Cell to Cell Communication and pH in the Frog Lens

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ABSTRACT Fiber cells of the lens are electrically and diffusionally interconnected through extensive gap junctions. These junctions allow fluxes of small solutes to move between inner cells and peripheral cells, where the majority of transmembrane transport takes place. We describe here a method utilizing two intracellular microelectrodes to measure the cell to cell resistance between fiber cells at any given distance into the intact lens. We also use ion-sensitive microelectrodes to record intracellular pH at various depths in the intact lens. We find that gap junctions connecting inner fiber cells differ in pH sensitivity as well as normal coupling resistance from those connecting peripheral cells. The transition occurs in a zone between 500 and 650 μm into the lens. Fiber cells peripheral to this zone have a specific coupling resistance of 1.1 Ωcm², whereas those inside have a specific coupling resistance of 2.7 Ωcm². However, when the cytoplasm of fiber cells is acidified by bubbling with CO₂, peripheral cells uncouple and the cell to cell resistance goes up more than 40-fold, whereas junctions inside this zone are essentially unaffected by changes in intracellular pH. In a normal frog lens, the intracellular pH in fiber cells near the lens surface is 7.02, a value significantly alkaline to electrochemical equilibrium. Our data suggest that Na/H exchange and perhaps other Na gradient-dependent mechanisms in the peripheral cells maintain this transmembrane gradient. Deep in the lens, the fiber cell cytoplasm is significantly more acidic (pH, 6.81) due to influx of hydrogen across the inner fiber cell membranes and production of H⁺ by the inner fiber cells. Because of the normally acid cytoplasm of interior fiber cells, their loss of gap junctional sensitivity to pH may be essential to lens survival.

INTRODUCTION

The primary function of the lens is to focus light on the retina. It is able to do so because of its shape, high index of refraction, and lack of light-scattering elements. Blood vessels and intracellular organelles scatter light, so the lens has evolved a physiological system (reviewed in Mathias and Rae, 1985, 1989) in which its fiber cells are able to survive without these structures. In brief, the surface epithelial and differentiating fiber cells provide the majority of the transport. They extrude sodium,
take in potassium, and regulate pH and calcium. The inner fiber cells are connected by gap junctions to these peripheral cells, thus allowing fiber cells to maintain transmembrane ion gradients, a resting voltage, and constant volume. Hence, gap junctions are essential to the survival of inner cells.

Active transport by surface cells and the tightly coupled syncytium of fiber and peripheral cells confer on the lens some properties like those of a giant single cell. However, a single cell is small (micrometer distances) and relies on simple intracellular diffusion of small solutes transported across its surface membrane. The lens is much larger (millimeter distances) and diffusion through gap junctions from surface to the inner cells is slow. Moreover, diffusion over long distances requires a significant concentration difference. Circulating ionic currents (Kinsey and Reddy, 1965; Candia, Bentley, and Mills, 1971a; Robinson and Patterson, 1983; Parmalee, 1986) driven by standing voltage gradients (Mathias and Rae, 1985; Candia, Bentley, Mills, and Toyofuku, 1971b) augment simple diffusion in the lens. Mathias (1985) suggested that fluid flow also assists in moving solutes through the lens. Nevertheless, standing concentration gradients for intracellular ions have been recorded (Paterson, 1972, 1973), and ions like hydrogen (Bassnett and Duncan, 1985) or calcium (Jacob, 1983), which are known to affect gap junctions, are at different concentrations in fiber cells at different depths within the lens. Moreover, the structure of lens gap junctional proteins changes as one looks from the peripheral cells to central cells (Lo and Harding, 1986; Takemoto, Takehama, and Horwitz, 1986; Kistler and Bullivant, 1987).

The purpose of this paper is to describe some of the functional properties of lens fiber cell gap junctions. Because of the above described variation in both environment and structure of junctions, we used a unique frequency domain impedance technique to study the junctions at specific locations within the intact lens.

Preliminary accounts of various aspects of this work have appeared in abstract form (Mathias and Rae, 1986; Mathias, Riquelme, and Rae, 1988).

METHODS

Small grass frogs (Rana pipiens) were killed and the lenses were dissected as described in Mathias, Rae, and Eisenberg (1979). The intact lens is left suspended by its zonules to retinal and scleral tissue, which is cut into four flaps that are subsequently pinned into a Sylgard-lined bath. As described in Mathias, Rae, Ebihara, and McCarthy (1985), the bath could be exchanged in ~3 s.

The composition of solutions is given below in millimolar. Normal Ringer: 108.5 Na⁺, 2.5 K⁺, 118 Cl⁻, 2 Ca²⁺, 1.5 Mg²⁺, and 0.1 PO₄³⁻; pH 7.4. High Ca²⁺ Ringer: 78.5 Na⁺, 2.5 K⁺, 124 Cl⁻, 20 Ca²⁺, 1.5 Mg²⁺, 5 HEPES, titrated to pH 7.4 with NaOH. pH calibration: 10 Na⁺, 100 K⁺, 110 Cl⁻, 5 HEPES, titrated to pH 6.2 or 8.0 with NaOH. Voltage electrode: 2,500 K⁺, 2,500 Cl⁻. Current electrode: 2,500 K⁺, 2,500 acetate.

Impedance Studies

Two intracellular microelectrodes of 3–10 MΩ resistance were positioned so that the current-passing electrode was as close to the center of the lens as possible and the voltage-recording electrode was at a known depth and angular distance from the anterior or posterior pole. A differential voltage electrode was placed in the bathing solution to subtract voltage drops in the bath and bath electrode. We utilized the circuits described in Mathias, Rae, and Eisenberg
A random current of selected average bandwidth was generated using a noise generator (model 3722A; Hewlett-Packard Co., Palo Alto, CA). The voltage and current waveforms were digitized on a Fourier analyzer (model 5420A; Hewlett-Packard Co.) and the frequency domain impedance was computed in real time. Data were collected as described in Mathias et al. (1981). We usually recorded three impedance data sets plus a calibration curve. The Fourier analyzer records and averages 256 frequency lines, which we selected to be between 0 and 12, 100, or 800 Hz. The calibration curve was 0-800 Hz. The impedance and calibration data were subsequently transferred to an IBM PC-AT for analysis.

Our data analysis procedure has changed very little from the procedure described in Mathias et al. (1979, 1981, 1985). The 800-Hz data were corrected using the calibration curve. The three data sets were then merged into one set, which was approximately equally spaced on a log frequency scale from 0.08 to 800 Hz. We collected many more frequency lines than were needed to specify our model; hence, we dropped the majority of the data during the merging process and used just 128 frequency lines for curve fitting. The merged data were properly scaled, transformed to magnitude and phase format, and wild point edited using a manual cursor--controlled program. Data at 60-Hz multiples are usually several standard deviations off of the curves, so we manually identified these points and the computer replaced them with the average value of several neighboring points on either side.

The final magnitude and phase impedance data were analyzed by one of two methods. In those designs to simply measure changes in cell to cell coupling, we used the magnitude at 800 Hz to determine the effective intracellular resistivity as described in the Theory section. In many circumstances we were interested in determining the fiber cell and surface cell membrane conductances. In these studies we used the Levenberg-Marquardt nonlinear curve-fitting algorithm (IMSI Inc., Houston, TX) as described in Mathias et al. (1979) to minimize the sum square error of impedance data minus theory (magnitude and phase at 128 frequency points between 0.08 and 800 Hz) by adjusting the parameters in the model. Mathias et al. (1979) describe the various tests we have applied to ensure that the curve-fitting procedure provides reliable estimates of the parameter values.

**Intracellular pH Studies**

Single-barreled microelectrodes were pulled from 1.5 mm o.d. microfilament glass (#6030; A-M Systems, Inc., Everett, WA). Immediately before pulling, the glass was thoroughly cleaned (Munoz, Deyhimi, and Coles, 1983). We first washed the glass pipettes in nitric acid, then sonicated them in ethanol for 2 rain, rinsed and boiled them in filtered distilled water, and finally dried them in an oven. The microelectrodes were silanized as described in Dresdner, Kline, and Witt (1989). In a covered beaker they were exposed to vapor of trimethylchlorosilane (Sigma Chemical Co., St. Louis, MO) for 15 min. They were then removed and baked for 2 h at 200°C. The silanized micropipette was back-filled with the pH-sensitive liquid ion exchange resin (World Precision Instruments, New Haven, CT) to a distance 200-500 μm from the tip. The filling process was monitored at ×400 magnification. Those electrodes that properly filled with resin were back-filled from a syringe with 2.5 M KCl (pH 7); their resistance was 1-4 GΩ.

Special circuits were needed to interface with the high resistance resin-filled electrodes. We fabricated a simple follower circuit (see the differential amplifier in Fig. 1 of Mathias et al., 1979), which utilized an OPA 111 (Burr Brown, NY) FET input operational amplifier (input bias current ≤ 1 pA and input resistance ~ 10⁶ GΩ). A similar circuit was used to interface with a second, normal KCl-filled microelectrode used to record the intracellular voltage. Each
electrode was connected to a resistor by a relay-controlled switch so a pulse of current could be passed at any time during the experiment and the resistance of the electrodes determined. Driven shields around the microelectrodes enhanced the bandwidth, which was at least 10 Hz (<20 ms response time constant).

The pH-sensitive microelectrodes were calibrated before and after the experiments. We used four solutions: normal Ringer, pH 7.4; pH calibration solutions, pH 6.2 and 8.0; and normal Ringer bubbled with 100% CO₂, pH 5.8. The average slope for the 14 electrodes we used was 53 ± 3 (SEM) mV per 10-fold pH change. The initial and final voltages in each calibration solution were always within 10 mV, or ±5 mV, from the average.

The pH-sensitive microelectrode and a voltage-recording microelectrode were inserted into opposite sides of the lens 45° from the posterior pole and positioned at the same distance from the surface. The voltage microelectrode recorded the resting potential and the difference between the voltage and pH-sensitive electrodes gave the intracellular pH.

**THEORY**

Equations for radial current flow in a spherically symmetric syncytial tissue (Eisenberg, Barcilon, and Mathias, 1979) are based on the equivalent circuit shown in Fig. 1. For simplicity, the effects of spherical convergence are not shown. The current I (amps) is injected into a cell at the center of the lens, r = 0. Current flows to the surface along two paths: \( R_i \) (\( \Omega \) cm) is the effective resistivity of the intracellular path, where current flows from cell to cell through gap junctions; \( R_e \) (\( \Omega \) cm) is the effective resistivity of the extracellular path between cells, where current follows a narrow tortuous path to the surface. The intracellular and extracellular compartments are separated by the fiber cell membranes whose specific conductance is \( g_m \) (S/cm²) and capacitance is \( \varepsilon_m \) (F/cm²). At the surface of the lens, \( r = a \), the extracellular space is

**FIGURE 1.** The equivalent circuit for radial current flow in a spherically symmetric syncytial tissue. For simplicity, the spherical convergence of the structures is not shown. Current is injected into a cell at the lens center \( (r = 0) \). Current flows radially to the surface \( (r = a) \) from cell to cell through gap junctions along the resistive path \( R_i \). At the surface, intracellular current must cross the surface cell membranes \( (G_s + j\omega C_s) \) to flow to the bath electrode. Current can also cross the membranes of the inner cells \( (g_m + j\omega \varepsilon_m) \), enter the extracellular compartment, and flow radially to the bath along the small tortuous extracellular spaces indicated by the resistive path \( R_e \).
open to the bath, where we assume the potential is zero, whereas the intracellular
compartment is insulated by the surface cell membranes, whose specific conductance
is $G_s$ (S/cm$^2$) and capacitance is $C_s$ (F/cm$^2$). The current density in the intracellular
and extracellular space, respectively, is:

$$i_i = -\frac{1}{R_i} \frac{d\psi_i(r)}{dr} \quad \text{(amp/cm$^2$)} \quad (1)$$

$$i_e = -\frac{1}{R_e} \frac{d\psi_e(r)}{dr} \quad \text{(amp/cm$^2$)} \quad (2)$$

where $\psi_i(r)$ (volts) is the voltage in the intracellular, extracellular space at a distance
$r$ (cm) from the lens center. Total current is

$$I = -4\pi^2 \left[ \frac{1}{R_i} \frac{d\psi_i}{dr} + \frac{1}{R_e} \frac{d\psi_e}{dr} \right] \quad \text{(amp)} \quad (3)$$

The transmembrane current equals the change in current per unit length along the
extracellular space. In spherical geometry this is given by

$$\frac{1}{r^2} \frac{d}{dr} \left( r^2 \frac{1}{R_e} \frac{d\psi_e}{dr} \right) = \frac{S_m}{V_i} \left( g_m + j\omega C_m \right) \left( \psi_e - \psi_i \right) \quad (4)$$

where $S_m/V_i$ (cm$^{-1}$) is the surface of membrane per unit volume of tissue and $j\omega$ (s$^{-1}$)
is the Fourier transform variable: $j = \sqrt{-1}$ and $\omega$ (rad/s) is angular frequency.

At the lens surface

$$\psi_e(a) = 0 \quad (5)$$

$$-\frac{1}{R_i} \frac{d\psi_i(a)}{dr} = (G_s + j\omega C_s)\psi_i(a) \quad (6)$$

The solution for the intracellular voltage depends on two terms, one that depends
on frequency and one that is frequency independent but depends on the location
within the lens.

$$\psi_i = Iz_i(j\omega, r) \quad (7)$$

$$Z_L = Z_m(j\omega) + R_s(r) \quad (8)$$

Eisenberg et al. (1979) derived $Z_i$ when $R_i$ and $R_s$ are constants. Mathias et al. (1981)
extended this analysis to allow $R_i$ to take on two values, one in the interior
($r \leq 0.67a$) and one in the periphery ($r > 0.67a$). Both of these studies used a
perturbation approach based on $R_i/(R_i + R_s)$ being a small parameter everywhere.

Under these conditions,

$$Z_m = \frac{1}{4\pi^2(G_s + j\omega C_s + y_e)} \quad (\Omega) \quad \frac{R_i}{R_i + R_s} \to 0 \quad (9)$$

$$y_e = \frac{\gamma}{R_i + R_s} \left( \coth \gamma a + \frac{1}{\gamma a} \right) \quad (\text{S/cm}^2) \quad (10)$$
Based on many previous impedance studies (Mathias et al., 1979, 1981; reviewed in Mathias and Rae, 1985) as well as data reported in this paper, typical values for the components in Fig. 1 are:

\[ G_s = 5 \times 10^{-4} \text{ S/cm}^2 \]
\[ g_m = 5 \times 10^{-7} \text{ S/cm}^2 \]
\[ R_i = 3.5 \times 10^5 \text{ cm} \]
\[ C_s = 5 \times 10^{-6} \text{ F/cm}^2 \]
\[ c_m = 1 \times 10^{-6} \text{ F/cm}^2 \]
\[ R_e = 50 \times 10^3 \text{ Om} \]

For \( r \leq 0.67a \), Mathias et al. (1981) found that \( R_i \) increased by a factor of 2 to 3.

In the studies reported in this paper, we induce uncoupling of fiber cells and cause \( R_i \) to become rather large, so the perturbation approach is not valid. Moreover, we find that \( R_i(r) \) is a continuous function of radial location. Under these conditions we could not find generally valid approximate solutions to these equations; however, at high frequencies the membrane impedance goes to zero and the following approximations are general and useful:

\[ \psi_e(r) \rightarrow \psi(r), \quad \omega \rightarrow \infty \] (12)

If Eq. 12 is substituted into Eq. 3, we find

\[ \frac{d \psi_i}{dr} = -I \frac{R_R}{4\pi r^2(R_i + R_e)} \quad \omega \rightarrow \infty \] (13)

The boundary conditions at the surface (Eqs. 5 and 6) become

\[ \psi(a) \rightarrow \psi_e(a) = 0 \quad \omega \rightarrow \infty \] (14)

Integration of Eq. 13 with boundary condition Eq. 14 yields

\[ \psi(r) = -I \int_c^a \frac{R_R}{4\pi(R_i + R_e) r^2} dr = IR_s(a, r) \quad \omega \rightarrow \infty \] (15)

From the mean value theorem,

\[ R_s(a, r) = \frac{R_R}{R_1 + R_e} \frac{1}{4\pi} \left( \frac{1}{r_1} - \frac{1}{a} \right) \] (16)

where \( R_1 (\Omega cm) \) is the value of \( R_i(r) \) for some \( r \) in the interval \((r_1, a)\). In a normal lens with a relatively constant \( R_i \ll R_e \), Eq. 16 simplifies to the point source series resistance defined in Eisenberg et al. (1979) or Mathias et al. (1981).

Eq. 16 is most useful when the interval \((r_1, a)\) is sufficiently small so that \( R_i \) is representative of the value of \( R_i(r) \) everywhere on the interval. To estimate \( R_i \) at locations deep within the lens, we measured the intracellular voltage at two different radial locations that are close together. At high frequencies, the voltage difference asymptotes to

\[ \psi(r_2) - \psi(r_1) = IR_s(r_1, r_2) \quad \omega \rightarrow \infty \] (17)
Assuming $r_1 > r_2$ so $R_s(r_1, r_2)$ is positive, we have

$$R_s(r_1, r_2) = \frac{R_z R_e}{R_z + R_e} \frac{1}{4\pi} \left( \frac{1}{r_2} - \frac{1}{r_1} \right)$$

(18)

where $R_z$ ($\Omega\text{cm}$) is the value of $R_i(r)$ for some $r$ in the interval $(r_2, r_1)$.

Eqs. 12–18 are valid without regard to the size of $R_j/(R_i + R_e)$ or radial variation in $R_i(r)$ or $R_e(r)$. The form of $Z_m$ in Eq. 8 is also generally valid; however, when $R_i(r)$ and $R_e(r)$ vary with radial location, we could not find an expression for $Z_m$.

**RESULTS**

The magnitude of the input impedance of the lens, $|Z_L(j\omega, r)|$, is defined as the peak intracellular voltage divided by peak injected current, when both are sinusoids of frequency $\omega = 2\pi f$. We inject the current into a cell near the lens center and record the voltage in a different cell at distance $r$ cm from the center. Fig. 2 illustrates $|Z_L|$ at $r = 0.9a$ as a function of frequency in either normal conditions or after 20 h in high calcium Ringer (A), or after 30 min in normal Ringer solution bubbled with 100% CO$_2$ (B). The dotted lines in Fig. 2 illustrate $R_z(a, r)$ in each condition. Eq. 16 of the Theory section relates $R_s$ to the effective intracellular resistivity $R_i$, which depends on cell to cell coupling by gap junctions.

The results in Fig. 2 A were the longest study we performed in high calcium. The uncoupling of cells was not large, whereas the deterioration of the lens was apparent. For the data in Fig. 2 A, the value of $R_i$ increased from 3.7 kΩ·cm to 6.8 kΩ·cm over a period of 20 h. The membrane-dependent component of the impedance, $Z_m$ in Eq. 8 or 9, dropped considerably. The input resistance ($Z_m(0)$) started at 8 kΩ but dropped to 2.65 kΩ, and the resting potential went from $-71$ to $-57$ mV over the course of the study. Moreover, the appearance of the lens was dramatically affected by the high calcium. Over the first few hours the lens became opaque, and by the end of the experiment it appeared that substantial cell death had occurred. Under similar conditions, Jacob (1983) reported a rise in intracellular calcium from 0.7 to 260 μM and uncoupling of fiber cell junctions. Our observations suggest that the rise in intracellular calcium is but one of several effects, many of which are irreversible.

From these data and the data in Fig. 3 we conclude that the effects of calcium on lens gap junctions is relatively small in comparison to pHi, at least over a range of intracellular concentrations of up to a few hundred micromolar.

Fig. 2 B shows the dramatic (and reversible) effect of bubbling the bathing solution with CO$_2$. Under these conditions, the bath pH drops to $\sim 5.8$, but more importantly, the CO$_2$ readily crosses cell membranes and causes acidification of the cytoplasm (see Fig. 6). The total impedance increases at all frequencies, primarily because $R_i$ increases from 200 to 5,200 Ω. In the normal Ringer solution the value of $R_i$ is 2.7 kΩ·cm (from Eq. 16 with $r = 0.14$ cm, $a = 0.16$ cm, and $R_e = 51$ kΩ·cm), whereas in 100% CO$_2$ the parallel resistivity of $R_i$ with $R_e$ is 60 kΩ·cm, so $R_i$ has increased enormously, $R_i \gg 60$ kΩ·cm, and $R_e = 60$ kΩ·cm. The membrane-dependent component of the impedance increased slightly and $Z_m(0)$ went from 5 kΩ in normal Ringer to 6.1 kΩ in 100% CO$_2$. This small increase in the membrane-dependent...
component of the lens resistance may actually reflect a reduction in the length constant for extracellular current flow due to the increase in $R_i$ and $R_e$ (analogous to decreasing $y_e$ in Eq. 10). In contrast to the small increase in $Z_m(0)$, the lens depolarizes significantly, going from $-64$ mV in normal Ringer to $-24$ mV in 100% CO$_2$. Mathias et al. (1985) suggested that the fiber cell membranes were selective for sodium and chloride and had an unusually small conductance for potassium. If so, then the negative resting voltage normally recorded in the fiber cells is due to coupling to the outer epithelial and differentiating cells, which have a relatively high potassium conductance. By closing the gap junctions with 100% CO$_2$, we may be uncoupling the K$^+$-selective cells without actually blocking K$^+$ channels. In this way a

![Figure 2](figure2.png)
large depolarization can occur without much change in membrane conductance; instead, the depolarization is due to the increase in $R_i$.

**Calcium Effects on Fiber Cell Coupling**

Fig. 3 A summarizes our data on lenses soaked in high Ca$^{2+}$ Ringer solution. As can be seen, the rate of uncoupling is slow and not monotonic, and the degree of uncoupling is much less than that in low pH. To achieve significant uncoupling, we had to wait ~6 h, a time that allowed irreversible damage to occur. We used the Ca$^{2+}$ iontophore A23187 to enhance the effect, but Fig. 3 B shows that the degree of uncoupling is still relatively small. The value of $R_i$ approximately doubles in 6 h; however, in the one experiment where we waited 10 h (dashed line in Fig. 3 B), there was little further increase in $R_i$. In one study, we used a pH-sensitive microelectrode during the exposure to 20 mM Ca$^{2+}$ with A23187 (data not shown). We detected no significant change in pH over a 1-h period, but small changes in pH over long time periods are difficult to detect with this method. In summary, owing to irreversible damage induced by long-term exposure to high bath Ca$^{2+}$, we could not reliably study the effect of elevated intracellular Ca$^{2+}$ on $R_i$ in intact lenses. We do conclude, however, that our data on pH-induced uncoupling are unlikely to be related to changes in [Ca].
pH Effects on Fiber Cell Coupling

Fig. 4A summarizes our data on coupling of peripheral fiber cells in lenses exposed to Ringer solution bubbled with 100% CO₂. In normal Ringer, \( R_{ri}/(R_{ri} + R_e) \approx R_i = 3.5 \text{kf} \text{cm} \). On exposure to 100% CO₂, \( R_{ri}/(R_{ri} + R_e) \) increases to an average value of 120 kΩ cm in a period of \( \sim 50 \text{ min} \). This requires that \( R_e \) must increase by a factor of 2 to 3, probably owing to fiber cell swelling and the accompanying reduction in volume of the extracellular space, and \( R_i \) has increased enormously to a value \( \gg 120 \text{kf} \text{cm} \) (i.e., a \( >40 \)-fold increase). Fig. 4B illustrates that the CO₂ effect on \( R_i \) is \( >95 \)% reversed after \( \sim 80 \text{ min} \) back in normal Ringer. Exposure to 100% CO₂ also caused the lenses to reversibly depolarize from \(-72 \text{ mV} \) in normal to \(-29 \text{ mV} \) in 100% CO₂. The depolarization followed the time course of the change in \( R_i \) (see Fig. 6A).

The average radius of the lenses we used for these studies was \( a = 0.168 \text{ cm} \), so the average point of recording was at \( r = 0.149 \pm 0.013 \text{ cm} \), which we round off to \( \sim 0.9a \). \( (B) \) An extended study from one lens showing the onset and reversibility of the 100% CO₂ effect.
the high frequency magnitude of the impedance at two neighboring depths, as indicated in Eq. 17, to obtain the average value of $R_i R_e/(R_i + R_e)$ between the two locations. The spatial resolution is thus ~200 μm. The amplitude is determined by subtracting two large resistances to obtain a relatively small one, so the results are subject to significant variance. Nevertheless, the data in Fig. 5 strongly suggest that the pH effect on $R_i$ does not extend beyond ~600 μm into the lens, regardless of how long we waited (in one experiment we waited 12 h but most of the data were recorded 1.5 h after exposure to CO₂).

**Spatial Variation in Cell to Cell Coupling**

Fig. 5 A shows the dependence of $R_i R_e/(R_i + R_e)$ on radial location in 100% CO₂. The data were recorded from five different lenses. In two lenses the voltage-recording microelectrode was inserted at 45° from the posterior pole and advanced toward the lens center. In the other three lenses, the point of insertion was 45° from the anterior pole. The lens is symmetric about an axis that goes from anterior to posterior pole, so the data are equivalent to measuring in a single lens along a line that enters the lens at 45° from the posterior pole, passes through the center, and exits at 45° from the anterior pole. To within the resolution of our measurement, the CO₂ effect seems to be the same in the anterior and posterior hemispheres, so we present all of our data as a function of distance from the lens center in Fig. 5 B.

Fig. 5 B compares the radial dependence of $R_i R_e/(R_i + R_e)$ in 100% CO₂ (anterior and posterior data) with posterior measurements in normal Ringer. Note that the resistivity is plotted on a log scale which amplifies the variance of the low resistivity data. The triangles are a scatter graph of all the data in Fig. 5 A. The open circles are a scatter graph of studies from 11 lenses in which we recorded the radial dependence in normal Ringer. The filled circles at $r = 0.9a$ are averages of the data at $r = 0.9a$. The filled circles at $r = 0.5a$ are averages of all the data at $r < 0.6a$. In normal Ringer, $R_i R_e/(R_i + R_e) = R_i$. Mathias et al. (1981) suggested that $R_i$ assumes two values, switching at $r = 0.67a$. Indeed, there seems to be a rather abrupt change in $R_i$ between $r = 0.6a$ and 0.7a, with $R_i = 3.5 \text{kΩcm}$ for $r$ peripheral to this zone and $R_i = 9 \text{kΩcm}$ for $r$ inside this zone. The change is too abrupt to be proportional to a diffusion gradient of an intracellular substance, though a very steep nonlinear dependence of the junctions on some substance cannot be ruled out. However, it seems more likely that the junctions undergo some structural modification. This hypothesis is consistent with the pH effect in 100% CO₂, since junctions inside this zone appear to have a different pH sensitivity than peripheral junctions.

There are at least three possible reasons for the lack of uncoupling of interior junctions: (1) communication between interior fiber cells could be mediated by cell to cell fusions (Kuszak, Macsai, Bloom, Rae, and Weinstein, 1985) rather than gap junctions, and fusions are presumably not sensitive to pH; (2) because of diffusion limitations, the CO₂ was not reaching the interior cells; or (3) the junctions between interior cells are not sensitive to pH. With regard to (1), if fusions provide a continuous path, each cell must fuse at least once with each of four to six neighbors. Assuming that fusions are semirandom, we estimate that a minimum of 10 fusions per fiber cell are needed to insure continuity between all interior cells. Mathias and Rae (1989) use the data in Kuszak et al. (1985) to calculate the number of fusions per fiber cell at various depths into the lens. The number is on the order of 1 fusion per
FIGURE 5. The dependence of $R_iR_j/(R_i + R_j)$ on radial location in 100% CO$_2$ and normal Ringer solutions. (A) These data are a scatter graph of $R_iR_j/(R_i + R_j)$ vs. radial location in five lenses exposed to 100% CO$_2$ for ~1.5 h. The distance from the surface of the lens appeared to be the most important factor, so we normalized the position of recording to $r/a$ and present the data accordingly. For these lenses, the average radius was $a = 0.167 \pm 0.013$ cm (SD). Two lenses were impaled through the posterior surface at a point 45° from the posterior pole. Three lenses were impaled through the anterior surface at a point 45° from the anterior pole. Given the symmetry of the lens about its polar axis, the data effectively fall along a line that goes from the posterior surface, through the center, to the anterior surface. To the accuracy of our measurement, the data appear symmetric, so we plot all values as a function of $r/a$ in B. (B) The resistivity is now graphed on a log scale to compare the radial variation in normal Ringer (circles) with that in 100% CO$_2$ Ringer (triangles). Over a zone of $r = 0.6a-0.7a$ the properties of the gap junctions appear to change. Junctions peripheral to this zone have a lower coupling resistance in normal conditions but can be induced to uncouple by acidification of the cytoplasm, whereas junctions inside this zone have little if any sensitivity to acidification. The filled circles at $r = 0.9a$ are mean ± SEM of $R_iR_j/(R_i + R_j)$ in 100% CO$_2$ from six lenses, and in normal Ringer from five lenses. The filled circles ± SEM at $r = 0.3a$ are from all the data at $r < 0.6a$.

10 fiber cells. These are much too sparse to have a significant effect on $R_i$. With regard to (2), we used ion-sensitive microelectrodes to record the intracellular pH (see Figs. 6–8), and conclude that pH changes do indeed occur in the inner fiber cells. Thus, hypothesis (3) appears to be the best one: the interior fiber cell junctions differ from those connecting peripheral cells.

Intracellular pH

Fig. 6 shows the time course of the change in pH$\dagger$ and intracellular voltage, recorded at $r = 0.9a$, during exposure to Ringer solution bubbled with 100% CO$_2$. The time
course of the change in \( \text{pH}_i \) is similar to that of \( R_i R_e/(R_i + R_e) \) shown in Fig. 4, though slightly faster. The change in intracellular voltage in Fig. 6 more closely parallels the change in resistivity in Fig. 4. Fig. 7 shows the result of the same kind of experiment, except that the pH and voltage are now recorded in a cell at \( r = 0.3a \). The voltage change still parallels the change in coupling between peripheral cells (see Fig. 4), whereas the pH change is much slower. The \( \Delta \text{pH} \) in the interior cells is only about half that in peripheral cells; nevertheless it is significant (\( \Delta \text{pH}[0.3a] = -0.4 \)) and

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\text{FIGURE 6. The dependence of pHi and intracellular voltage on time in normal Ringer bubbled with 100% CO}_2. \text{ These data were recorded in a cell at } r = 0.9a \text{ cm from the center of a lens of radius } a = 0.195 \text{ cm. The change in pH is large (0.8 units) and somewhat faster than the effect on coupling shown in Fig. 4. The delay could represent the time constant of channel gating, the time constant of binding H\(^+\) to the channels, or the occurrence of other events between the drop in pH, and closure of junctions. The change in intracellular voltage is somewhat slower and more closely parallels the closure of peripheral gap junctions.}
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should have caused significant uncoupling if the inner junctions had the same pH sensitivity as peripheral ones. This point is emphasized in Fig. 8 by comparing the dependence of peripheral and inner junctions on \( \text{pH}_i \).

Fig. 8 is a graph of the average value of \( R_i R_e/(R_i + R_e) \) from one group of lenses vs. the average \( \text{pH}_i \) from another. The three points in the upper curve (\( r = 0.9a \)) were determined using normal Ringer, 20% CO\(_2\), and 100% CO\(_2\). In the bottom curve (\( r = 0.3a \)) we used normal Ringer and 100% CO\(_2\). Though the change in \( \text{pH}_i \) in the inner cells is less than that in peripheral cells, it occurs over a range of \( \text{pH}_i \), where
peripheral cells are most sensitive. It seems evident that the inner fiber cell junctions are indeed much less sensitive to pH$_i$.

Fig. 9A shows the normal, steady-state chemical potential for intracellular H$^+$ ($E_m$) and the intracellular voltage ($\psi$). The average value of pH$_i$ in normal conditions

![Figure 7](image7.png)

**Figure 7.** The dependence of pH$_i$ and intracellular voltage on time in normal Ringer bubbled with 100% CO$_2$. These data were recorded in a cell at $r = 0.3a$ cm from the center of a lens of radius $a = 0.157$ cm. The change in pH$_i$ at $r = 0.3a$ is much slower and considerably smaller than that at $r = 0.9a$. Nevertheless, pH$_i$ drops ~0.4 units, which is a significant acidification. The time course of the change in intracellular voltage is much more rapid than the pH$_i$ change, suggesting that the voltage is determined by effects of pH$_i$ on peripheral cells, perhaps through uncoupling of peripheral gap junctions.

![Figure 8](image8.png)

**Figure 8.** A plot of $R_i R_e/(R_i + R_e)$ vs. pH$_i$ at either $r = 0.9a$ or $r = 0.3a$. At $r = 0.3a$, the resistivity data are from Fig. 5 for normal Ringer or 100% CO$_2$ Ringer. In a separate group of five lenses, we used ion-sensitive microelectrodes to determine pH$_i$ in normal Ringer and 100% CO$_2$ Ringer. These lenses had an average radius of $a = 0.169 \pm 0.018$ cm and the data were recorded at $r = 0.20a-0.48a$. At $r = 0.9a$, the resistivity data at the highest and lowest values of pH$_i$ are from Fig. 5 in normal Ringer and 100% CO$_2$ Ringer. The intermediate value of resistivity is from seven lenses studied in 20% CO$_2$ Ringer. The pH$_i$ data are from a separate group of six lenses exposed to normal or 100% CO$_2$ Ringer, and seven lenses exposed to normal or 20% CO$_2$ Ringer. The average radius of these lenses was $a = 0.176 \pm 0.022$ cm and the pH$_i$ data were recorded at $r = 0.86a-0.92a$. 


The normal steady-state chemical (Nernst) potential for intracellular H⁺ (EH) with respect to the bath and the intracellular voltage (ψ) are shown in A. (A) The chemical potentials for intracellular H⁺ are calculated from the normal Ringer values of pH shown in Fig. 8. The dashed line connecting the values of EH at r = 0.9a and 0.3a has no theoretical significance, but shows the gradient in pH with location. The normal intracellular voltage in the same lenses is illustrated by the lower two filled circles. The dashed curve connecting the voltage points is calculated as described in Mathias and Rae (1985). It illustrates the expected shape of the radial gradient in intracellular voltage due to radially circulating ionic current carried primarily by Na⁺. A curve of this shape accurately describes high resolution measurements of ψ vs. r as shown in Mathias and Rae (1985). These data illustrate that H⁺ at r = 0.9a is well out of electrochemical equilibrium. Moreover, the change in EH between r = 0.3a and 0.9a is opposite to the change in ψ. Thus the electrochemical potential for intracellular H⁺ (ψ - EH) is more negative at r = 0.9a, so there will be a radial flux of H⁺ from inner fiber cells, through gap junctions to the surface cells. (B) The value of R(Ri/R + Rg) in 20% CO₂ is used to detect changes in pH. When Na⁺ in the bath is replaced with either choline or tetramethyl ammonium (data not shown) there is a rapid (and reversible) increase in coupling resistance, suggesting a drop in pH. Similar results are obtained when amiloride (1 μM) is added to the bath instead of removing Na⁺. All of these studies were performed in peripheral fiber cells at the posterior surface. The results suggest that these cells use Na/H exchange as one mechanism of extruding the H⁺ that is moving from the lens center to peripheral cells. In the study shown, a = 0.190 cm, r = 0.156 cm, and exposure to 20% CO₂ caused the lens to depolarize from −87 to −49 mV.

drops from ~7.02 at r = 0.9a to 6.81 at r = 0.3a. If extrusion of hydrogen primarily occurs at the lens surface (Wolosin, Alvarez, and Candia, 1988, 1990; Bassnett, 1990), the intracellular pH at the center of the lens (r = 0) is probably even lower. Gap junctions between inner fiber cells are thus required to exist in a relatively acid environment. Their lack of sensitivity to low pH is therefore not likely to be fortuitous; rather, it seems essential to the performance of their normal function.

As can be seen in Fig. 9A, extrusion of hydrogen is against its electrochemical gradient (also see Discussion) so an active ATPase or secondary active cotransport...
system is required. Wolosin et al. (1988, 1990) have characterized a sodium-hydrogen cotransport system and a sodium-dependent bicarbonate transporter in the epithelial cells of the toad lens. Bassnett (1990) reported Na/H exchange in epithelial cells from the chick lens. Ye and Zadunaisky (1990) have reported evidence for Na/H exchange in vesicles made from fiber cell membrane, but the source of the membrane was not localized within the lens. The Na/H transporter (reviewed in Bashford, 1990) uses the electrochemical gradient for sodium as the source of energy to transport hydrogen out of the lens in an electrically neutral 1Na+ for 1H+ exchange. We tested for the presence of this transporter in the frog lens as shown in Fig. 9 B. By bubbling with 20% CO2 we partially close gap junctions and stimulate extrusion of H+. Under these conditions the coupling of peripheral fiber cells is very sensitive to any further modification of pH, (see Fig. 7). When sodium is removed from the bath, there is an immediate increase in coupling resistance, suggesting a drop in pH due to a sodium-dependent transport system or systems. Amiloride does not affect membrane conductance in the frog lens but should block sodium/hydrogen exchange, so we repeated the protocol in Fig. 9 B except that amiloride was added instead of removing sodium. The result was the same, suggesting that sodium/hydrogen exchange is one mechanism for regulating pH in the frog lens. The rapidity of the effect on Ri of removing bath sodium (or adding amiloride) suggests that this mechanism is primarily localized to surface cells. These studies were performed in peripheral fiber cells (r = 0.9a) at the posterior surface of the lens; hence these fiber cells as well as the epithelial cells appear to utilize Na/H exchange.

DISCUSSION

To summarize, we have shown that gap junctions connecting peripheral fiber cells are different from those connecting inner fiber cells. The differences we observe are in pH sensitivity and in the average cell to cell coupling resistance.

A rather abrupt change in the cell to cell resistance occurs in a zone that is between 650 and 500 μm from the lens surface (r = 0.7a to 0.6a in these small lenses, where a = 0.16 cm). The effective intracellular resistivity Ri is on average 3,500 Ω cm in cells peripheral to this zone and 9,000 Ω cm in the interior cells. The cell to cell resistance in a unit area of membrane can be calculated by multiplying Ri by the average cell width (~3 μm for fiber cells). The cell to cell resistance thus calculated varies with radial location from 1.1 to 2.7 Ω cm², so all of the cells are exceptionally well coupled. The change in cell to cell resistance could be due to a change in the density of junctions, a change in the number of open channels per junction, or a change in the single channel conductance. The last two possibilities require some modification of protein structure or state within the junctions. Moreover, the dramatic loss of pH sensitivity in roughly the same zone cannot be attributed to a simple change in the number of junctions. Rae, Thomson, and Eisenberg (1982) also found that dinitrophenol caused uncoupling of fiber cells peripheral to this zone but not inside it. A hypothesis consistent with all of these observations is that the peripheral differentiating fiber cells synthesize the gap junctional proteins that connect peripheral fiber cells. These junctions are internalized as the lens grows and ages and the protein structure is modified by this process. The modified proteins form higher resistance gap junctions, which do not close during acidification. This modification is essential
to the survival of inner cells since the physiological mechanism of handling hydrogen ion balance cannot keep the inner cells at neutral pH.

Hydrogen ions are transported out of the lens across the membranes of surface epithelial and differentiating fiber cells. We do not know all of the transport mechanisms but, at a minimum, sodium-hydrogen exchange appears to be utilized. The transport mechanisms must be energy dependent because they maintain internal hydrogen well out of electrochemical equilibrium. The transmembrane electrochemical gradient for hydrogen, $\psi_i - \psi_e - E_H$, equals about $-50$ mV near the surface ($r = 0.9a; \psi_i = -72$ mV; $\psi_e = 0; E_H = -22$ mV). The electrochemical gradient across inner fiber cell membranes is not known since pH$_e$ in the extracellular spaces between fiber cells has not been measured; however, as described below, we can be reasonably certain that hydrogen is out of equilibrium by at least $-27$ mV.

Our view of the handling of hydrogen by the lens can be visualized by looking at Fig. 1. Hydrogen leaks into the lens cells through $g_m$ and $G_s$ down its electrochemical gradient. Hydrogen is also produced in the fiber cells (the $R_i$ compartment) as a byproduct of anaerobic metabolism (Bassnett, Croghan, and Duncan, 1987). The change in electrochemical potential for hydrogen between cells at $r = 0.3a$ to $r = 0.9a$ is $22$ mV ($\psi_i[0.3a] = -62$ mV; $\psi_i[0.9a] = -72$ mV; $\Delta pH_i = 0.21$). A flux of hydrogen moves down this gradient from inner cells through gap junctions to surface cells where it is extruded. If hydrogen is moving into fiber cells through $g_{an}$, then there must be a flux of hydrogen along the extracellular clefts ($R_e$ in Fig. 1), moving from the surface toward the center. We can bound the value of $pH_e$ by considering two limiting situations: (1) Assume that metabolic production of hydrogen is zero so the intracellular flux from center to surface is equal and opposite to the extracellular flux. The value of $R_e$ is about five times greater than $R_i$; hence, the extracellular gradient would have to be about five times greater than the intracellular. The electrochemical potential for extracellular hydrogen at $r = 0.3a$ would therefore have to be $-110$ mV with respect to the bath. From other studies (Mathias and Rae, 1985) we know that $\psi_e(0.3a) = -30$ mV, so pH$_e$ would have to be $\sim 8.8$. However, the transmembrane electrochemical gradient becomes outward for pH$_e$ greater than $\sim 7.4$, which is contrary to our initial assumption, so the intracellular flux from central to peripheral fiber cells must be partially due to metabolic production of hydrogen. (2) Assume that the intracellular flux of hydrogen is entirely due to metabolic production so there is no flux along the extracellular spaces. Hydrogen would then have to be in equilibrium with $\psi_e$, so at $r = 0.3a$, $\psi_e = -30$ mV implies that pH$_e$ is $\sim 6.9$. However, in this situation the transmembrane gradient at $r = 0.3a$ is not zero; rather, it is about $-27$ mV, which should cause a transmembrane flux contrary to our initial assumption. We can therefore assume that pH$_e$ at $r = 0.3a$ is greater than 6.9 but less than 7.4. We also conclude that metabolic production and a transmembrane flux of hydrogen both contribute significantly to the observed intracellular gradient.

One issue not addressed by this study is the angular variation in the electrical properties of the lens. As mentioned earlier, the lens is symmetric about an axis that goes from the posterior pole through the center to the anterior pole. However, the anterior surface consists of a layer of epithelial cells, whereas the posterior surface consists of foot-like projections at the ends of differentiating fiber cells. The value of
surface membrane conductance we report is the average of the anterior and posterior values. Preliminary studies on the rat lens reported in abstract form (Baldo, Mathias, and Hagstrom, 1990) found that the gap junctional resistance coupling peripheral cells at the equator is much lower than at either pole. The studies reported here measured \( R_\text{g} \) halfway between the pole and equator, which is where it takes on a value equal to its angular average. We are currently working on a quantitative description of both the angular and radial dependence of \( R_\text{g} \) in the rat lens, but the theoretical analysis is much more complex than that presented here.

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