Regional distribution of the Na\(^+\) and K\(^+\) currents around the crystalline lens of rabbit

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Candia, Oscar A., and Aldo C. Zamudio. Regional distribution of the Na\(^+\) and K\(^+\) currents around the crystalline lens of rabbit. Am J Physiol Cell Physiol 282: C252–C262, 2002; 10.1152/ajpcell.00360.2001.—Early studies described asymmetrical electrical properties across the ocular lens in the anterior-to-posterior direction. More recent results obtained with a vibrating probe indicated that currents around the lens surface are not uniform by showing an outwardly directed K\(^+\) efflux at the lens equator and Na\(^+\) influx at the poles. The latter studies have been used to support theoretical models for fluid recirculation within the avascular lens. However, the existence of a nonuniform current distribution in the lens epithelium from the anterior pole to the equator has never been confirmed. The present work developed a modified short-circuiting technique to examine the net flows of Na\(^+\) and K\(^+\) across arbitrarily defined lens surface regions. Results indicate that passive inflows of Na\(^+\) occur at both the anterior polar region and posterior lens surface, consistent with suggestions derived from the vibrating probe data, whereas K\(^+\) efflux plus the Na\(^+\)-K\(^+\) pump-generated current comprise the currents at the equatorial surface and an area anterior to it. Furthermore, Na\(^+\)-K\(^+\) pump activity was absent at the posterior surface and its polar region in all lenses examined, as well as from the anterior polar region in most lenses. The latter unexpected observation suggests that the monolayered epithelium, which is confined to the anterior surface of the lens, does not express an active Na\(^+\)-K\(^+\) pump at its anterior-most aspect. Nevertheless, this report represents the first independent confirmation that positive currents leave the lens around the equator and reenter across the polar and posterior surfaces.

Using-type chamber; short-circuit current; ion transport

Because of its high [K\(^+\)], low [Na\(^+\)], and general gross properties, early observations compared the crystalline lens to a giant spherical cell (17, 18). From this paradigm, numerous physiological studies described the overall functional characteristics of the lens, e.g., intralenticular potentials, input resistances, and impedance analyses (11, 12, 15, 22, 24–26). In contrast, other early investigations emphasized the influence of the lens epithelium, which comprises a single layer of cuboidal cells on the anterior side only, in conferring to the lens asymmetrical electrophysiological properties (7–9, 14, 19). When the lens is isolated in a double chamber that separates the anterior from the posterior lens surfaces, an anteriorly directed positive electrical potential develops, in all studied species, as a result of an electrogenic Na\(^+\)-K\(^+\) pump located in the surface, basolateral membrane of the epithelium (13). Logically, it was assumed that the activity of this monolayer was relatively uniform and that a positive current must emanate from the anterior pole to the equator. However, Patterson and coworkers (23, 27, 29, 30), using the vibrating probe, showed in frog and rat lenses a nonhomogeneous distribution of currents around the lens surface.

The findings of Patterson’s group were not widely received, and no other laboratory attempted to reproduce their results. Nevertheless, on the basis of Patterson’s initial model, in which ionic currents exited around the equator and reentered the lens at the poles, Mathias et al. (21) developed a theoretical model for electrolyte and fluid circulation inside the lens. That model also included information obtained from impedance analysis (5, 20).

Our experience with the vibrating probe in the rabbit lens suggested that this device was unreliable. We present here an alternative, novel approach to empirically examine the ionic currents about the lens surface. It entails the use of three O-rings of different sizes to separate the anterior surface from the posterior surface of the lens at three different parallels between either the anterior or the posterior pole and the equator, thereby enabling the study of the currents at seven predefined zones.

In this article we report that in the isolated rabbit lens, a positive current leaves from around the equator and an area anterior to it and reenters the lens across its posterior face as well as a small area around the anterior pole. The current across this anterior polar area is insensitive to ouabain despite the presence of the lens epithelium. Our results represent the first independent confirmation of initial observations of Patterson and collaborators and identify additional asymmetrical aspects in this remarkable organ.

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METHODS

Theoretical considerations. To simplify the analysis, we performed all experiments in Cl−-free solutions where the predominant movement of charges between lens and solutions is due to Na+ and K+ fluxes. Furthermore, when the isolated lens is placed in a bath, the net interchange of these charges between solution and lens must be zero; i.e., the total charges leaving the lens must be equal to the total charges entering. However, this balance can be accomplished not necessarily because there is no charge movement across the lens surface but because a net charge movement across a particular area of the lens surface is compensated by an equal and opposite charge movement across another lens area.

We will adopt the convention for the lens immersed in a bath that positive currents from the bath to the lens are positive and that positive currents from the lens to the bath are negative. Thus a net K+ flow from the lens to the bath will have a negative sign.

When the lens is immersed in a saline solution, it is essentially short-circuited by the low-resistance solution around its surface, and no potential difference (PD) can be detected between electrodes placed on any two points in the proximity of the lens surface. However, if the lens is isolated in an Ussing-type chamber (7–9) and two separate areas in the anterior-posterior direction are electrically isolated, there are two possible outcomes: 1) the total current at each one of the two areas is independently balanced, in which case the translens PD (PDt) will be zero; or 2) such individual balance does not occur, in which case one side will develop a PD with respect to the other as a consequence of the asymmetry between the two delimited areas.

Despite the separation of the two areas, short-circuiting restores the distribution of currents to the open-bath condition. The short-circuit current (ISC) represents the difference between inward and outward currents at each surface. However, the ISc is a current that circulates through the lens and an external circuit, thereby completing a closed loop. Because of this, the ISc enters the lens across one surface and leaves across the other. In this situation, the sign of the current must be based on the direction of circulation (e.g., clockwise or counterclockwise within the loop) and not on whether the current enters or leaves the lens. Thus the sign of the ISc should be based on its direction in the external circuit. We have chosen to define the ISc as positive when a positive current flows from the anterior to the posterior pole in the external circuit and, consequently, from the posterior to the anterior pole through the lens. Of course, the polarity of the ISc will depend on the natural polarity of the lens and how it is mounted in the chamber. As shown later, this precise definition of the ISc is necessary to determine the direction in which current and ion fluxes traverse the surface of the lens. When a “flat” epithelium is mounted in a bicameral chamber, the only detectable asymmetry is that between the apical and basolateral sides. Because of its semispherical shape, the lens offers the opportunity for a comparison between multiple pairs of its total surface and in doing so allows for a determination of the transport and permeability properties of various arbitrarily defined zones. To exploit the advantages inherent in lens geometry, we designed a special chamber that enabled separation of six pairs of areas.

Chambers. Two chambers were used in the experiments to be described. One, a three-compartment chamber, shown diagrammatically (Fig. 1), was exclusively utilized to determine the resistance ratio between near equal areas of the posterior and anterior surfaces (RP/RA). The lens was initially supported between two centrally perforated Lucite disks with O-rings as surfaces of contact. Each O-ring touched the lens with just enough pressure to prevent leaks, without distorting the shape of the lens. This was accomplished by advancing four screws on the disk that brought them closer together against the resistance of a large O-ring that sealed a center compartment. The two-disk lens assembly was mounted between the halves of an Ussing-type chamber with conventional PD-measuring and current-sending electrodes. A third PD electrode was placed in the center compartment through an opening in the large O-ring. This opening also served to fill the center compartment with Ringer’s solution. The surface area in contact with the outer solution given by the diameter of the small O-rings (7.9 mm) was 0.65 cm² on each side. The equatorial surface bathed by the solution in the center compartment was about 2.04 cm² for a typical ~10.5-mm equatorial diameter lens. With this arrangement the PD drop between the center and external compartments could be determined. The ratio of PDs is equal to the resistance ratio between the respective surfaces.

The second chamber, used in all the other experiments, is shown in Fig. 2. It consists of a lower Lucite hemichamber with a glass-made recirculation attachment, a Lucite upper hemichamber with its glass-made recirculation device, and a center spacer that fits between both hemichambers. The spacer served as an atrium around the lens to prevent the upper chamber from touching the organ. Lenses were placed on one of three O-ring assemblies (Fig. 2 and inset) with the anterior epithelial side either up or down. This O-ring lens assembly was then positioned on the lower hemichamber. The spacer was placed on the top of the O-ring assembly, and the upper chamber was in turn situated over the spacer. The whole system was secured by two nylon thumbnuts screwed onto threaded rods also used as guides. Once the hemichambers were filled with the bathing solutions, the upper hemichamber was at atmospheric pressure. The fluid in the
Translenticular electrical measurements. Lenses from adult albino rabbits that had been killed by CO₂ asphyxiation were mounted in either of the chambers described. Agar-NaCl-filled polyethylene tubing served as a salt bridge connecting each bathing compartment to Ag-AgCl electrodes for PD measurements. A second pair of bridges, connected to an automatic voltage-clamp system (28), was used to short-circuit PD, with the current needed to keep PD at 0 mV (i.e., the Isc) continuously recorded. For R₀ measurements, the current needed to offset the short-circuited condition by ±10 mV was measured every 2–5 min for a few seconds.

Description of lens zones. The three O-ring separations resulted in the seven zones Z₁–Z₇ (Fig. 3), in which Z₁ with Z₇, Z₂ with Z₆, and Z₃ with Z₅ constituted symmetrical pairs. Z₄ was without a pair. The general dimensions of these zones for an idealized spherical lens of 11 mm in diameter also are shown in Fig. 3. Regarding the mounting positions, P₁, P₂, and P₃ were symmetrical to P₆, P₅, and P₄, respectively. Regardless of the O-ring used and the zones included, the anterior side is defined as the one that includes the anterior pole, and by default, the area on the other side of the O-ring is the posterior side. Figure 3 also shows the equivalent resistance circuit when the O-ring is in P₂, separating Z₁ and Z₂ from Z₃, Z₄, Z₅, Z₆, and Z₇.

Solutions. The bathing medium used during the dissection and bathing of lenses in the chambers lacked Cl⁻ and had the following composition (in mM): 1.8 calcium gluconate, 1.2 MgSO₄, 2 K₂HPO₄, 119 sodium gluconate, 25 NaHCO₃, and 10 glucose. The pH of this solution when bubbled with 5% CO₂–95% air was ~7.45. Its osmolality measured 285 mosmol/kgH₂O. Lenses were incubated in the Cl⁻-free solution for at least 1 h before being mounted in the chamber.

In some experiments described in RESULTS, a Cl⁻-free, elevated-K⁺ solution with reduced Na⁺ was used. For this, potassium gluconate replaced an equimolar amount of the Na⁺ salt.

Determination of resistances of individual zones. When resistance across the lens isolated in an Ussing-type chamber with the anterior and posterior surfaces separated by a circular O-ring (Fig. 2) is measured, the obtained value is the series combination of the resistance of both surfaces with the lower chamber, because its level was about 10 cm below that of the fluid in the upper chamber, exerted a negative pressure on the lower face of the lens that kept it in place and provided a seal that electrically isolated the two surfaces of the lens bathed by the upper and lower solutions. The O-ring assembly had a center aperture of either 4.8, 6.4, or 7.9 mm in diameter. The lens, with an equatorial diameter between 10 and 12 mm, was not subjected to an external pressure, since the internal diameter of the spacer was 15 mm. From the placement of the lens on either of the three O-ring assemblies, with its anterior or posterior pole facing the O-ring, six positions (P₁–P₆) were defined that allowed for the separation of seven lens zones that are described below.

A common protocol was to start with the posterior lens pole on the smallest ring (P₁) to study zone 1 (Z₁; the posterior polar region); after recording in this position, open the chamber and change to the larger rings (P₂ and P₃, respectively), revert the lens (P₄), and then sequentially change to the smaller rings with the anterior pole now facing downward. Many other sequences were tested. In most experiments, each individual lens was examined in at least three positions. As shown in Fig. 2, the chamber has inlets in which to insert agar bridges for the measurements of PD, Isc, and translens resistance (R₀).
associated lens fibers. With the use of smaller O-rings, the resistance of one side becomes larger, whereas the other becomes smaller (see Fig. 3). In general, the total measured resistance is larger as the size of the O-ring decreases and the separation of the anterior zones moves away from the equator toward one of the poles.

In our experimental approach, the lens surface is divided into seven zones, but there are only six O-ring positions from which to measure the combined resistances. The seventh value necessary to solve the equation is provided by the resistance value obtained with the three-compartment chamber. The two 7.9-mm O-rings used in the three-compartment chamber separate the lens into three zones: A (anterior), E (equatorial), and P (posterior). This O-ring placement corresponds to P3 and P4 in the two-compartment chamber. Thus the resistances measured at these positions in the two-compartment chamber are

$$R_{P3} = \left( \frac{RE \cdot RA}{RE + RA} \right) + RP$$

and

$$R_{P4} = \left( \frac{RE \cdot RP}{RE + RP} \right) + RA$$

where $R_{P3}$ and $R_{P4}$ are the resistances measured at positions P3 and P4, respectively, and RE, RA, and RP are the resistances of the equatorial, anterior, and posterior zones defined for the three-compartment chamber.

Also, in this chamber the resistance ratio is $r = RP/RA$. It can be shown that

$$\frac{(R_{P3} - RP) \cdot RA}{RA + RP - R_{P3}} = \frac{(R_{P4} - RA) \cdot RP}{RA + RP - R_{P4}}$$

which can be simplified to

$$\frac{(R_{P3} \cdot r \cdot RA) - (R_{P4} \cdot RA \cdot r \cdot RA^2)}{RA + r \cdot RA - R_{P4}}$$

which can be converted into a quadratic equation

$$(R_{P3} - R_{P4} \cdot r^2) \cdot RA^2 + (R_{P3} - R_{P4} \cdot r - R_{P4}) \cdot RA = 0$$

and solving for RA

$$RA = \frac{R_{P3} \cdot R_{P4} \cdot r - R_{P2} \cdot R_{P4}}{R_{P3} - R_{P4} \cdot r^2}$$

and then

$$RP = RA \cdot r$$

and

$$RE = \frac{(R_{P3} - RP) \cdot RA}{(RP + RA - R_{P3})}$$

RE is identical to the resistance of zone 4, $R_4$.

Once this value is known, the resistance of the other six zones, $R_1$, $R_2$, $R_3$, $R_5$, $R_6$, and $R_7$, can be obtained. The combined resistances of $Z4-Z7$ ($R_{4-7}$) is the parallel combination of RA and RE. To obtain $R_{3a}$, the resistance of Z3, the O-ring is placed in P2 in the two-compartment chamber. This position is shown in Fig. 3. The measured resistance between points $a$ and $p$, $R_{P2}$, is equal to

$$R_{P2} = \frac{R_{4-7} \cdot R_3 + R_{1-2}}{R_{4-7} + R_3}$$

where $R_{1-2}$ is the combined resistance (in parallel) of Z1 and Z2. Then

$$R_{1-2} = R_{P2} - \left( \frac{R_{4-7} \cdot R_3}{R_{4-7} + R_3} \right) = \frac{R_3 \cdot RP}{R_3 - RP}$$

and

$$(R_{P2} \cdot R_{4-7} + R_{P2} \cdot R_3 - R_{4-7} \cdot R_3) \cdot (R_3 - RP)$$

which can also be converted into a quadratic equation

$$(R_{P2} - R_{4-7} - RP) \cdot (R_3)^2 + [(R_{4-7} - RP) \cdot R_{P2} \cdot R_3] \quad - (R_{P2} \cdot R_{4-7} \cdot RP) = 0$$

and solving for $R_3$

$$R_3 = \frac{R_{P2} \cdot (RP - R_{4-7}) \pm \sqrt{(R_{P2} \cdot (RP - R_{4-7}))^2 + 4(R_{P2} - R_{4-7} - RP) \cdot R_{P2} \cdot R_{4-7} \cdot RP}}{2(R_{P2} - R_{4-7} - RP)}$$

By changing the position of the O-ring and following the same procedure, the resistances of the remaining zones can be determined.

**RESULTS**

Determination of the posterior-to-anterior surface resistance ratio. Six experiments were performed with the three-compartment chamber with the lens bathed in Cl−-free Ringer’s. The average PDt was 19 ± 4 mV, with the anterior side positive. The electrical potential difference between the posterior and center compartment (Fig. 1, PDp) was 17 ± 5 mV. This observation confirms the presence of the Na⁺-K⁺ pump in the epithelium covering the anterior face and equatorial region. However, the main reason for the use of this chamber was the determination of RP/RA, a value needed for the processing of data obtained with the bicameral chamber. When a pulse of current was sent across the lens between the outer compartments (no current flow in to or out of the center compartment), a potential deflection of 3.00 mV across the lens was simultaneously recorded as a 1.79 ± 0.16-mV deflection across the posterior-to-center electrodes. These values correspond to an RP/RA of 1.48 ± 0.12. The $R_t$ was 1.87 ± 0.30 kΩ·cm². This value represents the series resistance of the 0.65-cm² surface in contact with the external solutions plus that of the lens fibers between them. Because the O-rings used in this chamber were the larger size used for P3 and P4 of the two-compartment chamber, the area of the lens surface in contact with the bathing solutions was the addition of Z1, Z2, and Z3 or Z5, Z6, and Z7 for the posterior and anterior side, respectively.

Determination of $I_{sc}$ at each position. Although it was not always possible to obtain information from every position for practical reasons of time or because the lens deteriorated during the manipulation necessary to change O-rings, this was accomplished in 11 experiments. Two such experiments are shown in Figs. 4 and 5; resistances were examined in the six possible positions.
Experiment 19, shown in Fig. 4, was started in P1 where the lens is sitting with its posterior polar region on the smallest O-ring. As expected, the $I_{sc}$ indicated that a positive current was entering the posterior pole and leaving across the much larger surface isolated above the O-ring. Changing from P1 to P2 increased the $I_{sc}$, indicating that a positive current that had been entering the lens anterior to the O-ring across $Z_2$ was now part of the posterior inward current. The $I_{sc}$ increased further at P3. Also, the electrical conductance progressively increased from P1 to P3. This also was expected, because the largest O-ring more evenly divides the anterior and posterior surfaces. The change to P4 reduced the $I_{sc}$ to $\sim 6$ $\mu$A from 13.4 $\mu$A, indicating that across the equatorial $Z_4$, a net positive $I_{sc}$ was leaving the lens. In P5, the $I_{sc}$ became negative. This indicates that a net positive current was entering the lens across its anterior pole ($Z_7$) and its surrounding $Z_6$, and leaving throughout $Z_1$–$Z_5$. Because we already know (from determination at P1, P2, and P3) that a positive current enters $Z_1$–$Z_3$, a much larger positive current must be leaving $Z_4$ and $Z_5$. This is actually expected, because the Na$^+$-K$^+$ pumps that produce an outward current are concentrated in the equatorial zone ($Z_4$) and anterior epithelium (6, 10). Finally, with the use of the smallest O-ring with the anterior pole down (P6), the $I_{sc}$ remained negative. This result, which is a novel finding, indicates that a positive cur-

Fig. 4. Representative short-circuit current ($I_{sc}$) recordings obtained upon the sequential remounting of the rabbit lens at 6 different separations between the anterior and posterior surfaces as defined by the O-ring position. Upward deflections are the points at which the translens resistance was recorded. The length of the deflection is proportional to the electrical conductance. Toward the end of the experiment shown, the lens was returned to P5. In this situation, the additions of BaCl$_2$ and ouabain to the lower chamber bathing the anterior side of the lens were without effect. Such additions to the upper hemichamber exposed the posterior-most and equatorial lens surfaces to the drugs, resulting in elimination of the $I_{sc}$.

Fig. 5. Experiment in which the rabbit lens was sequentially remounted in symmetrical positions, i.e., P1 with P6, P5 with P2, and P3 with P4 to illustrate the reversals in the current flow across the lens. At the end of the experiment, in P4, BaCl$_2$ plus quinidine were added first to the posterior bath and then to the anterior bath.
rent entered across the anterior polar region delimited by the 4.8-mm-diameter O-ring. One could expect that the Na⁺-K⁺ pumps known to be present at the epithelium covering the anterior pole would produce an outwardly directed positive current. Although an unlikely possibility, K⁺ entering the anterior pole may produce the observed \( I_{sc} \). However, after the lens was returned to P5, BaCl₂ had absolutely no effect on the \( I_{sc} \) when added to the anterior bath. Even more perplexing was the fact that ouabain did not affect this \( I_{sc} \) either. BaCl₂ and ouabain had their usual effects when added to the posterior-side solution bathing Z1–Z5 in this position.

Although anterior-side ouabain had a minor effect in some experiments done with P5, which includes Z6 and Z7, in 10 experiments in which the glycoside was added to an isolated Z7, there was no effect in the \( I_{sc} \). Clearly, the Na⁺-K⁺ pump was physiologically inactive in this region of the epithelium. One must conclude that the \( I_{sc} \) is carried by a Na⁺ current that was recirculating across the anterior pole.

Experiment 112, shown in Fig. 5, also was started in P1, but the lens was then inverted so that the anterior polar region sat on the smallest O-ring (P6). The \( I_{sc} \) continued to flow in the same direction in the external circuit, but across the lens it now went from the anterior to the posterior side, thus requiring the reversal of the scale according to the definition in METHODS. Compared with P6, P5 extends the area of the anterior surface limited by the O-ring. The orientation of the lens was then again inverted while the same O-ring was used, resulting in P2. In comparison with P1, one can see that the \( I_{sc} \) was larger with P2, indicating that additional current was entering the lens across Z2. By using the largest O-ring, the \( I_{sc} \) at P3 was obtained, which was usually the largest compared with that of the other positions. As observed here, in 30% of lenses the \( I_{sc} \) oscillates because of the concerted opening and closing of the K⁺ channels, which we have examined in previous publications (2, 3). Although it was necessary to interrupt the \( I_{sc} \), open the chamber, and turn the lens over to reposition it in P4, the oscillations continued. Additions of 5 mM BaCl₂ plus 10⁻⁴ M quinidine to the posterior side in this position, which includes Z1–Z4, reduced the \( I_{sc} \) and stopped the oscillation. This can be interpreted as a reduction of K⁺ current leaving via the K⁺ channels in Z4 that were closed by the blockers.

Adding the blockers to the anterior side (Z5–Z7) produced an increase in \( I_{sc} \). In this position the \( I_{sc} \) is given by the Na⁺ current entering across Z5–Z7 minus the K⁺ current leaving the lens across these zones. Clearly, from the response of the \( I_{sc} \) to the blockers, the K⁺ current was larger across the posterior side (which includes in this position equatorial Z4) than across the anterior side.

**Determination of the \( I_{sc} \) at each lens zone.** With the posterior polar region on the smaller O-ring (P1), one measures directly the \( I_{sc} \) of Z1. In P2, one measures the \( I_{sc} \) of Z1 plus Z2 and can calculate the \( I_{sc} \) of Z2 by subtraction. In general, the following matrix can determine the \( I_{sc} \) across each zone:

\[
\begin{align*}
I_{sc,Z1} &= I_{sc,P1} - 0 \\
I_{sc,Z2} &= I_{sc,P2} - I_{sc,P1} \\
I_{sc,Z3} &= I_{sc,P3} - I_{sc,P2} \\
I_{sc,Z4} &= I_{sc,P4} - I_{sc,P3} \\
I_{sc,Z5} &= I_{sc,P5} - I_{sc,P4} \\
I_{sc,Z6} &= I_{sc,P6} - I_{sc,P5} \\
I_{sc,Z7} &= 0 - I_{sc,P6}
\end{align*}
\]

Based on the sign definition for \( I_{sc} \) (see METHODS), this matrix is consistent with the convention that positive currents entering the lens are positive and positive currents leaving the lens are negative.

Table 1 shows currents measured in 47 lenses from at least 2 positions. In some experiments, the \( I_{sc} \) for a given position was determined for a second time, and an average value was entered in Table 1. The average \( I_{sc} \) for every position (shown at the bottom of Table 1) was used in the matrix above to calculate the \( I_{sc} \) at every zone. These values are summarized in Table 2 and Fig. 6. As can be seen, current gets into the lens not only across the three posterior regions, as always thought (4, 7), but also across the anterior pole (Z7). This is a new finding for the rabbit lens, confirmatory of the prediction in rat and frog lenses (29). It should be noted that the equatorial zone is by far the largest, and it is possible that the low density of the current is simply the result of a transition from an inward current at its most posterior part to outward current at the equator proper and its anterior part. A similar situation may exist in Z6, where in some experiments a positive current entering the lens was found. Its density may be an indication of a transition from outward to inward current as the anterior pole is approached. In any case, the matrix dictates that the total current leaving the lens (negative) must be equal to the current entering the lens.

A close examination of the experiments in Table 1 shows that in some cases (e.g., experiments 47, 62, and 79), the \( I_{sc} \) in P6 was positive. This is an indication that, contrary to the average, a positive current was leaving across Z7. As shown in Fig. 7, in these cases the addition of BaCl₂ to the anterior side reversed the polarity of the \( I_{sc} \). This is an indication that there was a K⁺ current leaving the lens across K⁺ channels (presumably driven by an electrochemical gradient) that was larger than the inward Na⁺ current.

To ascertain the presence of K⁺ channels in Z7 and to compare their permeability with that of Na⁺ channels, the following protocol was implemented in P6. The solution bathing the anterior polar region was replaced by one in which the K⁺ concentration was increased by 30.7 mM (to 34.7 mM), and the Na⁺ concentration was reduced by the same amount to 109.3 mM. If the permeability of Na⁺ and K⁺ of the zone bathed by the solution in which the change was made were the same, and assuming a minimal effect on the pump, there would be no immediate change in the \( I_{sc} \) because the same number of Na⁺ ions are replaced.
by K⁺, and the reduction in the unidirectional Na⁺ influx would be compensated with an equal increase in K⁺ influx. If the permeability of K⁺ were larger than that of Na⁺, the I_{sc} would become more negative or less positive because the additional K⁺ current is larger than the reduced Na⁺ current entering the lens. This is what happened in six experiments shown in Table 3.

Making the same ionic change in the posterior solution produced the opposite change in the I_{sc}, indicating that the overall K⁺ permeability of the other six zones was larger than that of the Na⁺. Also shown in Table 3 is the effect of the same ionic change when the O-ring is in P1, separating Z1 from the other zones. Increasing the K⁺ concentration (with an equal reduction in Na⁺) in the posterior solution increased the I_{sc} in four experiments, indicating that the K⁺ permeability of Z1 was larger than that of Na⁺. In the other two experiments in which the I_{sc} decreased, one must conclude that the Na⁺ permeability of Z1 was larger. This is consistent with the increase in resistance in these experiments compared with the decrease in the other four. When the change is done on the anterior side that includes Z2 through Z7, the I_{sc} decreased, indicating an overall larger K⁺ permeability around this area of the lens surface.

**Determination of the electrical resistance of each zone.** Table 4 shows the mean resistance values of lenses determined at the six previously defined positions. An initial comparison can be made between values registered at symmetrical positions P1 and P6. P1 had a larger resistance than P6. Because Z2 through Z6 are common in these positions, the resistance ratio P1/P6 therefore indicates the resistance ratio of Z1/Z7, although their values are not revealed. A similar comparison can be made between P2 and P5. The ratio now is larger (1.88 vs. 1.42), indicating that the resistance of the now included Z2 is even larger than that of Z6, making the ratio larger. This type of analysis gave a general idea of the resistances of various zones but cannot determine their exact values. Using the analysis described in METHODS, we have calculated the resistances of each zone, which also are shown in Table 4. The lowest resistance was found in Z4, followed by that of Z6 and Z7; the highest was that of Z2. When these values were normalized for an area of 1 cm² (kΩ·cm²), the resistances of Z4, Z6, and Z7 were still the lowest, but the difference between the other zones was greatly reduced. Of course, these resistances are mainly given by Na⁺ and K⁺ currents driven by the prevailing gradients. Analysis of these currents is outside of the scope of this presentation.

**DISCUSSION**

The crystalline lens, with its high-protein-containing fibers representing almost its total mass and the single-layered epithelium on its anterior face, has been the subject of innumerable biochemical, cell biology, and molecular biology studies (18, 21). Only a handful of investigators have studied the electrophysiology of the lens. Although certain elements of asymmetry in ionic movement had been described (6, 19), our laboratory was the first to isolate the toad lens in an Ussing-type chamber and record an I_{sc} that was originated by the activity of the Na⁺-K⁺ pump in the lens epithelium (7–9). We, as well as others, assumed that the activity of the Na⁺-K⁺ pump was relatively uniform on the

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**Table 1. Short-circuit currents recorded at 6 different O-ring positions separating the anterior from the posterior surfaces of isolated rabbit lenses**

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>1.5</td>
<td>4.2</td>
<td>13.4</td>
<td>5.9</td>
<td>−9.6</td>
<td>−8.6</td>
</tr>
<tr>
<td>20</td>
<td>2.0</td>
<td>6.2</td>
<td>10.8</td>
<td>−1.6</td>
<td>−2.7</td>
<td>−1.2</td>
</tr>
<tr>
<td>21</td>
<td>3.5</td>
<td>6.1</td>
<td>17.0</td>
<td>6.5</td>
<td>−2.0</td>
<td>−6.5</td>
</tr>
<tr>
<td>22</td>
<td>2.4</td>
<td>4.1</td>
<td>13.1</td>
<td>6.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>3.8</td>
<td>10.5</td>
<td>21.9</td>
<td>8.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>1.5</td>
<td>4.0</td>
<td>8.5</td>
<td>4.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>2.6</td>
<td>4.6</td>
<td>7.5</td>
<td>2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>3.3</td>
<td>9.3</td>
<td>10.1</td>
<td>6.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values for short-circuit current (I_{sc}) are μA per lens. A positive value indicates a positive I_{sc} directed across the lens in the posterior-to-anterior direction. A negative value indicates a positive I_{sc} directed across the lens in the anterior-to-posterior direction. P1–P6, positions 1–6 of O-ring.
Table 2. Currents entering or exiting the rabbit lens across 7 predefined zones

<table>
<thead>
<tr>
<th>Zone</th>
<th>Current, μA</th>
<th>SE</th>
<th>Zonal area, cm²</th>
<th>Current per zonal area, μA/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z1</td>
<td>3.9</td>
<td>0.4</td>
<td>0.19</td>
<td>20.7</td>
</tr>
<tr>
<td>Z2</td>
<td>2.9</td>
<td>0.7</td>
<td>0.17</td>
<td>16.9</td>
</tr>
<tr>
<td>Z3</td>
<td>6.4</td>
<td>1.1</td>
<td>0.24</td>
<td>26.6</td>
</tr>
<tr>
<td>Z4</td>
<td>-10.8</td>
<td>2.0</td>
<td>2.15</td>
<td>-5.0</td>
</tr>
<tr>
<td>Z5</td>
<td>-2.1</td>
<td>1.9</td>
<td>0.24</td>
<td>-8.7</td>
</tr>
<tr>
<td>Z6</td>
<td>-1.2</td>
<td>0.9</td>
<td>0.17</td>
<td>-7.1</td>
</tr>
<tr>
<td>Z7</td>
<td>0.9</td>
<td>0.5</td>
<td>0.19</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Values were calculated from means in Table 1, and currents are expressed first as μA per zone and then as μA/cm² for each zone (Z1–Z7). A positive value indicates a positive current entering the lens across the surface of the zone, while conversely, a negative value indicates a positive current leaving the lens across the zone.

The results from Patterson’s group (23, 27, 29, 30) indicated a totally new concept for lens electrophysiology: ionic currents leaving the lens at the equatorial region and reentering at each pole. However, the reports by Patterson and coworkers did not account for all the currents that should be moving across the lens surface. We designed the present experiments to empirically reexamine Patterson’s model with a more direct method in which the $I_{sc}$ was recorded at various arbitrarily defined zones. Our initial results were in accord with Patterson’s conclusions, suggesting that his model was, in general, correct. This is based on the belief that the methodology that we have used is sound and the theoretical analysis valid. Also in support of this, Gao et al. (16) estimated that the pump current density at the frog lens equator was about 20 times larger than that at the anterior pole.

The O-ring provided an excellent seal that separated two areas of the lens surface without damage. Our results were reproducible after the lens was removed and repositioned on various O-rings. The short-circuiting technique, as applied in this work, simulates the condition of a freely submerged lens, with the $I_{sc}$ being a measure of the net current entering on one side of the O-ring and leaving on the other. These currents are originated by the activity of the Na⁺-K⁺ pumps located mainly in the epithelial cells by two mechanisms: the current produced directly by the pump and indirectly by the passive flow of K⁺ and Na⁺ due to gradients created by the pump. We chose Cl−-free solutions to simplify the identification of currents that could produce the $I_{sc}$.

Table 2 and Fig. 6 show the average currents and their direction recorded across the seven zones. It should be emphasized that these zones were chosen on the basis of sizes of O-rings that could support the lens with a certain degree of reproducibility. Although we tried to use lenses of uniform size (~10.5-mm diameter), the separation of zones was clearly arbitrary. This was particularly evident in P4, P5, and P6, where inward and outward currents were registered (see Table 1). Our previous work with a simple separation of anterior from posterior surfaces determined that across the posterior surface of the lens, the inward current was essentially a Na⁺ current (4). The division of this surface into Z1, Z2, and Z3 did not change this conclusion. The current was inward and rather uniform among these three zones (see Table 2). Ouabain and BaCl₂ had only minor or no effect when added to
Table 3. Effects of equal but opposite changes in bathing solution \([K^+]\) and \([Na^+]\) on \(I_{sc}\) and electrical resistance across rabbit lenses isolated in P6 and P1

<table>
<thead>
<tr>
<th>(I_{sc}), (\mu A)</th>
<th>Electrical Resistance, (k\Omega)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P6c</td>
<td>P6 – K(_c)</td>
</tr>
<tr>
<td>-1.4</td>
<td>-5.8</td>
</tr>
<tr>
<td>Mean</td>
<td>0.7</td>
</tr>
<tr>
<td>SE</td>
<td>0.83</td>
</tr>
</tbody>
</table>

P6 and P1 denote positions 6 and 1, respectively, with subscript c indicating the control values. The designations K\(_a\) and K\(_p\) indicate the inward and outward \(K^+\) current, respectively. The densities of the \(Na^+\) and \(K^+\) pump currents were added to the side that included Z4.

Examination of the \(I_{sc}\) in P5 and P6 revealed a finding unique to this article. The currents across Z5 and Z6 remained negative (outward) but in some experiments reversed to a positive (inward) current. The effects of ouabain and BaCl\(_2\) were variable, suggesting that in these zones, three currents may coexist: the 

\(Na^+-K^+\) pump current, the \(K^+\) diffusion current, and the \(Na^+\) diffusion current, with the net dictated by their relative contributions. In the majority of experiments, the \(I_{sc}\) in P6 was negative. This indicates an inward current across Z7 that exits across all the other zones to complete the circuit around the external loop. Notice that according to the adopted convention, a negative \(I_{sc}\) in P6 denotes a positive inward current across Z7 \((I_{sc}, Z7 = 0 – I_{sc}, P6)\). Although this inward current was the norm, in some experiments the current was outwardly directed. This was due to the fact that the outward \(K^+\) current was larger than the opposite \(Na^+\) current. Blocking the \(K^+\) current with BaCl\(_2\) reversed the outward current or increased the inward current. In the majority of cases, ouabain had no effect on the current across Z7. This novel result indicates that the lens epithelium, in an area of about 3–4 mm in diameter concentric to the anterior pole does not translocate \(Na^+\) for \(K^+\). The reason for this deficiency is not clear. Possibly, this region contains an inactive pump or the enzyme is simply absent.

The density of the \(Na^+\) current in Z7 is at least 4.7 \(\mu A/cm^2\) (Table 2) and could be larger because an outward \(K^+\) current may be subtracting from the \(Na^+\) current in some cases. As we have observed repeatedly before, amiloride had no effect on the \(Na^+\) currents of the lens (data not shown). The activity of the \(Na^+-K^+\) pump

Table 4. Electrical resistance of the isolated rabbit lens across predefined zones

<table>
<thead>
<tr>
<th>Resistance Measured at P1–P6, (k\Omega)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>SE</td>
</tr>
<tr>
<td>(n)</td>
</tr>
</tbody>
</table>

Calculated Resistance for Individual Zones

<table>
<thead>
<tr>
<th>Z1</th>
<th>Z2</th>
<th>Z3</th>
<th>Z4</th>
<th>Z5</th>
<th>Z6</th>
<th>Z7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per zone, (k\Omega)</td>
<td>4.55</td>
<td>10.00</td>
<td>4.52</td>
<td>0.23</td>
<td>6.19</td>
<td>3.13</td>
</tr>
<tr>
<td>Normalized for zonal area, (k\Omega\cdot cm^2)</td>
<td>0.91</td>
<td>1.90</td>
<td>1.78</td>
<td>0.46</td>
<td>1.61</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Fig. 8. Summary of the flows of \(Na^+\) and \(K^+\) across the anterior, equatorial, and posterior surfaces. Although not indicated, there is a circulation of ions around the lens between the points of exit and entry.

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pumps is concentrated in Z4, Z5, and Z6. The total outward current of these zones is 14.1 μA. Although the largest contributor is Z4, this is due to its largest area, because its density is lower than that of Z5 and Z6. The lower density may be due to the fact that Z4 included a subequatorial posterior area without pumps.

Previous studies (2, 4) and our present experiments show that the $I_{sc}$ can be totally inhibited by BaCl$_2$ and ouabain when applied to Z4, Z5, and Z6. The ouabain component is ~40%, so the total pump current is ~5.64 μA (14.1 × 0.40) and the outward Na$^+$ current of the pump is 16.9 μA (5.64 × 3, for a 3:2 Na$^+$/K$^+$ ratio). The inward current across Z1, Z2, Z3, and Z7 totals, on average, 14.1 μA. Because 16.9 μA of Na$^+$ must get back into the lens across these zones, 2.8 μA must be carried by K$^+$ leaving across these zones. Because the K$^+$ influx translocated by the Na$^+$/K$^+$ pump should be 11.25 μA (16.9 × 2/3), 8.46 μA should be diffusing out across Z4, Z5, and Z6. Notice that 8.46 μA is 60% of the 14.1 $I_{sc}$. Figure 8 shows schematically vectors representing the currents originated by the diffusion of Na$^+$ and K$^+$ and the Na$^+$/K$^+$ pump across three main areas of the lens surface. This diagram accounts for all currents entering and leaving the lens and indicates that small fractions of the K$^+$ outflow maybe across Z1 through Z3 and Z7.

The electrical resistance of the different lens zones is not uniform when normalized by unit area. Z2 and Z3 had the largest resistances, and Z4, Z6, and Z7 had the lowest. The low resistance of those zones is probably a reflection of a high density of K$^+$ channels. The equimolar replacement of Na$^+$ by K$^+$ demonstrated that the K$^+$ permeability of Z2 was larger than that of Na$^+$.

### References


