Human Lens Membrane Cation Permeability Increases with Age

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Parallel studies of the ionic balance and membrane permeability characteristics of normal human lenses were carried out in three countries (USA, England, and Italy). Similar age-related changes were found in each laboratory. The lens membrane potential and resistance declined markedly with age while internal Na⁺ and free Ca²⁺ increased. There was a concomitant stimulation of Na⁺ and K⁺ transmembrane fluxes. These data indicate that in the ageing process there is an increasing contribution to membrane ionic traffic from a channel, or channels, that permit Na⁺, K⁺ and Ca²⁺ to pass. The increase in permeability coincides exactly with the increase in optical density that occurs in the ageing human lens. Invest Ophthalmol Vis Sci 30:1855–1859, 1989

Changes in internal tissue cation content occur in a number of age-related diseases, including cataract¹ and arteriosclerosis.² In the commonly occurring cortical cataract, for example, there is a progressive alteration of ion content with Na⁺ and Ca²⁺ increasing together.³ Both the Na⁺ and K⁺ permeability of lenses with this type of cataract appear to have increased.⁴⁻⁵ Little is known, however, of the changes in membrane physiology that occur normally with age and predispose the tissue to an eventual decline in function. The lens would appear to provide an excellent model to study ageing since not only can function be accurately assessed both in vivo and in vitro by photographic methods, but it is uniquely accessible for physiological studies. For example, the whole organ can easily be removed from donor eyes and it survives well in culture.⁵⁻⁶ It has, moreover, minimal extracellular space so cellular ion concentrations can readily be measured. As all of its cells are well coupled electrically, the membrane properties of the whole lens can be conveniently measured using only two internal microelectrodes.⁷

In this report we present data obtained in three laboratories concerning the changes in membrane permeability and cation content that accompany the ageing process in the normal human lens. The underlying membrane permeability changes explain why the human lens becomes increasingly susceptible to cortical cataract with age.

Materials and Methods

Lens Preparation

Donor eyes were obtained within 1–24 hr of death and after dissection lenses were placed in artificial aqueous humor (AAH) solution (35°C) with the following composition: NaCl, 130 mM; KCl, 5 mM; MgCl₂, 0.5 mM; CaCl₂, 1 mM; NaH₂CO₃, 10 mM; glucose, 5 mM; buffered with 10 mM HEPES to pH 7.3. Any lenses with visible opacities were discarded. Cataract lenses were placed in AAH immediately after removal by cryosurgery.

Electrical Measurements

Lenses were placed anterior face down in a perspex chamber and continuously perfused with AAH (35°C). Two microelectrodes were inserted through the posterior capsule and the two voltage readings obtained never differed by more than 5 mV. The individual voltages were stable during several hours of perfusion.

One of the electrodes was then used to inject current into the lens in order to measure the membrane resistance. Because of the importance of junctional pathways in the lens⁶ it is necessary to measure lens impedance over a wide frequency range. Sine waves of current in the frequency range 0.01 to 100 Hz were injected and the impedance calculated from the amplitude and phase relationships of the resulting voltage responses.⁸

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Supported by SERC (UK), NIH Grant EY-03681-09 (USA), CNR and MPI (Italy), and by travel Grants from EURAGE and NATO.

Submitted for publication: November 15, 1988; accepted January 10, 1989.

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In order to measure free calcium an additional electrode was inserted into the lens with the tip filled with calcium-sensitive resin. The membrane-recording electrode served as a reference and the free calcium was computed from electrode calibration graphs.9

Flux Measurements

For 22Na influx studies, individual lenses were placed in 36 cm² petri dishes and incubated for 2 hr at 35°C in AAH solution. They were then removed from the solution, blotted on filter paper, weighed and homogenized in 5% TCA. After centrifugation, the lenses were assayed by liquid scintillation techniques and the influx calculated as described previously.9 For 86Rb efflux studies, lenses were preincubated for 4 hr in AAH containing 1 μCi/ml of 86Rb as a tracer for potassium. They were then transferred to nonradioactive AAH to obtain the efflux kinetics. The nonradioactive solution was changed every 10 min and the efflux rate (min⁻¹) was determined over a 2 hr period.6

Ion Analyses

Lenses were perifused with AAH for approximately 4 hr during electrophysiological or radiotracer experiments. They were homogenized in 5% TCA, centrifuged as described above and Na⁺ and K⁺ measurements were carried out on the supernatant by flame photometry. The dried pellet was used to calculate the weight of water in the lens and the results expressed on a mM basis.

Results

The membrane potential of the isolated, perifused human lens declines from a value of approximately -50 mV at 20 years to about -30 mV at 60 years of age (Fig. 1A). Accompanying this depolarization is an increase in lens sodium content from around 25 mM at 20 years to approximately 40 mM at 70 years (Fig. 1B). Interestingly, the K⁺ content remains relatively constant throughout the ageing process. From the ion concentration and voltage data it is possible to predict the underlying changes in the sodium–potassium permeability ratio. The relationship between membrane
potential \((E_m)\), permeability \((P_K\) and \(P_{Na}\)) and ion concentration is given by the Goldman equation\(^{10}\):

\[
E_m = -\frac{RT}{F} \ln \frac{P_K K_o + P_{Na} Na_0}{P_K K_o + P_{Na} Na_0}
\]

The values for \(E_m\) were obtained from Figure 1A and the Parma ion concentration data from Figure 1B were used as both sodium \((Na_0)\) and potassium \((K_o)\) were assayed on the same lens. \(Na_0\) and \(K_o\) are defined by the AAH solution and RT/F has the value 25.4 mV at 35°C.

The Goldman calculation (Fig. 1C) shows that there is a 6-fold increase in \(P_{Na}/P_K\) between the ages of 20 and 80 years. The curve passing through the data is taken directly from Weale\(^{11}\) and is in fact the change in optical density of the human lens measured at 490 nm.

The ionic mechanisms contributing to the membrane ageing processes were investigated directly by measuring \(K^+\) \((^{86}\text{Rb})\) and \(Na^+\) \((^{22}\text{Na})\) fluxes. In the isolated lens it is equally convenient to measure both influx and efflux. However, in common with most cells, the lens \(Na^+\) efflux and \(K^+\) influx are largely active processes, while \(Na^+\) influx and \(K^+\) efflux mainly take place by passive diffusion.\(^5\)\(^6\) In order, therefore, to estimate lens permeability, the \(^{22}\text{Na}\) influx and \(^{86}\text{Rb}\) efflux were measured over a 2 hr period and in both cases an increase in ion movement occurs with age (Fig. 2A, B). The stimulated fluxes do not appear to be due to a change in extracellular space as we have found that the \(^{14}\text{C}\) sucrose space is identical in 20- and 80-year-old lenses (data not shown). Instead, the data suggest that increases in both \(Na^+\) and \(K^+\) permeability have occurred. The ion concentration values, however, only show a significant change in \(Na^+\) (Fig. 1B). This is to be expected, as \(Na^+\) ions are distributed much further from electrochemical equilibrium than \(K^+\). A change in \(Na^+\) permeability will therefore produce a proportionately much greater increase in ion content than a comparable change in \(K^+\) permeability.

A further estimate of the overall changes in membrane permeability with age was obtained by measuring the electrical resistance directly. The lens is ideal for such a study as all of the fiber cells are electrically coupled and therefore current injected into one point spreads through every cell in the lens.\(^7\) We found that an age-related decrease in lens resistance occurred in the low frequency range where the membrane, rather than gap junction resistance dominates the response (Fig. 3). The resistance data therefore reinforce the ion flux measurements, again indicating an overall increase in cation permeability with age. The most economical explanation for this increase is the activation of a cation channel that would permit both sodium and potassium to pass.

There is evidence for such "nonspecific" cation channels in a wide range of lens species\(^{12}\) and this type of channel also allows \(Ca^{2+}\) to enter.\(^13\) We therefore looked for possible changes in internal calcium by inserting into the lens an additional calcium-sensitive microelectrode. The free calcium values for lenses below the age of 40 were uniformly low, but lenses above that age had, in general, increased free calcium (Fig. 4).
the lens appear to be most pronounced after the age of 40. These include a decrease in lens glutathione, an increased temperature sensitivity of glutathione reductase and an accelerated decline in the accommodative amplitude of the lens as well as a general increase in optical density and light scatter. The membrane potential and free calcium data from the current study support these observations (Figs. 1A, 4) and indeed not only do our present permeability ratio data and Weale’s optical density data show the same trend with age but they are superimposable using the same axes (Fig. 1C)! This congruence of two quite different sets of data suggests either that one common mechanism is responsible for both or that the alteration in cation distributions resulting from the increase in permeability ratio actually initiates the rise in optical density. The change in O.D. could follow either from an increase in absorbance or from a combination of absorbance and scatter.

We propose that the permeability ratio $P_{Na}/P_K$ increases not because of a decrease in $P_K$ but rather because of a relative increase in $P_{Na}$ through the age-related activation of nonspecific cation channels. These channels can be activated by membrane deformation and, more interestingly in view of the change in lens membrane oxidation state with age, by membrane sulphydryl group oxidation. Since calcium can move through nonspecific cation channels, the increase in free calcium that we observed could occur through two processes, both resulting from the increasing activation of a nonspecific cation channel. The first would simply be the direct entry of Ca through this pathway, but a secondary increase could also occur in exchange for Na that has previously entered through the channel.

Calcium plays a critical role in cataract, as it does in a number of diseases, and in the lens the molecular mechanisms have begun to be elucidated: opacification follows calcium-induced aggregation and proteolysis. Since internal calcium concentration and cation permeability would appear to be related, these mechanisms may well explain the increase in optical density and light scatter that occur with age in the normal lens.

We have therefore shown that comparable aging data can be obtained in three countries. More interestingly, however, the data indicate that the major ionic events in cortical cataract, namely the increase in Na and Ca are initiated in many noncataractous lenses as a result of aging. The alterations become increasingly apparent after the age of 40 and we suggest that they could occur as the result of a common membrane mechanism, namely, the increased activation of a nonspecific cation channel. As similar channels have been reported in a very wide range of

**Discussion**

It has previously been noted that many of the age-related optical and biochemical changes that occur in

![Fig. 3. Insert. Typical response of the resistance of the lens as a function of frequency. The high-frequency response is dominated by the cytoplasmic and bath (bulk) resistances, while the low-frequency response contains additional resistance and capacitance contributions predominantly from the superficial membrane system. See refs. 7 and 8 for further details.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933379/)

**Main Graph.** Resistance of the human lens as a function of frequency. The membrane resistance can be computed from the difference between the resistance at 0.01 Hz and that obtained at the highest frequencies (>10 Hz). Four other lenses (age 20-65 years) measured fitted exactly with this trend. The data in this case were obtained in Norwich, England.

![Fig. 4. Free calcium in the human lens as a function of age. The data were obtained in Rochester, Michigan.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933379/)
tissues, we suggest that they provide a major route for age-related Ca\(^{2+}\) overload.

**Key words:** human, lens, membrane, age, permeability

**Acknowledgments**

We wish to thank the staff of the Hospital Ophthalmology Departments in the three countries for their assistance in providing donor eyes. We also acknowledge rewarding discussions with Dr. Peter C. Croghan.

**References**