effect at rest.

The significance of the extracellular resistance should increase as the conductivity of the Ringer solution is decreased. Experiments to test this expectation were done by partially replacing NaCl of the Ringer solution with sucrose. This replacement will alter the gradients of sodium and chloride concentration within the tissue and across membranes but, at least initially, will leave the potassium gradient unchanged. Replacing NaCl with sucrose should dramatically increase the extracellular resistance within the lens, and the membrane conductance should change as well, if there is a significant membrane conductance for sodium or chloride. Impedance measurements are able to separate extracellular resistance from membrane conductance because they have reasonably distinct effects on the plot of phase vs. frequency. Figure 5 shows the result of a typical experiment. The impedance of the lens in normal Ringer is plotted along with the impedance of the same lens, determined 30 min after the replacement of 50 % of the NaCl with sucrose. The effective extracellular resistivity R_e (units ohm. cm; R_e is related to r_e in fig. 7 of Mathias et al., 1979) increased from 51 to 103 kohm. cm when the resistivity of the two solutions was changed from 87 to 161 ohm. cm. These results show that the extracellular resistivity varies with the resistivity of the bathing solution; they show also that the resistance of the extracellular solution can be a significant impediment to current flow through the membranes within the lens.

The extracellular resistance can also be estimated qualitatively without impedance measurements. If electronic feedback is used, the voltage *at the tip of the voltage recording microelectrode* (but not necessarily anywhere else) can be controlled. If that voltage is driven through a step, the resulting current is quite sensitive to the value of the resistance of the extracellular space as can be seen from a qualitative analysis of a lumped equivalent circuit of the lens (Fig. 6) in which the potential within the extracellular space in the lens is treated as uniform but different from the bath potential. (Note that quantitative analysis of this circuit is not useful since it ignores the distributed nature of the lens, which, as we have seen, is of great practical importance.) Qualitatively, the response to a step voltage should be a current transient with a time course primarily determined by the time to charge the capacitance of the inner membranes. If the extracellular effective resistance is high, the time course of the current response should be slow, extending over hundreds of milliseconds; if the extracellular effective resistance were low, the time course would be much shorter (see equation A2 of Mathias, Levis and Eisenberg, 1980). The time to charge the inner membranes observed in the typical experiment illustrated in Fig. 7, curve R, is some 800 msec, a result indicating a large value of the extracellular series resistance.

Increase in the resistivity of the Ringer solution should significantly alter the peak value and time course of this transient, if current flow through the inner membranes

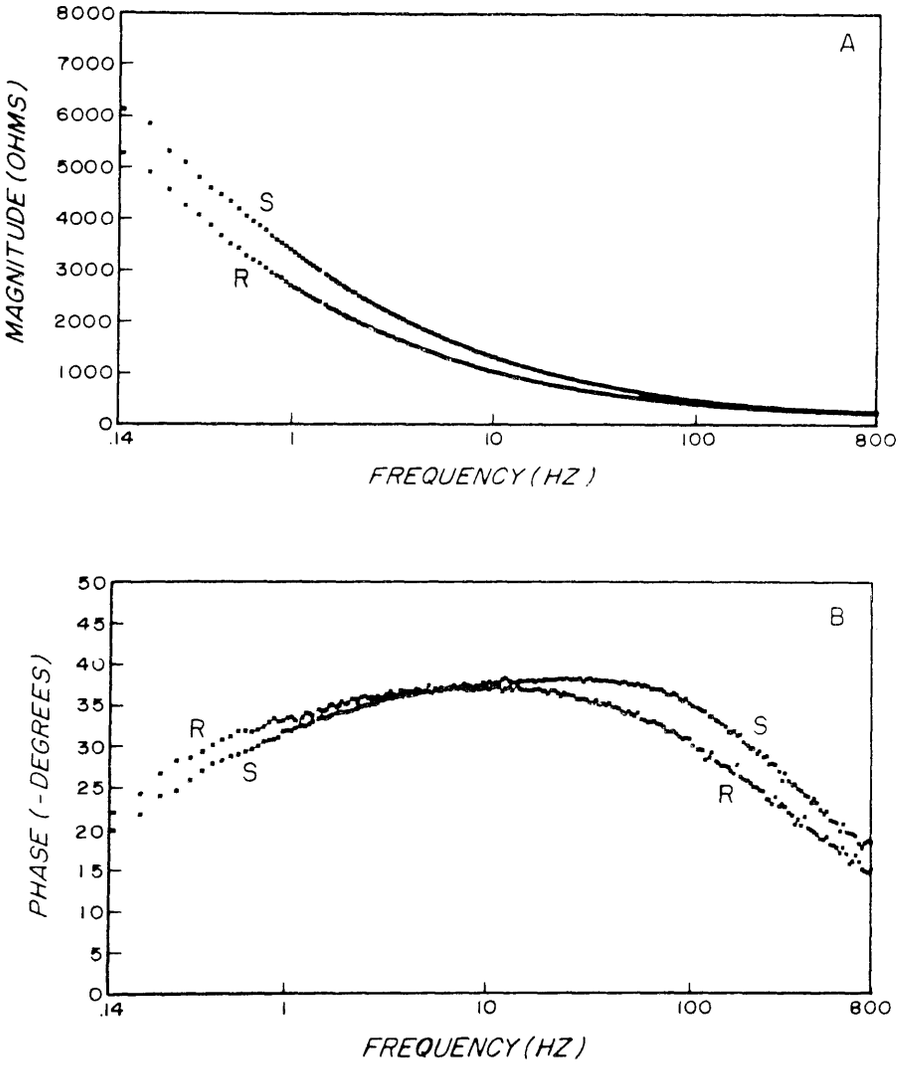

FIG. 5. Overlay of magnitude (A) and phase (B) plots of impedance data gathered from a lens of 0·33 cm equatorial 'diameter' placed first in Ringer solution (R) and then in a solution (S) in which half of the sodium had been replaced by sucrose. The measurements were made using the procedure and electronics of Mathias et al. (1981). The current microelectrode was placed in the center of the lens and the voltage electrode was 230 μm beneath the posterior surface. The result shown is one of seven similar experiments.

is significantly affected by the resistance of the extracellular space within the lens. Figure 7(a) shows the current transients produced in a Ringer in which half the NaCl is replaced with sucrose. Note the reduction in the amplitude of the slow current transient. Figure 7(b) shows that the integral of the current flow, a measure of the total charge applied to the lens, is reduced when extracellular resistivity is increased.

These results indicate that current transients from the lens depend strongly on the extracellular resistance whereas current transients from simple tissues do not.

The structural complexity of the lens produces this difference. Even in the oversimplified lumped model (Fig. 6), the total charge (i.e. the effective capacitance) depends on the conductance of membranes and the conductivity of extracellular space, as well as on the capacitive properties of membranes (see appendix of Mathias et al., 1980). But the effect of extracellular resistivity cannot be quantitatively analyzed with a simple lumped circuit model of the lens, because the potential within the extracellular

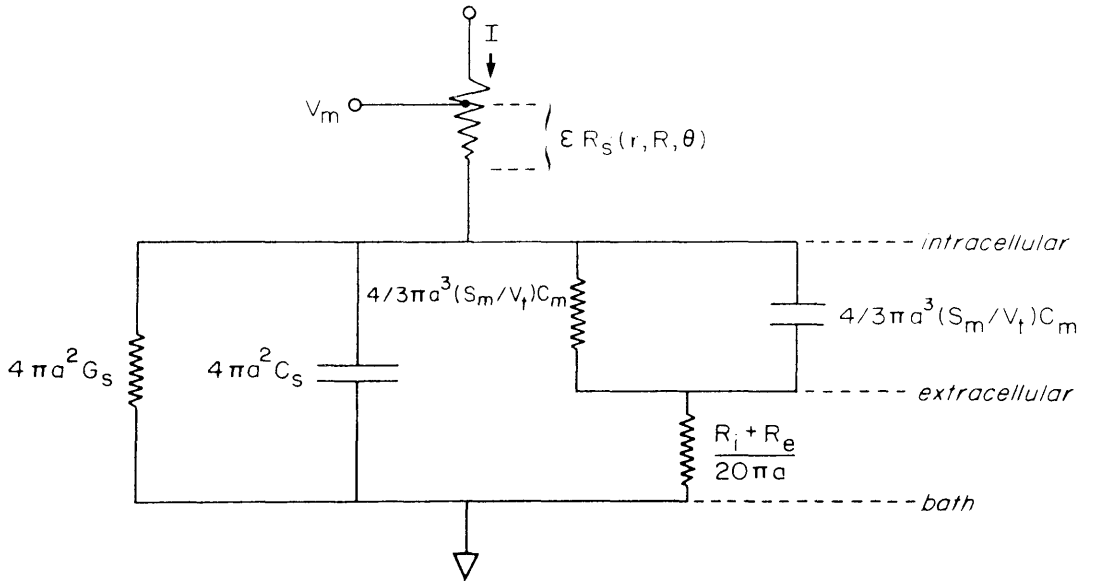

FIG. 6. A lumped approximation to the lens equivalent circuit. The current to charge the inner membrane capacitance C_m must flow through the extracellular resistance. Typical values for these parameters are given in Mathias et al. (1979). This lumped circuit is a poor approximation to the distributed admittance of the lens: even in tiny frog lenses at d.c., the decrement of potential within the extracellular space is significant.

space has significant radial variation. In that case, the response to a step voltage, and the effective capacitance, depend on the area of inner membrane across which current is flowing, as well as on the membrane and solution properties included in the lumped model. Since the area of membrane across which current flows is a function of solution resistivity, a distributed theory, like syncytial cable theory, is needed if the experiments of Fig. 7 are to be analyzed quantitatively.

While such quantitative analysis is not attempted here, qualitative analysis of the effects of extracellular resistance remains useful. Figure 7 shows that the extracellular resistance has significant effects on the peak value of the current transient and the charge flowing into the lens. The experiments thus show directly, if qualitatively, that properties of the extracellular space modify the overall properties of the lens.

4. Discussion

These results have implications for the understanding of lens function and the design of experiments to determine function. First, we find that the input resistance of the lens depends on the properties of two membrane systems, which have quite different specific membrane resistances. Given the difference in resistance of the inner and outer membranes (Mathias et al., 1979), we think it unlikely that their permeability properties are identical, whether those properties are measured electrically or by flux measurements. The overall properties of the lens must be interpreted as the composite of the properties of at least two different membranes.

We have also shown that an appreciable fraction of the input resistance of the lens depends on the extracellular resistance. This resistance arises in the tortuous, restricted clefts between lens fibers. Because the flux of ions, solutes and water are significantly impeded by the extracellular space, as well as by the inner membranes, physiological properties of the lens are expected to depend on extracellular parameters,

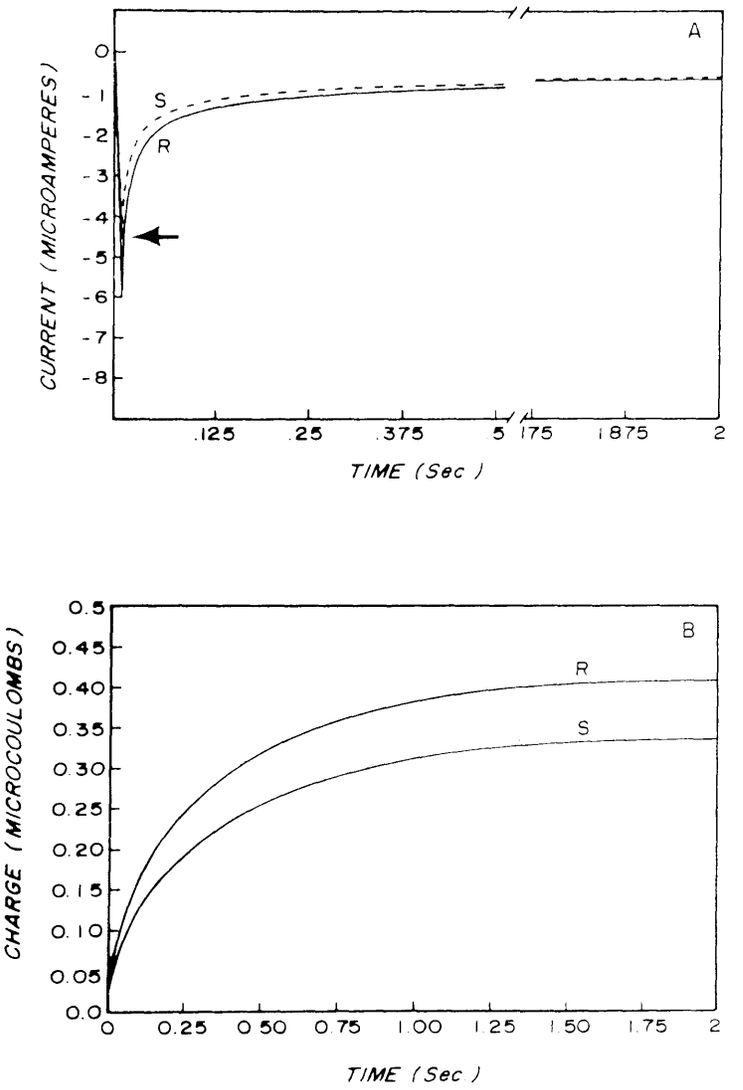

FIG. 7. The effect of sucrose on the transient current. The lens was voltage clamped *only* in the sense that the potential at the tip of the voltage microelectrode was controlled, using the circuit shown in Fig. 1. The command potential was a 5 mV hyperpolarizing step from resting potential. Panel (a) is an overlay of the current transients from the lens in Ringer (R) and in a solution (S) in which half of the sodium chloride is replaced by sucrose. The arrow identifies the peak of the transient in the sucrose Ringer. Panel (b) is an overlay of the charge necessary to change the lens voltage determined by integrating the transient in Panel (a).

as well as membrane parameters. For this reason physiological properties, even of the lens in normal Ringer, cannot be interpreted as the property of two membrane systems in parallel; the effects of the extracellular space must be included as well.

When experimental interventions are made, one must remember the geometry and resistance of the extracellular clefts. Osmotic shrinkage or structural rearrangement of the space between cells is probable, if the tonicity of the Ringer is changed, or if the structure of the lens is altered, for example, during cataract formation. Structural changes in the extracellular space would change its resistance, modifying the flux of solutes across the inner membrane even if the properties of the inner membrane were constant. In other words, one cannot interpret flux measurements solely as properties of membranes until one has shown that they are *not* limited by properties of the extracellular space.

Isotopic fluxes from the lens (references in Rae, 1978) have often been interpreted as fluxes through the membrane of a giant spherical cell. We believe these isotopes also flow across inner membranes and are impeded by flow through the extracellular space, just as current flows through the inner membranes and is impeded by the extracellular resistance. If our belief is correct, observed isotopic fluxes will have a rather complex relation to the flux across individual membranes: (1) The flux observed will be a composite of fluxes across two different systems of membranes. The composite flux cannot be analyzed as if it came from one membrane, because such analysis could not distinguish between two simple transport systems, one in each membrane, and a single complex transport system, in just one such membrane. Nor can the composite flux be fractionated into its components by pure thought; rather specific experiments designed to emphasize or suppress one component or the other are needed. (2) The isotopic flux across the inner membranes will be affected by the resistance of the extracellular space. If this effect is ignored, properties of the extracellular space will be attributed to a membrane conductance system. (3) The extracellular space within the lens forms a severely restricted volume, in which isotope will accumulate and thus be available for backflux. Attwell, Eisener, and Cohen (1979) have shown the difficulties in analyzing backflux in syncytial preparations in which the extracellular space is considerably less restricted than in the lens.

It seems unlikely that these difficulties will prove trivial; we believe that in combination they will make measurements of isotopic fluxes from syncytial tissues difficult to interpret with uniqueness. Certainly, ignoring these difficulties will confuse properties of different membrane systems, extracellular space and artifacts with the properties of membrane transport systems.

Physiological measurements made in non-isosmotic solutions are particularly likely to be misleading unless the role of the extracellular space is explicitly included. Hyperosmotic solutions are likely to modify the spacing between lens fibers, changing the extracellular resistance and the accessibility of the inner membranes, thus changing the input resistance of the lens. For example, Patmore and Duncan (1980) showed that solutions made hypertonic with tetraethylammonium ion increased the input resistance of the lens and attributed this to a pharmacological action of the ion. On the other hand, Jacob and Duncan (1980) found a similar result, using solutions made hypertonic with sucrose.

The high resistance of the extracellular space also has important implications for the analysis of electrical properties, particularly in voltage clamp experiments. The presence of a large distributed radial resistance in series with a membrane guarantees that the membrane is not voltage clamped, when the voltage in the cytoplasm is clamped. Workers in skeletal, smooth and cardiac muscle have found this situation too complex to analyze uniquely, particularly if the membrane has non-linear properties. (The classical reference to this problem is Johnson and Lieberman, 1971; Noble, 1975, p. 45 has a useful succinct discussion.) The problem is that the non-linear current flowing across inner membranes changes the extracellular voltage. The current flowing across the inner membranes must also flow through the extracellular resistance, because the membrane conductance is in series with the extracellular resistance. The resulting potential drop in the extracellular space changes the membrane potential across the inner membranes; hence, the inner membranes are *not* voltage clamped. Current flow which seems to reflect the properties of voltage clamped membranes really depends on the time course of voltage across the inner membranes and on the extracellular resistance. In tissues with substantial radial resistance, current flow

measured with voltage clamp methods cannot be interpreted as current flow through a single voltage clamped membrane.

Measurements of potential made with microelectrodes are measurements of the potential difference between the cytoplasm, at the tip of the microelectrode, and the bathing solution outside the lens, where the indifferent bath electrode is placed. This potential difference closely approximates the sum of the potential drop across the inner membrane (the trans-membrane potential) and the potential drop within the extracellular space inside the lens. The potential measured with microelectrodes gives the potential on only one side of the inner membranes; as such it does not estimate the trans-membrane potential, which depends on the potential on the inside and outside of the fiber membrane. If the extracellular space around a membrane is restricted, as it is in cardiac, skeletal and smooth muscle, and the lens, the potential in the extracellular space will be spatially non-uniform, even if the intracellular potential within the cytoplasm were uniform. Thus, spatial uniformity of cytoplasmic potential does *not* ensure or imply spatial uniformity of trans-membrane potential. Measurements of potential must be made on both sides of a membrane if one is to evaluate the spatial uniformity of trans-membrane potential. And only when the trans-membrane potential is spatially uniform can currents measured with voltage clamp techniques be directly interpreted as membrane currents.

The cytoplasmic potential in the lens has been shown to be uniform, away from the current microelectrode (Eisenberg and Rae, 1976). Delamere, Paterson and Holmes (1980b), and Duncan, Patmore and Pynsent (1981) have argued that such uniformity of cytoplasmic potential implies uniformity of trans-membrane potential across the inner membranes of the lens. This argument fails on logical grounds, as just discussed. Furthermore, Eisenberg et al. (1979), have shown that the impedance measurements of Mathias et al. (1979), imply a spatial non-uniformity of extracellular potential within the lens, even a spatial non-uniformity of the d.c. potential. The effect of such standing gradients in extracellular voltage is not well understood in electrophysiology; we are unaware of intracellular measurements or analysis of any tissue with such gradients. The standing gradients of potential will be accompanied by standing gradients of concentration, and standing currents; they may be accompanied by standing gradients of hydrostatic pressure and standing water flow. Current voltage relations of the lens, such as those measured in voltage clamp experiments, must be expected to depend on all these processes. Relating the non-linearity of such current voltage relations to membrane properties thus will require analysis of electrodiffusion (i.e. current flow, diffusive flow, and water flow) in the extracellular space within the lens. The required analysis is closely related to the analysis of concentration gradients in the lateral intercellular space of leaky epithelia. (See Weinstein and Stephenson, 1981, for a recent discussion and references to this complex and controversial subject.)

Recognition of these facts might avoid the wasteful recapitulation of history. As recently put by Mullins (1981): '...voltage clamp methods were applied to cardiac fibers long before an appropriate analysis of the errors that might be involved. Thus a substantial literature concerning artifacts was built up and only recently have there been realistic efforts to analyze ionic currents in cardiac fibers.'

Appendix 1. Polarization Capacitance

We present a simple analysis to show that, to a first approximation, accumulation of ions in a small space outside a membrane contributes a current analagous to a capacitive current through a membrane dielectric.

When ions carry current across a membrane into a restricted volume, the concentration of the current carrier will necessarily change. The change in concentration depends on the total number of ions which cross the membrane compared to the number which diffuse away from the restricted volume. If the current is large, or persists long enough, or if diffusion is sufficiently restricted, the change in concentration can have quite dramatic physiological effects (Frankenhaeuser and Hodgkin, 1956; Orkand, 1979).

The change in concentration accompanying current flow reduces the driving force for the movement of the ion, because of the inherent stability of electrochemical systems as described by 'The Principle of LeChatelier' (derived for equilibrium situations by Callen, 1960, and for non-equilibrium situations by DeGroot and Mazur, 1962). If a voltage is applied to a membrane, the flow of an ion will thus tend to decrease with time, as the concentration of that ion changes on each side of the membrane (most significantly on the side with restricted volume). The decrease in current will be similar to the decrease of current in a capacitor after voltage is applied. In the capacitor the accumulation of charge opposes the current flow; in the restricted volume, the accumulation of ions (i.e. the change in concentration) opposes the current flow. If the accumulation or depletion of ions is small, we can perform a linearized analysis and compute the circuit parameters, the resistors and capacitors, which model the accumulation process.

Assume that the membrane potential $v(t)$ is perturbed about the resting potassium equilibrium potential $\bar{E}_K$, i.e. $v(t) = \bar{E}_K + \delta v(t)$. Furthermore, assume that diffusion or convection of K^+ out of or into the restricted extracellular volume is negligible. Then, whatever ions cross the membrane, carrying current, change the concentration in the extracellular space. Accordingly, the change in extracellular potassium concentration is $\delta K_e(t)$, where

$$\delta K_e(t) = \frac{1}{F V_e} \int_0^t i_K(\hat{t})\, \mathrm{d}\hat{t}, \tag{A 1-1}$$

where V_e is the volume of extracellular space (assumed far more restrictive than the intracellular space on the other side of the membrane) and F is the Faraday constant.

Because the initial membrane potential is assumed to be the potassium equilibrium potential, the current is

$$i_K(t) = S_m\, g_K\{v(t) - E_K(t)\} = S_m g_K\{\delta v(t) - \delta E_K(t)\}, \tag{A 1-2}$$

where S_m is the surface of membrane enclosing the restricted volume.

The equilibrium potential varies with time because the potassium concentration varies.

$$E_K(t) = \bar{E}_K + \delta E_K(t) = \frac{RT}{F} \ln \frac{\bar{K}_E + \delta K_E(t)}{K_i} = \frac{RT}{F} \ln \frac{\bar{K}_e}{K_i} + \frac{RT}{F} \ln \left(1 + \frac{\delta K_e}{\bar{K}_e}\right), \tag{A 1-3}$$

where $\bar{K}_e$ is the resting potassium concentration, R is the gas constant and T the absolute temperature. If the change in potassium concentration is small, the logarithm can be expanded, giving

$$E_K(t) \simeq \bar{E}_K + \frac{RT}{F} \delta_{K_e} \equiv \bar{E}_K + \delta E_K. \tag{A 1-4}$$

Thus, the change in equilibrium potential is linearly related to the change in potassium concentration

$$\delta E_K \simeq \frac{RT}{F} \frac{\delta K_e}{\bar{K}_e}, \tag{A 1-5}$$

and the current is given by

$$i_K(t) = S_m\, g\, \delta v(t) - S_m g_K \frac{RT}{F} \frac{\delta K_e}{\bar{K}_e}. \tag{A 1-6}$$

Equation (A 1-1) relates the change in potassium concentration δK_e to the current flow i_K, giving

$$i_K(t) = S_m\, g_K\, \delta v(t) - S_m\, g_K \frac{RT}{F\bar{K}_E} \frac{1}{FV_e} \int_0^t i_K(\hat{t})\, d\hat{t}, \tag{A 1-7}$$

or

$$\delta v(t) = \frac{1}{S_m g_K}\, i_K(t) + \frac{RT}{F^2 \bar{K}_e V_e} \int_1^t I_K(\hat{t})\, d\hat{t}. \tag{A 1-8}$$

It is convenient to rewrite equation (A 1-7) in terms of the current density $J_K(t) \equiv i_K(t)/S_m$,

$$\delta v(t) = \frac{1}{g_K} J_K + \frac{S_m}{V_e} \frac{RT}{F^2 \bar{K}_e} \int_0^t J_K(\hat{t})\, d\hat{t}. \tag{A 1-9}$$

Equation (A1-8) describes the properties of the equivalent circuit with specific series resistance $1/g_K$ (units: ohm . cm^2) and specific polarization capacitance (S_m/V_e) $(RT/F^2\bar{K}_e)$ (units: F/cm^2).

Appendix 2. Time Course of Diffusion within the Extracellular Space of the Lens

The syncytial model of the lens (Eisenberg et al., 1979) can be used to estimate diffusion times within the extracellular space of the lens, just as distributed electrical models of the t-system have been used to estimate diffusion times in the t-system of skeletal muscle (Nakajima, Nakajima and Bastian, 1975; Kirsch, Nichols and Nakajima, 1977). In both cases, the effects of membrane permeation, water flow, and standing gradients of potential or pressure are ignored to make the problem tractable.

The essential relation between diffusion and current flow in the extracellular space is given by the Nernst–Einstein relation (Atkins, 1978),

$$D_e = \frac{RT}{F^2} \frac{1}{R_e C_e}, \tag{A 2-1}$$

where D_e is the effective diffusion constant of the ions in the extracellular space, R_e is the effective resistivity of the solution in the extracellular space; and C_e is the total concentration of the ions in the extracellular space. The effective diffusion constant and effective resistivity are related to the bulk diffusion constant and bulk resistivity by equation (A–1) of Mathias et al., 1979.

The diffusion of solute applied suddenly to the outside of a tissue has been analyzed by Hill (1928) and published in textbooks (Crank, 1975). If the diffusion constant of a solute within the extracellular space of the lens is taken as the diffusion constant in free solution times the tortuosity factor (Mathias et al., 1979), equation 6.18 Crank (1975) can be used directly.

$$\frac{C(t,r)}{C(0,a)} = \frac{a}{r} \sum_{n=0}^{\infty} \left\{ \operatorname{erfc} \frac{(2n+1)a - r}{2\sqrt{D_e t}} - \operatorname{erfc} \frac{(2n+1)a - r}{2\sqrt{D_e t}} \right\}, \tag{A 2-2}$$

where $C(t,r)$ is the concentration at radial location r and time t following application of a concentration $C(0,a)$ at the outside of a sphere at time zero; erfc is the complementary error function.

The plot of equation (A 2–2) given in Crank (1975, fig. 6.1) shows that the concentration of an ion with the free solution mobility of potassium reaches 80 % of its final value in a time of 30 min at a depth of 800 μm, in a lens of radius $a = 1{\cdot}6$ mm. If we assume that the surface density of membranes (surface per unit volume: S_m/V_T; units cm^2/cm^3) is more or less uniform within the lens, it is easy to estimate how much membrane is within 800 μm of the surface. Because the volume of a sphere varies with the cube of radius, only a small fraction of the membranes, in fact $12{\cdot}5\,\% = [(S_m/V_T) \cdot (4/3)\,\pi(a/2)^3] \div [(S_m/V_T)\,(4/3)\,\pi a^3]$, are further than a half-radius from the outside. Thus, solute applied to the outside of the lens reaches nearly 90 % of the membranes within 30 min.

ACKNOWLEDGEMENT

It is a pleasure to acknowledge the programming assistance of Ms J. Rae and to thank Dr B. Eisenberg for her critical reading of the manuscript.

The work was supported principally by NIH grants EY 03095, EY 03282 and HL 20230. and the Louise C. Norton Fund.

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