Spontaneous Fluid Transport across Isolated Rabbit and Bovine Ciliary Body Preparations

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Purpose. To quantify spontaneous fluid transport across the isolated ciliary bodies of rabbit and bovine and to determine their osmotic permeabilities.

Methods. A complete annulus of ciliary body was mounted in a custom-designed chamber appropriate for detecting net fluid movement across the in vitro preparation.

Results. A net fluid flow in the blood-to-aqueous direction was measured. It was generally observed that tissue freshness is a critical parameter for detection of such flow. The spontaneous, baseline fluid transport rate lasted, on average, ≈4 hours. This flow solely reflects the secretory activity of the isolated ciliary epithelium, since the in vitro arrangement precludes contributions from ultrafiltration. Both the isolated rabbit and bovine ciliary body epithelia transported fluid in the absence of an external osmotic or pressure gradient. After corrections for area and possible collapse of the processes, a total flux rate of approximately 23 μL/hour or 13% of the in vivo flow in rabbit was estimated. This value agrees with predictions of ionic fluxes and short-circuit current measurements, which are also obtained in vitro. The fluid flow is bicarbonate dependent in rabbit and chloride dependent in bovine, consistent with ionic transport mechanisms described in these species. Ouabain inhibited the fluid flow across both species, indicating dependence on active ionic transport. Irrespective of the spontaneous fluid transport, a flow elicited by an osmotic gradient allowed for a calculation of the osmotic permeability coefficient (P_f; $\approx 10^{-3}$ cm/s) in line with reports in other epithelia. In addition, mannitol permeability (5.6×10^{-6} cm/sec) was similar to that measured in "tight" epithelia, as determined by measurements of radiolabeled fluxes of the sugar across rabbit ciliary bodies mounted in the chambers used for the present fluid transport study.

Conclusions. This work demonstrates that isolated ciliary epithelial preparations transport fluid in the blood-to-aqueous direction. The present observations suggest that mounting arrangements for measuring volumetric fluid flow across the ciliary epithelium is suitable for future studies directed toward the pharmacological control of secretion. (*Invest Ophthalmol Vis Sci.* 2005;46:939–947) DOI:10.1167/iovs.04-0950

Intraocular pressure (IOP) is the result of a balance between fluid secretion into and efflux from the eye's chambers. The driving force for fluid efflux is almost exclusively the IOP through the trabecular meshwork and the nonconventional uveoscleral pathway, whereas driving forces for fluid production across the ciliary epithelium are the osmotic gradient (created by ionic transport mechanisms) and a possible hydrostatic pressure difference. The relative contribution of the latter two forces to the production of aqueous humor is still an open question, although most researchers accept a contribution from both.[1,2] The fact that a pseudofacility can be measured indicates that a change in IOP influences not only the efflux but also the influx of aqueous humor. During tonometry, when an external pressure is applied to the eye, an increased outflow, as well as a decreased inflow, is observed. Thus, there is a pressure-dependent component in the aqueous humor inflow.

The importance of the osmotic force developed by active ionic transport became apparent from the initial work of Cole.[3,4] After this, several investigators were able to isolate the ciliary body epithelia of toad,[5] shark,[6,7] rabbit,[8,9] and bovine[10,11] in which active transport mechanisms for Cl^-, Na^+, and HCO_3^- were described. Complementing this work, other investigators have used the isolated tissue to look for transporters with intracellular electrodes,[12,13] fluorescent probes,[14–16] electron probe,[17,18] and patch clamp techniques,[19–22] or, in cultured ciliary epithelial cells, they have looked for channels by using microelectrodes[23] or patch-clamping[24–27] and for transporters by using radioisotopes[28–30] or fluorescent techniques.[31–33] As a result of this wealth of information, several models have been proposed to explain aqueous humor production by the ciliary epithelium.[34–36] Although the potential ions responsible for creating the necessary osmotic gradient have been identified, a quantitative accounting of the rate of ionic transport necessary for a fluid production of approximately 2 to 3 μL/min is still elusive. Indeed, it has been calculated[8,37] that the measured rate of ionic transport in the isolated rabbit ciliary epithelium could account for approximately only 15% of the observed fluid production in vivo. It is therefore possible that aqueous humor production is a concerted combination of both active and passive processes.

Despite the intensive effort just described, a satisfying model consistent with all the available experimental data does not exist. This situation may be due in part to the complexity of the ciliary epithelium and the variations in transport elements among the species studied. In the rabbit tissue, for example, the negative polarity of the aqueous side of the epithelium may be due to a blood-to-aqueous net transport of HCO_3^-, since removal of HCO_3^- from both bathing solutions reverses the electrical potential difference (PD),[8,38,39] whereas in the bovine tissue, a Cl^- transport from blood-to-aqueous seems to be responsible for the same PD orientation.[10,11,40] Moreover, with the isolated rabbit ciliary epithelium, Kishida et al.[41] and Crook et al.[42] also have described a net Cl^- transport as a component of the short-circuit current (I_{sc}) across the

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tissue, although in the latter study there was a large discrepancy between the net Cl^- flux and the I_{sc}.

It is widely known that the composition of the aqueous humor of different species varies, particularly with regard to the Cl^- and HCO_3^- concentrations.[43–46] In humans, for example, Cl^- concentration is higher and HCO_3^- concentration is lower in the posterior chamber than their concentrations in the plasma, whereas in the rabbit the reverse is true.[43–46] Most transport studies of the isolated rabbit ciliary epithelium have yielded results consistent with the in vivo higher-than-plasma concentration of HCO_3^- in the aqueous.[8,39,47,48] Even in the recent paper by Crook et al.,[42] bumetanide inhibited only 43% of the I_{sc}, the remainder of which may be a net HCO_3^- flux. Evidence indicative of a net HCO_3^- flux across the rabbit ciliary epithelium was provided by Wolosin et al.,[14,15] and Butler et al.,[16] who demonstrated an asymmetry in the expression of HCO_3^- transporters in the nonpigmented and pigmented epithelia. The latter exhibited the alkali-loading Na^+-dependent Cl^-/HCO_3^- exchanger, whereas the dominant bicarbonate transporters of the former resulted in HCO_3^- efflux. In the isolated bovine ciliary body, in contrast, previous findings show a net Cl^- transport toward the aqueous,[10,40,49] which is consistent with a higher-than-plasma concentration of Cl^- in the aqueous humor, as found in humans.

Only one study on the ciliary epithelium has been reported to date that measured the actual spontaneous movement of fluid across the isolated tissue,[50] as done elsewhere across other epithelia.[51–53] This earlier work quantified a fluid transport rate ≈10-fold lower than the generally accepted rate of in vivo aqueous formation. Thus, to examine this aspect of ciliary epithelial physiology, we performed measurements in two preparations, the rabbit and bovine, on which active ionic transport and membrane permeability studies have concentrated.

Materials and Methods

Adult albino rabbits of either sex weighing 3 to 4 kg were purchased commercially and killed by CO_2 asphyxiation. Barbiturates and other agents that could alter aqueous humor secretion were not used. Before death, the rabbits' care conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Experiments using bovine eyes were performed in the laboratories of authors in Hong Kong (C-HT) and Corrientes, Argentina (RMG), given the ready access to abattoirs at these locales. The bovine eyes were obtained and transported on ice by car to the respective laboratories within 0.5 to 2 hours after death, a requirement for these physiological experiments.

The dissection protocols for rabbit and bovine have been described.[8,10,40] Briefly, the eyes were placed in a modified Tyrode's solution, and the globe was bisected horizontally 4 to 5 mm posterior to the corneal limbus. The anterior half containing the iris-ciliary body (I-CB) was immediately placed corneal surface down in Tyrode's solution. Using a surgical microscope at 10× magnification, the choroid-retina was separated from the sclera to the ora serrata, the lens and its capsule were removed by cutting the zonules, and the ciliary body was separated with a lamellar dissecting blade (Beaver blade no. 66) from the sclera. The I-CB was transferred on a flat spatula and placed ciliary-process-side-up on a circular Lucite hemichamber covered with tightly stretched nylon (Fig. 1 for an illustration of the hemichamber surface that interfaced with the tissue), and was centered, using the choroid-retina for manipulation, over the chamber opening. A second piece of nylon was gently placed over the tissue followed by a second complementary hemichamber.

In the case of the rabbit I-CB, the Lucite chambers were fabricated by milling circular troughs into circular Lucite blocks. As such, the circular troughs, which defined the chamber walls within each hemichamber, retained a central post (4 mm in diameter) that was capped by an O-ring. The outside diameter of each circular trough,

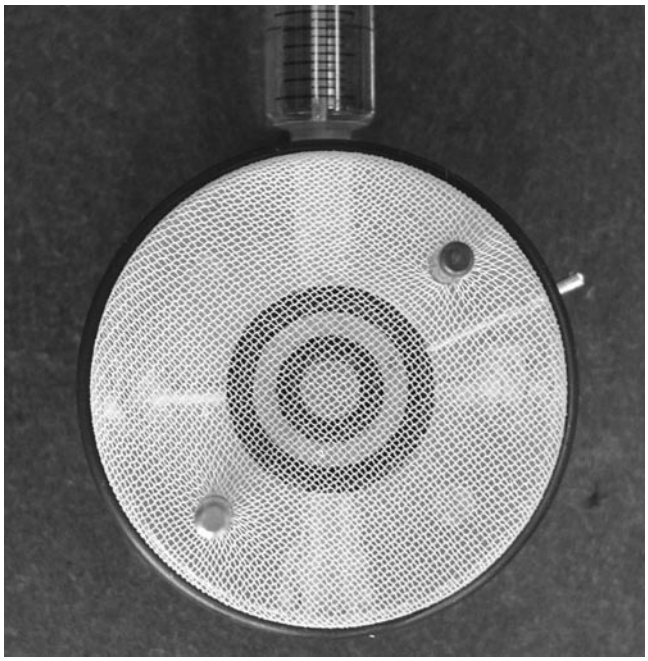

Figure 1. The nylon-covered hemichamber surface on which the complete annulus of rabbit I-CB was positioned. The concentric O-rings define a circular trough of 0.5 mL that bathe one side of the tissue. The inner O-ring sits within a notch on a central post of 4-mm diameter that occludes the pupil. After the I-CB was centered on the O-rings, a complementary hemichamber with matching O-rings (Fig. 2) was lowered on the alignment pins shown in the photograph.

which accommodated a fluid volume of 0.5 mL, was notched to accommodate an O-ring that aligned in the same plane as the O-ring capping the central post. In the final assembly, the central posts within each hemichamber occluded the pupil and only the ciliary body interfaced with the troughs, so that the tissue was bathed by 0.5 mL of solution on each side of the preparation. Each hemichamber had been further modified by drilling ports into the sidewalls of the Lucite to connect the fluid in the troughs to external vessels. In this regard, the hemichambers were not identical (Fig. 2). On one side, the hemichamber contained two ports connecting the trough to an external glass bubbler that enabled drug additions, changing of bath solutions and delivery of various gases, as well as placement of a closed "heating loop" through which thermostatically controlled water was circulated. On the other side, the Lucite hemichamber contained a port to a connector that fitted a 25-μL capillary with graduations allowing for visual detection of 0.25-μL changes in compartment volume. This hemichamber was also fitted with a leakproof port through which a probe from a thermocouple digital thermometer (Cole-Parmer Instrument Co., Vernon Hills, IL) was placed. Because the heating source solely resided within the glass bubbler on the contralateral side, the bath temperature within the capillary-containing hemichamber was approximately 2° lower, but constant.

After emplacement of the tissue between the hemichambers, bathing medium that had been prewarmed (36°C) and pre-equilibrated with the appropriate gas (typically, 5% CO_2 and 95% air) was pipetted into the glass bubbler (7 mL total volume) and into the port containing the connector (0.8 mL total) followed by the attachment of the capillary to the connector. The capillary was pretreated with a hydrophobic agent (Rain-X; SOAPUS Products, Houston, TX; available at www.rainx.com) to prevent fluid crawl within the capillary. Preliminary observations using a dialysis membrane in lieu of the I-CB determined that no fluid movement between the chamber compartments could be detected in the absence of a biological tissue. The fluid level within the capillary could be reset during the experiments by introducing a very thin needle that fit within the capillary barrel (0.46 mm in diameter).

FIGURE 2. The hemichambers used to isolate a complete annulus of rabbit iris-ciliary body between concentric O-rings. The internal O-rings are situated on 4-mm diameter posts that occlude the pupil in the complete assembly. The hemichamber affixed with the external glass bubbler is shown on the *left*, and the contralateral vessel has the connected graduated capillary. Within the enclosed chamber, the ciliary epithelium interfaces with the bathing solutions. The iris is pressed against the Lucite walls holding the tissue in place, so that there is minimal or no contact between the iris and the bath. Each hemichamber also has ports for the necessary salt bridges for transepithelial electrical measurements. In most experiments these openings were closed with leakproof plastic plugs.

In experiments in which increases in the capillary levels were recorded, the capillary volume was usually lowered throughout the experiment, and the total volume change as a function of time was recorded.

In separate experiments, the complete annulus of I-CB was oriented between the hemichambers so that either the stromal side or the ciliary process side interfaced with the chamber containing the capillary. Because of the enclosed nature of the capillary-containing hemichamber, solution changes and drug introductions were made only on the side of the preparation containing the glass bubbler.

Bovine I-CB preparations were isolated and mounted identically with that described for rabbit. However, because the bovine I-CB is larger and somewhat asymmetrical with a slit-like pupil, the Lucite hemichambers were machined appropriately to accommodate its geometry. The bovine chambers were made at Mount Sinai in New York and provided to the laboratories in Hong Kong and Corrientes. As in the case of the rabbit, the bovine tissue was mounted as a complete annulus that was bilaterally exposed to the bathing media. The exposed area of the bovine annulus was $\approx$1.36 cm^2 versus 1.02 cm^2 for the rabbit. Because the membrane surface area of the ciliary processes is larger than the area of the annulus exposed to the bathing solutions, fluid transport rates are expressed in terms of transmural movement of fluid in microliters per hour per preparation.

Bathing Solutions

The medium used during the dissection and bathing of the preparations during experiments was a modified Tyrode's solution of the following composition (in mM): 1.8 $CaCl_2$, 1.2 $MgCl_2$, 4.5 KCl, 103 NaCl, 30 $NaHCO_3$, 10 HEPES (hemi-Na salt), 1 Na_2HPO_4, 5.6 glucose, and 0.3 reduced glutathione. When pre-equilibrated with 5% CO_2, the pH of this solution was 7.5, and its osmolality measured 280 mOsmol/kg water. For tissues bathed in the absence of CO_2/HCO_3^-, $NaHCO_3$ was excluded, the HEPES (hemi-Na salt) was increased to 30 mM, and the osmolality was compensated for by adding sucrose. For a Cl^--free medium, SO_4^{2-} salts replaced those containing the halide, and the osmolality was adjusted with sucrose. When ouabain was applied, the glycoside was diluted into the hemichamber containing the glass bubbler from a 10 mM aqueous stock solution.

Data Analysis

The significance of experimentally elicited changes in fluid transport rates were analyzed with Student's *t*-test as either paired or unpaired data, depending on the situation, as described below.

Mannitol Fluxes

For an assessment of the integrity of the preparation within the chambers designed for the present study, ^{14}C-mannitol fluxes were measured across rabbit ciliary bodies mounted in the chamber detailed above. However, in this case, the capillary was replaced by a glass bubbler so that both sides of the tissue were bathed by rapidly circulating solutions, and electrical bridges were inserted into the bathing solutions through ports in the Lucite walls to quantify the transepithelial electrical parameters, as described in detail earlier.[8] For the flux measurements, unlabeled mannitol was added bilaterally at a concentration of 1 mM, followed by D-mannitol-1-^{14}C ($\approx$1 μCi/mL of bathing solution; Sigma-Aldrich, St. Louis, MO) to the aqueous-side hemichamber. Samples (2 mL) were then taken from the unlabeled bath and replaced with fresh Tryrode's solution (maintaining the chamber volume) at 15-minute intervals for 4 hours. During the course of the experiment, the labeled side was sampled (25 μL) periodically to determine the specific activity and assure that the unlabeled side activity was $<$5% of that of the labeled side. All samples were mixed with a modified Bray's solution and the beta emission was counted in a liquid scintillation detector.

RESULTS

Measurements of Spontaneous Fluid Movement

The chamber design was a critical facet for the detection of a spontaneous fluid movement across the ciliary epithelium isolated in a divided chamber. Although the overall mounting of the preparation resembles that described earlier for in vitro transepithelial electrical measurements in an Ussing-type arrangement,[8] the essential aspect that promoted success in the present study was the avoidance of leaks and the control of the bath temperature in the enclosed hemichamber containing the capillary. In addition, the preparations were not short-circuited, as happens under Ussing-type conditions, so that the transmural potential difference (PD) developed by the ions actively transported across the preparation could drive a companion ion paracellularly to create the osmotic gradient necessary for water flow.

Under these conditions using the rabbit ciliary body, a plot showing the average increases (n = 10 preparations) in aqueous-side capillary volume as a function of time is provided (Fig. 3A). The calculated flow rates (expressed on a per hour basis) during the course of the experiment are also plotted in the figure (solid squares). A gradual decline in the rate of fluid transport was commonly observed. In the case of the calculated rate of water movement in the blood-to-aqueous direction, for example, the flow decreased from 2.74 μL/hour per preparation (the initial rate between time 0 and 15 minutes; Fig. 3A) to 1.80 for the rate measured between 135 and 150

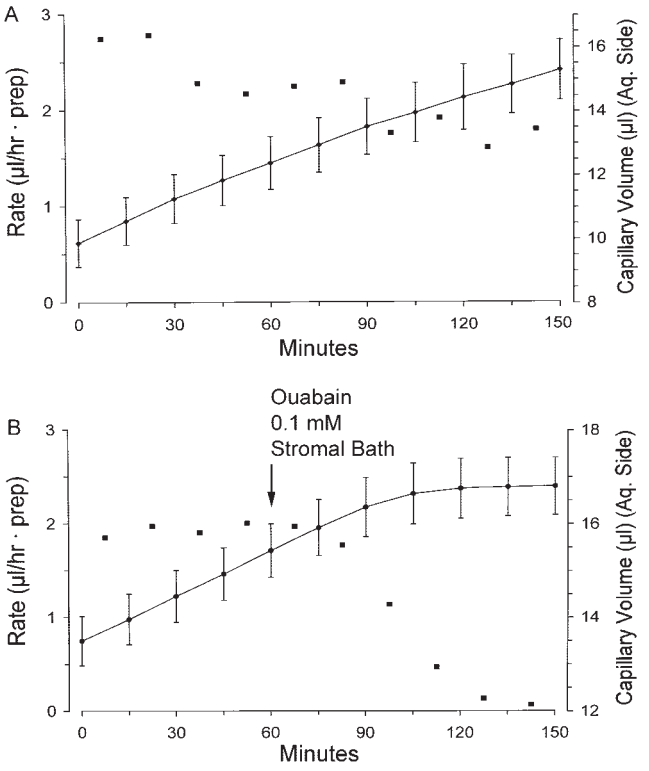

FIGURE 3. Spontaneous fluid movement across the isolated rabbit ciliary epithelium in the blood-to-aqueous direction. *Left axis*: the calculated flow rate, expressed in microliters per hour, during the course of the experiment plotted as *squares*. *Right axis:* the measured change in the capillary level on the aqueous side of the preparation plotted as a function of time, with the data points connected by a *solid line*. The initial capillary volume was arbitrarily positioned at a level 1 to 2 cm above that of the opposite-side bath within the glass bubbler (Fig. 2). (**A**) Data points are the mean ± SEM of results in 10 control experiments. (**B**). Effect of ouabain. The Na^+-K^+ ATPase inhibitor was present in the blood-side bathing solution from the point indicated (*arrow*). Points are the mean ± SEM of results in six rabbit preparations exposed to ouabain.

minutes. Thus, over a period of 2.5 hours, the fluid transport rate was at least 65% of the initial rate.

In contrast, with rabbit preparations exhibiting for 60 minutes a spontaneous fluid transport in the blood-to-aqueous direction, the introduction of ouabain to the stromal-side bath (Fig. 3B) induced a more pronounced decline in flow so that detectable transport rates between 135 and 150 minutes were virtually unobservable.

Given additional time (beyond 4 hours), fluid transport by control preparations also ceased. Nevertheless, the more marked decline in fluid transport with preparations exposed to the glycoside probably occurred due to increased epithelial Na^+ levels, which in turn reduced the driving force for secondary active transport presumably via the Na^+-dependent Cl^-/HCO_3^- exchanger[14–16] and the Na^+-K^+-$2Cl^-$ cotransporter.[42]

In other experiments (not shown), introducing the metabolic poison NaCN (2 mM) to the blood-side bath ($n = 3$ preparations) produced inhibitions of fluid transport with a time course similar to the more rapid decline observed with preparations exposed to ouabain ($n = 6$).

Not all preparations exhibited detectable levels of fluid transport (≈1/3). An exact explanation for this was not immediately apparent. The most probable cause was a leaky tissue that did not totally seal the two sides of the chamber. It was also observed that the shortest time that elapsed between the death of the animal and the isolation of the ciliary body in the chamber was, in general, the best predictor of experimental success. Suitable preparations displayed fluid movement within 15 minutes of mounting.

A mean plot ($n = 6$ preparations) for blood-to-aqueous fluid movement across the bovine ciliary body (Fig. 4A) indicated an in vitro rate of 3.04 µL/h per preparation. For this, the two initial rate measurements were discarded and the remaining relatively stable data were averaged. The final rate measurements determined between 120 and 150 minutes were 2.55 and 2.72 µL/h per preparation, or at least 80% of the mean level, consistent with a gradual decline after the initial stabilization period between time 0 and 30 minutes. Similar to results with the rabbit, the addition of ouabain led to a more rapid decline in flow, which reached zero approximately 60 minutes after treatment with the inhibitor (Fig 4B), than that observed in control preparations, which still transported fluid within this time frame.

Given indications for the transepithelial transport of HCO_3^- across the rabbit ciliary epithelium,[14–16] a set of experiments was conducted whereby the tissues were bathed bilaterally with a HEPES-buffered, HCO_3^--free medium with air bubbling of the blood-side bath and the capillary on the aqueous-side hemichamber (data included in Table 1). Under these conditions, the fluid transport rate in the blood-to-aqueous direction was 0.48 ± 0.10 µL/h per preparation (mean ± SEM, $n = 4$). The flow immediately increased approximately fivefold to 2.52 ± 0.29 µL/h per preparation ($n = 4$, $P < 0.01$ as paired data), on replacement of the blood-side bathing medium (aque-

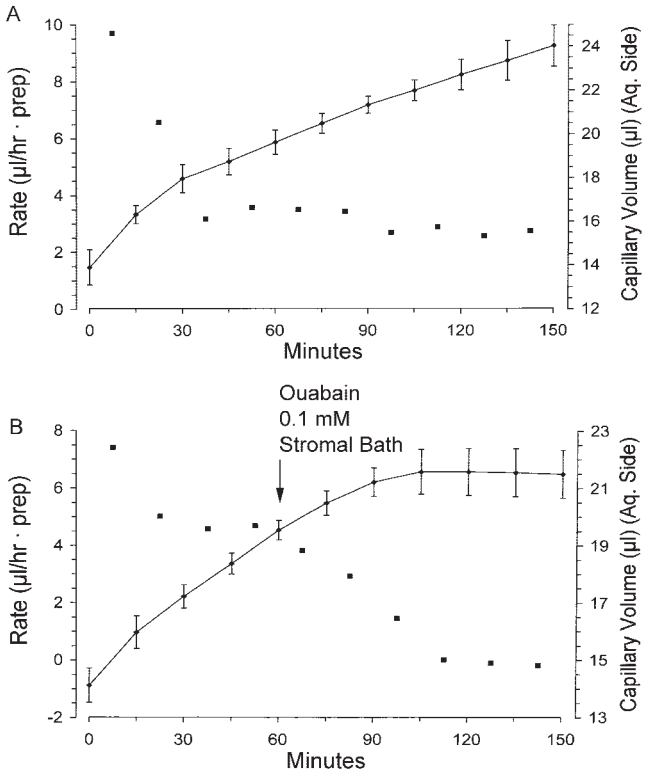

FIGURE 4. Spontaneous fluid movement across the isolated bovine ciliary epithelium in the blood-to-aqueous direction. *Left axis:* calculated flow rate plotted as *squares*. *Right axis:* the measured change in the capillary level on the aqueous side of the preparation, with data points connected by a *solid line*. Conditions were identical with those described in Figure 3. Data points are the mean ± SEM of results in (**A**) six control preparations and (**B**) eight bovine preparations exposed to ouabain.

TABLE 1. Spontaneous Volumetric Fluid Flow across the Isolated Rabbit Ciliary Epithelium

Capillary on Aqueous Side			Capillary on Blood Side	
Exp.	HCO_3^- Free (Blood Side)	HCO_3^- Rich (Blood Side)	Exp.	HCO_3^- Rich (Both Sides)
H1	0.68	3.08	B5	1.34
H3	0.32	2.94	B6	1.54
H5	0.62	2.28	B8	2.51
H7	0.30	1.80	B9	1.57
B1		1.06	B18	2.81
B2		3.25		
B3		0.79		
B4		1.78		
B10		4.26		
B11		4.74		
B12		1.47		
B14		1.21		
B16		0.58		
B17		2.49		
Mean	0.48	2.27		1.95
SEM	0.10	0.34		0.29
n	4	14		5

Data are expressed in microliters per hour per preparation and represent flow in the blood-to-aqueous direction. With the capillary on the aqueous side, results indicate that the capillary level increased; conversely, with the capillary on the blood side, the capillary level decreased. Experiment numbers labeled with an H denote tissues pre-equilibrated and bathed bilaterally in the divided chamber with HEPES-buffered solution plus air bubbling of the hemichamber opposite to that containing the capillary. In these cases, after quantifying the baseline rate of fluid transport, the blood-side bathing medium was replaced by an HCO_3^--containing solution with CO_2 gassing, the aqueous side compartment containing the capillary was not disturbed, and capillary levels were continuously recorded. In all other experiments (labeled B), tissues were bathed bilaterally with HCO_3^--buffered solutions with CO_2 bubbling limited to the side opposite the capillary-containing hemichamber.

ous-side left undisturbed), with Tyrode's solution containing 30 mM HCO_3^- plus 5% CO_2 gas bubbling. Experiment number H3 (Table 1) is shown as representative of the set (Fig. 5).

In 10 other preparations that were mounted directly with bilateral HCO_3^- containing solution plus 5% CO_2 gassing of the blood-side bath and the capillary on the aqueous side (i.e., the conditions illustrated in Fig. 3A), the fluid transport rate was 2.16 ± 0.47 μL/h per preparation, so that overall the mean for the 14 experiments in the presence of HCO_3^- was 2.27 ± 0.34 μL/h per preparation (Table 1). This rate was also measured (1.95 ± 0.29 μL/h per preparation; $n = 5$) under conditions in which the capillary-containing hemichamber interfaced with the blood side of the preparation and the bubbling was limited to the aqueous-side bath. Under these conditions the level of the capillary decreased as a consequence of fluid transport toward the aqueous-side hemichamber. Nevertheless, the calculated rates of transepithelial fluid movement were virtually identical ($P = 0.31$ as unpaired data), thereby suggesting that the unstirred layers that develop in close proximity to the epithelial cells (presumably in the lateral spaces between the cells) were not affected by the circulation within the hemichamber. The reversal of the chamber flow, which coincided with the reversed mounting of the tissue, is an indication that the flow was not an experimental artifact.

Analogous electrolyte-substitution experiments were performed with the bovine preparations. With this tissue the effect of the presence or absence of Cl^- in the blood-side bath on blood-to-aqueous fluid movement was determined (Table 2), given the demonstration of a net Cl^- flux across the isolated bovine ciliary epithelium and the absence of a net HCO_3^- flux.[49] In seven preparations in which SO_4^{2-} was used to replace Cl^- in the blood-side bath, fluid movement decreased from 3.68 ± 0.63 to -0.10 ± 0.64 μL/h per preparation ($P < 0.01$, as paired data). On reintroducing the control Tyrode's solution to the blood-side hemichamber, the fluid transport in the blood-to-aqueous direction recovered to 4.24 ± 0.58 μL/h ($n = 7$), a level indistinguishable from the original baseline flow ($P > 0.5$, as paired data). In four of the seven preparations, the absence of the halide elicited a reversal in the flow rate so that a net aqueous-to-blood flow was observed (Table 2), as exemplified with experiment number C22 (Fig. 6).

With the capillary on the blood side of the bovine preparation and CO_2 bubbling of the aqueous-side hemichamber, the measured blood-to-aqueous fluid movement (2.25 ± 0.20 μL/h per preparation, $n = 9$) was sensitive to the presence of aqueous-side ouabain, which was applied in eight of the nine experiments (Table 2). The fact that a clear inhibition was obtained within 90 minutes suggests that Na^+ levels also increased in the pigmented epithelium (PE) so that the Na^+-dependent secondary active transport mechanisms in this cell layer that are thought to drive transepithelial Cl^- transport were impeded.

Measurements of Osmotically Elicited Fluid Movement

To determine the osmotic permeability of the rabbit ciliary body, the osmolality of either bathing solution was increased by 100 and 200 mOsM. A typical experiment with the rabbit tissue, in which the osmolality of the blood-side bath was increased by sequential additions of sucrose is illustrated (Fig. 7). Notably, a gradient of approximately 100 mOsM (blood-side hypertonic) eliminated the spontaneous flow, suggesting that active transport creates an unstirred layer on the basolateral side of the nonpigmented epithelium (NPE), ≈100 mOsM larger than the blood-side solution. Such gradient may be the driving force for fluid secretion, as it would be continuously dissipated and replenished by active transport.

This tonicity maneuver immediately increased or reversed (depending on the side of addition) the spontaneous flow proportionally. Based on the elicited changes in flow, the osmotic permeability coefficient, P_f (expressed in centimeters per second) was calculated for the rabbit (Table 3) and bovine

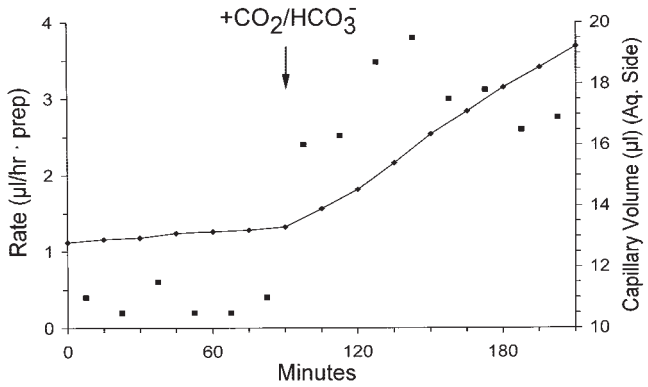

FIGURE 5. Spontaneous fluid movement across the isolated rabbit ciliary epithelium in the blood-to-aqueous direction. Representative experiment showing effect of bicarbonate introduction. *Left axis:* calculated flow rate plotted as *squares*. *Right axis:* the measured change in the capillary level on the aqueous side of the preparation, with data points connected by a *solid line*. Conditions as described in the footnote to Table 1.

TABLE 2. Spontaneous Volumetric Fluid Flow across the Isolated Bovine Ciliary Epithelium

Capillary on Aqueous Side				Capillary on Blood Side		
Exp.	Cl^--Rich (Both Sides)	Cl^--Free (Blood Side)	Cl^--Rich (Both Sides)	Exp.	Cl^--Rich (Both Sides)	Ouabain 0.1 mM (Aqueous Side)
HK1	1.77	1.02	4.17	HK4	1.89	0.01
HK2	3.21	1.29	4.41	HK5	2.00	
HK3	3.42	2.34	7.26	HK6	2.03	0.03
C10	2.61			HK7	1.09	0.00
C12	2.37			C16	2.10	0.07
C14	7.11			C23	2.53	0.01
C15	2.37			C30	2.78	0.00
C22	4.54	−2.21	3.52	C40	3.03	0.00
C31	6.88	−0.53	2.61	C43	2.78	0.05
C39	2.25	−1.74	3.03			
C55	3.70	−0.86	4.67			
C101	1.07					
C106	2.71					
Mean	3.38	−0.10	4.24		2.25	0.02
SEM	0.51	0.64	0.58		0.20	0.01
n	13	7	7		9	8

Data are expressed in microliters per hour per preparation and represent flow in the blood-to-aqueous direction. With the capillary on the aqueous side, values indicate that the capillary level increased; conversely, with the capillary on the blood side, the capillary level decreased. All tissues were pre-equilibrated and bathed bilaterally in the divided chamber with HCO_3^--buffered Tyrodes's solution plus CO_2 bubbling of the hemichamber opposite to that containing the capillary. Experiments numbered with an HK and C were performed in Hong Kong and Corrientes, respectively. Each condition lasted between 60 and 75 minutes. The recorded values were obtained after the new flow rates were stable or in the case of ouabain, 90 minutes after its addition.

tissues (Table 4, using 90 mM sucrose with the bovine specimen) from the following expression

$$P_f = J_v/(A \cdot V_w \cdot \Delta C_s),$$

where, J_v, is the net fluid flow (in cubic centimeters per second), A is the area of the membrane (in square centimeters), V_w is the partial volume of water (in cubic centimeters per mole), and ΔC_s is the difference in solute concentration (moles per cubic centimeters). The data in Tables 3 and 4 were obtained using the cross-sectional areas of the rabbit and bovine chambers (1.02 and 1.36 cm^2, respectively). In general, these results are similar to those in the unstimulated toad bladder, a tissue that exhibits increased water permeability when stimulated by anti-diuretic hormone.[53] If a larger, effective cross-sectional area were assumed, P_f would be proportionally lower, but still within a reasonable range for an epithelium. In addition, the existence of unstirred layers, which likely are also present in vivo, may play a role in the calculation of P_f.

Measurements of Mannitol Fluxes under Short-Circuited Conditions

To obtain information on the "tightness" of the ciliary body preparation within the presently used chambers, the permeability of the paracellular pathway across the rabbit tissue was measured with ^{14}C-mannitol in short-circuited conditions. The absolute value of the mannitol permeability was $5.6 \pm 3.8 \times 10^{-6}$ cm/s (mean ± SEM, $n = 5$) for a total 6-cm^2 area,[37,50] which is similar to that obtained across the rabbit ciliary body

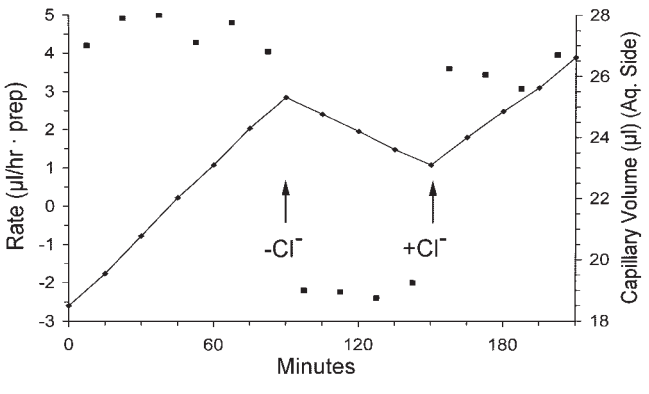

FIGURE 6. Spontaneous fluid movement across the isolated bovine ciliary epithelium in the blood-to-aqueous direction. Representative experiment showing the effect of chloride removal and reintroduction. *Left axis:* calculated flow rate plotted as *squares*. *Right axis:* The measured change in the capillary level on the aqueous side of the preparation, with data points connected by a *solid line*. Conditions as described in the footnote to Table 2.

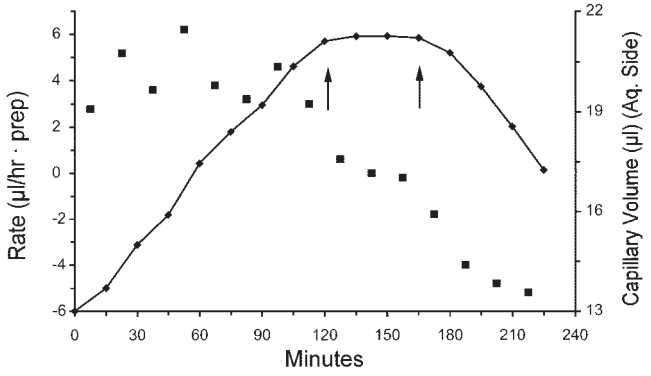

FIGURE 7. Fluid movement across the isolated rabbit ciliary epithelium in the blood-to-aqueous direction. Representative experiment showing the effect of increases in tonicity of the blood-side bathing solution. *Left axis:* calculated flow rate plotted as *squares*. *Right axis:* the measured change in the capillary level on the aqueous side of the preparation, with data points connected by a *solid line*. *Left arrow:* introduction of 100 mM sucrose to the blood-side bathing solution. *Right arrow:* the stromal-side sucrose concentration was increased to 200 mM, eliciting a reversal in the direction of fluid movement.

TABLE 3. Osmotic Permeability (P_f) of the Isolated Rabbit Ciliary Epithelium

Exp.	Control	200 mM Sucrose Blood Side	Change
21	5.87	−29.47	35.33
23	4.33	−32.93	37.27
29	23.67	−26.0	49.67
Mean	11.29	−29.47	40.76

Values are fluxes (J_v expressed in cubic centimeters per second $\times 10^{-7}$). $P_f = 11.1 \times 10^{-4}$ cm/s.

using a different mounting arrangement[37] and, as reported before, is independent of the relatively low transepithelial electrical resistance. These results are putatively consistent with the large transcellular electrolyte fluxes described earlier.[37] In agreement with these findings, the addition of amphotericin B (10 μM) to the aqueous-side bath at the end of the flux-sampling protocol, to permeabilize the basal membrane of the NPE, resulted in a rapid I_{sc} increase (from 0.6 ± 1.7 to 11.1 ± 2.3 μA/preparation; $n = 5$) that was sustained for an hour before a gradual decline (not shown). These elevated currents were promptly inhibited when ouabain was added to the stromal-side bath. Such amphotericin B-elicited I_{sc} changes have been described in detail.[38] These currents could only arise across an epithelium with intact cell membranes and functional ion transport systems. The fact that I_{sc} stimulation and subsequent inhibition is elicited by adding agents sequentially to the aqueous and blood sides indicates the viability of both cell layers.

DISCUSSION

With a volumetric technique that we developed, spontaneous fluid flows across isolated rabbit and bovine ciliary body preparations were measured. From the effects of electrolyte substitution experiments and Na^+-K^+ pump inhibition with ouabain, the detected transmural volume changes represent net fluid transport secondary to active transport mechanisms in the ciliary epithelia of these species.

In approximately one third of the isolated preparations, such fluid transport was not detectable. It was generally observed that tissue freshness was a critical factor favoring the development of spontaneous fluid movement. When obtained, such flow typically lasted for ≈3 to 4 hours. The detected fluid movement solely reflects the secretory activity of the isolated ciliary epithelium, since the in vitro arrangement precludes contributions from ultrafiltration, as well as externally applied osmotic or pressure gradients.

The gradual decline in fluid transport observed under control conditions (Figs. 3A, 4A) is not necessarily a consequence of a deterioration of the excised tissue within this time frame. There was no increase in the mannitol fluxes measured over a 4-hour period suggesting no decline in tissue integrity. Moreover, transepithelial electrical parameters of control preparations were stable over 3 to 4 hours, indicating that the driving force for spontaneous fluid transport is not lost within this period in vitro. If so, the decline in fluid transport may result from a loss in membrane water permeability. Hypothetically, water channels could close or be removed from the membrane.

Measurements across the bovine ciliary body were performed in Hong Kong and Corrientes because of the practicality of obtaining the eye globes from local abattoirs and mounting the preparations in vitro within 2 hours of the death of the animal. The net flow rate across the bovine ciliary body was markedly reduced on the removal of Cl^- from the blood-side bath; readdition of the anion to the bathing medium restored the flow.

In the case of the rabbit ciliary epithelium, a fluid flux in the blood-to-aqueous direction of approximately 2 μL/h per preparation was measured (Table 1). This rate was reduced to ≈0.5 μL/h by the removal of bicarbonate.

Overall, the sensitivity of fluid movement across the bovine preparation to chloride and that of the rabbit preparation to bicarbonate is consistent with the respective anionic requirements for maintaining a transepithelial potential difference across these tissue specimens in electrophysiological experiments.[8,49] The fundamental difference between these two animal models is the directionality of the Cl^-/HCO_3^- exchanger at the basolateral side of the PE. In the rabbit, it is the Na^+-dependent Cl^-/HCO_3^- exchanger that pumps HCO_3^- into the PE and extrudes H^+ and Cl^- from the PE into the stroma.[14–16] In the bovine, the Na^+-independent, acid loading Cl^-/HCO_3^- exchanger moves Cl^- into the PE and removes HCO_3^-. As a consequence of this reversal in Cl^-/HCO_3^- transport, there is no transepithelial movement of HCO_3^- in the bovine,[49] which is replaced by an additional influx of Cl^-,[49] resulting in a much larger transepithelial Cl^- flux from blood-to-aqueous in the bovine than in the rabbit.

The two-compartment chamber used in this work exposed a 1.02-cm^2 (rabbit) and 1.36-cm^2 (bovine) annulus of ciliary body to the bathing solutions. It has been estimated that the total surface of the ciliary processes in the rabbit is approximately 6 cm^2.[37,50,54] Such data have not been reported for the bovine, but a 40% larger area, for a total surface of the processes of ≈8.5 cm^2 seems a reasonable assumption. In vivo, the processes are turgid due to blood circulation. When isolated in the present chamber arrangement, the processes are partially collapsed so that the sectional ≈1-cm^2 area may represent only a fraction of the active surface.

The in vivo rate of rabbit aqueous humor formation is approximately 180 μL/h,[55,56] or ≈90-fold larger than that measured in the present study. Because not all the processes and pars plana are included in the surface exposed to the bathing medium, it is difficult to compare the in vitro and in vivo rates accurately. The average in vitro measurement was 2.27 μL/h, with a high rate of 4.74 μL/h (Table 1). Assuming the largest rate is more representative of a robust preparation and that the in vitro collapse of the processes, as well as the incomplete epithelial surface exposed to the bathing solutions, contribute to reduce the measured fluid transport rate by a factor of 10, then the maximum estimated in vitro rate is 47 μL/h or as low

TABLE 4. Osmotic Permeability (P_f) of the Isolated Bovine Ciliary Epithelium

Exp.	Control	90 mM Sucrose Aqueous Side	90 mM Sucrose Blood Side	Change
66	5.6		−21.1	26.7
68	3.6	14.4		10.8
69	1.7		−17.8	19.4
71	13.9		−13.3	27.2
74	5.6	15.6		10.0
77	27.8		4.2	23.6
79	15.3		−8.3	23.6
83	20.0		−5.0	25.0
94	11.1	26.4		15.3
Mean	11.6	18.8	−10.2	20.2
SEM	2.9	3.8	3.8	2.2

Values are fluxes (J_v expressed in cubic centimeters per second $\times 10^{-7}$). $P_f = 9.2 \times 10^{-4}$ cm/s.

as 23 μL/h—thus, between 13% and 26% of the in vivo rate. Based on earlier I_{sc} measurements and ionic fluxes across the rabbit ciliary body,[8,37,38] it has been estimated that active transport could not contribute more than 15% to the fluid flow considered to result from secretion, which roughly corresponds to the present rates obtained. As such, the fluid transport rates measured in these experiments are generally consistent with the rate of active transport reported earlier.

The large discrepancy between in vitro and in vivo fluid transport also suggests the importance of additional factors. One obvious consideration is that hydrostatic pressure may be an important driving force in vivo by inducing ultrafiltration, as previously proposed.[2,8] Alternatively, it is also possible that in the isolated preparation, without blood supply, the rate of active transport decreases. Perhaps the blood circulation provides hormones or other paracrine agents that stimulate active ionic transport, which are obviously not present in the in vitro bathing solutions. These putative shortcomings also existed under the conditions of an earlier attempt to quantify spontaneous fluid transport across the isolated rabbit ciliary body.[50]

Ostensibly, not all aqueous humor production may arise from fluid movement across the ciliary processes. For example, in the human and rabbit, the reported rates of aqueous humor production are ≈165 and 180 μL/h, respectively.[55–57] Given estimations for the total surface areas of the ciliary processes in these species of approximately 2 and 6 cm^2, respectively,[54] the fluid transport rates exhibited by these species are ≈83 and 30 μL/h per square centimeter, if all fluid solely traversed the ciliary epithelium. The fact that cogent arguments limit the entry pathway for proteins to the anterior surface of the iris and thus direct entry to the anterior chamber,[58] implies that fluid may also passively enter via this route.

Nevertheless, based on the measured P_f, which was consistent with those obtained in other epithelia,[51–53,59] it is apparent that the ciliary epithelium was not physically compromised by the present mounting arrangement. Had there been structural damage to these cells, the imposition of the transepithelial osmotic gradient would have resulted in a larger fluid displacement from one hemichamber to the other, and a nonphysiological osmotic permeability would have been calculated.

Similarly, structural damage to the tissue would have resulted in larger mannitol fluxes than those observed. It is well established that mannitol does not penetrate cell membranes. Therefore, mannitol fluxes are routinely used as a marker for paracellular permeability. That both cell layers were capable of transport was indicated at the end of the mannitol experiments by sequentially adding amphotericin B and ouabain. Amphotericin B on the aqueous side shunts the Na^+-K^+ pumps at the basolateral membrane of the NPE, thereby uncovering the large I_{sc} produced by the PE, as seen before using another mounting arrangement.[38] If either cell layer were not viable, this effect would not be observed. After the addition of ouabain to the blood side, the Na^+-K^+ pumps of the PE are inhibited and the I_{sc} rapidly declines toward zero.

An interesting alternative arrangement for characterizing the mechanisms underlying aqueous humor formation involves the use of an in vitro perfused bovine eye.[60] With this methodology, a perfusion pressure is applied, and 40% of aqueous humor formation was ouabain insensitive,[60] suggesting that this may represent a proportion of the formation due to passive ultrafiltration. However, neither that study nor the present one validates a large role for ultrafiltration in the in vivo production of aqueous humor. Some other factors, as discussed above, may be involved, so that ultrafiltration does not by necessity represent the large difference between the in vivo and in vitro fluid transport values.

Overall, the present observations suggest that the mounting arrangement for measuring volumetric fluid flow across the ciliary epithelium is suitable for future studies directed toward the pharmacological control of the secretory activity of the epithelium. It would be important to determine the effects of pharmacological agents on fluid transport to confirm their postulated effect in vivo. Hypothetically, drugs known to reduce aqueous humor formation as determined by fluorophotometry, may or may not affect fluid transport. A demonstration of a similar effect in vitro would establish that their action is mediated by inhibition of ionic transport mechanisms. A reduced or absent effect on in vitro fluid transport would be a strong indication that in vivo there are additional important components involved in the production of aqueous humor.

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References

1. Bill A. The role of ciliary blood flow and ultrafiltration in aqueous humor formation. *Exp Eye Res*. 1973;16:287–298.
2. Beneyto Martin P, Fernandez-Vila PC, Perez TM. Determination of the pseudofacility by fluorophotometry in the human eye. *Int Ophthalmol*. 1996;19:219–223.
3. Cole DF. Evidence for active transport of chloride in ciliary epithelium of the rabbit. *Exp Eye Res*. 1969;8:5–15.
4. Cole DF, Nagasubramanian S. Substances affecting active transport across the ciliary epithelium and their possible role in determining intraocular pressure. *Exp Eye Res*. 1973;16:251–264.
5. Saito Y, Watanabe T. Relationship between short-circuit current and unidirectional fluxes of Na and Cl across the ciliary epithelium of the toad: demonstration of active Cl transport. *Exp Eye Res*. 1979;28:71–79.
6. Wiederholt M, Zadunaisky JA. Effects of ouabain and furosemide on transepithelial electrical parameters of the isolated shark ciliary epithelium. *Invest Ophthalmol Vis Sci*. 1987;28:1353–1356.
7. Wiederholt M, Flugel C, Lütjen-Drecoll E, Zadunaisky JA. Mechanically stripped pigmented and non-pigmented epithelium of the shark ciliary body: morphology and transepithelial electrical properties. *Exp Eye Res*. 1989;49:1031–1043.
8. Krupin T, Reinach PS, Candia OA, Podos SM. Transepithelial electrical measurements on the isolated rabbit iris-ciliary body. *Exp Eye Res*. 1984;38:115–123.
9. Krupin T, Wax MB, Carre DA, Moolchandani J, Civan MM. Effects of adrenergic agents on transepithelial electrical measurements across the isolated iris-ciliary body. *Exp Eye Res*. 1991;53:709–716.
10. To CH, Mok KH, Do CW, Lee KL, Millodot M. Chloride and sodium transport across bovine ciliary body/epithelium (CBE). *Curr Eye Res*. 1998;17:896–902.
11. To CH, Mok KH, Tse SK, et al. In vitro bovine ciliary body/epithelium in a small continuously perfused Ussing type chamber. *Cell Struct Funct*. 1998;23:247–254.
12. Chu TC, Green K. Bicarbonate and DIDS effects on intracellular potential difference in rabbit ciliary epithelium. *Curr Eye Res*. 1990;9:233–239.
13. Wiederholt M, Zadunaisky JA. Membrane potentials and intracellular chloride activity in the ciliary body of the shark. *Pflugers Arch*. 1986;407(suppl 2):S112–S115.
14. Wolosin JM, Bonanno JA, Hanzel D, Machen TE. Bicarbonate transport mechanisms in rabbit ciliary body epithelium. *Exp Eye Res*. 1991;52:397–407.
15. Wolosin JM, Chen M, Gordon RE, Stegman Z, Butler GA. Separation of the rabbit ciliary body epithelial layers in viable form: identification of differences in bicarbonate transport. *Exp Eye Res*. 1993;56:401–409.
16. Butler GA, Chen M, Stegman Z, Wolosin JM. Na^+- Cl^-- and HCO_3^--dependent base uptake in the ciliary body pigment epithelium. *Exp Eye Res*. 1994;59:343–349.

17. Bowler JM, Peart D, Purves RD, Carre DA, Macknight AD, Civan, MM. Electron probe X-ray microanalysis of rabbit ciliary epithelium. *Exp Eye Res*. 1996;62:131–139.
18. Macknight AD, McLaughlin CW, Peart D, Purves RD, Carre DA, Civan MM. Formation of the aqueous humor. *Clin Exp Pharmacol Physiol*. 2000;27:100–106.
19. Chen S, Sears M. A low conductance chloride channel in the basolateral membranes of the non-pigmented ciliary epithelium of the rabbit eye. *Curr Eye Res*. 1997;16:710–718.
20. Chen S, Wan XL, Sears M. pICln can regulate swelling-induced Cl^- currents in either layer of rabbit ciliary epithelium. *Biochem Biophys Res Commun*. 1998;246:59–63.
21. Edelman JL, Loo DD, Sachs G. Characterization of potassium and chloride channels in the basolateral membrane of bovine nonpigmented ciliary epithelial cells. *Invest Ophthalmol Vis Sci*. 1995; 36:2706–2716.
22. Wang L, Chen L, Jacob TJ. The role of ClC-3 in volume-activated chloride currents and volume regulation in bovine epithelial cells demonstrated by antisense inhibition. *J. Physiol* (*Lond*). 2000;524: 63–75.
23. Helbig H, Korbmacher C, Wohlfarth J, Coca-Prados M, Wiederholt M. Electrical membrane properties of a cell clone derived from human nonpigmented ciliary epithelium. *Invest Ophthalmol Vis Sci*. 1989;30:882–889.
24. Yantorno RE, Carre DA, Coca-Prados M, Krupin T, Civan MM. Whole cell patch clamping of ciliary epithelial cells during anisosmotic swelling. *Am J Physiol*. 1992;262:C501–C509.
25. Chen S, Inoue R, Inomata H, Ito Y. Role of cyclic AMP-induced Cl conductance in aqueous humour formation by the dog ciliary epithelium. *Br J Pharmacol*. 1994;112:1137–1145.
26. Coca-Prados M, Anguita J, Chalfant ML, Civan MM. PKC-sensitive Cl^- channels associated with ciliary epithelial homologue of pICln. *Am J Physiol*. 1995;268:C572–C579.
27. Coca-Prados M, Sanchez-Torres J, Peterson-Yantorno K, Civan MM. Association of ClC-3 channel with Cl^- transport by human nonpigmented ciliary epithelial cells. *J Membr Biol*. 1996;150:197–208.
28. Helbig H, Korbmacher C, Kuhner D, Berweck S, Wiederholt M. Characterization of Cl^-/HCO_3^- exchange in cultured bovine pigmented ciliary epithelium. *Exp Eye Res*. 1988;47:515–523.
29. Helbig H, Korbmacher C, Erb C, et al. Coupling of 22Na and 36Cl uptake in cultured pigmented ciliary epithelial cells: a proposed role for the isoenzymes of carbonic anhydrase. *Curr Eye Res*. 1989;8:1111–1119.
30. Helbig H, Korbmacher C, Nawrath M, Erb C, Wiederholt M. Sodium bicarbonate cotransport in cultured pigmented ciliary epithelial cells. *Curr Eye Res*. 1989;8:595–598.
31. Tao W, Prasanna G, Dimitrijevich S, Yorio T. Endothelin receptor A is expressed and mediates the [Ca2+]i mobilization of cells in human ciliary smooth muscle, ciliary nonpigmented epithelium, and trabecular meshwork. *Curr Eye Res*. 1998;17:31–38.
32. Hou Y, Pierce WM Jr, Delamere NA. The influence of ascorbic acid on active sodium transport in cultured rabbit nonpigmented ciliary epithelium. *Invest Ophthalmol Vis Sci*. 1998;39:143–150.
33. Hou Y, Delamere NA. Studies on H(+)-ATPase in cultured rabbit nonpigmented ciliary epithelium. *J Membr Biol*. 2000;173:67–72.
34. Jacob TJ, Civan MM. Role of ion channels in aqueous humor formation. *Am J Physiol*. 1996;271:C703–C720.
35. Civan MM. Transport by the ciliary epithelium of the eye. *News Physiol Sci*. 1997;12:158–162.
36. Civan MM, Macknight AD. The ins and outs of aqueous humour secretion. *Exp Eye Res*. 2004;78:625–631.
37. Chu TC, Candia OA. Electrically silent Na^+ and Cl^- fluxes across the rabbit ciliary epithelium. *Invest Ophthalmol Vis Sci*. 1987;28: 445–450.
38. Pesin SR, Candia OA. Na+ and Cl− fluxes, and effects of pharmacological agents on the short-circuit current of the isolated rabbit iris-ciliary body. *Curr Eye Res*. 1983;2:815–827.
39. Sears ML, Yamada E, Cummins D, Mori N, Mead A, Murakami M. The isolated ciliary bilayer is useful for studies of aqueous humor formation. *Trans Am Ophthalmol Soc*. 1991;89:131–152.
40. Do CW, To CH. Chloride secretion by bovine ciliary epithelium: a model of aqueous humor formation. *Invest Ophthalmol Vis Sci*. 2000;41:1853–1860.
41. Kishida K, Sasabe T, Iizuka S, Manabe R, Otori T. Sodium and chloride transport across the isolated rabbit ciliary body. *Curr Eye Res.* 1982–83;2:149–152.
42. Crook RB, Takahashi K, Mead A, Dunn JJ, Sears ML. The role of NaKCl cotransport in blood-to-aqueous chloride fluxes across rabbit ciliary epithelium. *Invest Ophthalmol Vis Sci*. 2000;41:2574–2583.
43. Cole DF. Aqueous and Ciliary Body. In: Graymore, CN, ed. *Biochemistry of the Eye.* New York: Academic Press; 1970;115–125.
44. Cole DF. Ocular Fluids. In: Davson H. ed. *The Eye.* New York: Academic Press; 1984;269–390.
45. Davson H, Matchett PA, Roberts JRE. Comparative studies of the distribution of chloride between plasma and aqueous humour. *J Physiol.* 1952;116:47P–48P.
46. Kinsey VE. Comparative chemistry of aqueous humor in posterior and anterior chambers of rabbit eye. *Arch Ophthalmol*. 1953;50: 401–417.
47. Carre DA, Tang CS, Krupin T, Civan MM. Effect of bicarbonate on intracellular potential of rabbit ciliary epithelium. *Curr Eye Res*. 1992;11:609–624.
48. McLaughlin CW, Peart D, Purves RD, Carre DA, Macknight AD, Civan MM. Effects of HCO_3^- on cell composition of rabbit ciliary epithelium: a new model for aqueous humor secretion. *Invest Ophthalmol Vis Sci*. 1998;39:1631–1641.
49. To CH, Do CW, Zamudio AC, Candia OA. Model of ionic transport for bovine ciliary epithelium: effects of acetazolamide and HCO_3. *Am J Physiol*. 2001;280:C1521–C1530.
50. Burstein NL, Fischbarg J, Liebovitch L, Cole DF. Electrical potential, resistance, and fluid secretion across isolated ciliary body. *Exp Eye Res*. 1984;39:771–779.
51. Fischbarg J, Maurice DM. An update on corneal hydration control. *Exp Eye Res*. 2004;78:537–541.
52. Candia OA. Electrolyte and fluid transport across corneal, conjunctival and lens epithelia. *Exp Eye Res*. 2004;78:527–535.
53. Hays RM, Leaf A. Studies on the movement of water through the isolated toad bladder and its modification by vasopressin. *J Gen Physiol*. 1962;45:905–914.
54. Silverman RH, Lizzi FL, Ursea BG, et al. High-resolution ultrasonic imaging and characterization of the ciliary body. *Invest Ophthalmol Vis Sci*. 2001;42:885–894.
55. Maren TH, Godman DR, Pancorbo BM, Vogh BP. Timolol decreases aqueous humor flow but not Na^+ movement from plasma to aqueous. *Invest Ophthalmol Vis Sci*. 1997;38:1274–1277.
56. Murray DL, Bartels SP. The relationship between aqueous humor flow and anterior chamber protein concentration in rabbits. *Invest Ophthalmol Vis Sci*. 1993;34:370–376.
57. Brubaker RF. Flow of aqueous humor in humans. *Invest Ophthalmol Vis Sci*. 1991;32:3145–3166.
58. Freddo TF. Shifting paradigm of the blood-aqueous barrier. *Exp Eye Res*. 2001;73:581–592.
59. Andreoli TE, Schafer JA. Effective luminal hypotonicity: the driving force for isotonic proximal tubular fluid absorption. *Am J Physiol*. 1979;236:F89–F96.
60. Shahidullah M, Wilson WS, Yap M, To CH. Effects of ion transport and channel-blocking drugs on aqueous humor formation in isolated bovine eye. *Invest Ophthalmol Vis Sci*. 2003;44:1185–1191.