Transport of fluid by lens epithelium

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Fischbarg, Jorge, Friedrich P. J. Diecke, Kunyan Kuang, Bin Yu, Fengying Kang, Pavel IsEROVICH, Yansui Li, Heinz RosskothEn, and Jan P. Koniarek. Transport of fluid by lens epithelium. Am. J. Physiol. 276 (Cell Physiol. 45): C548–C557, 1999.—We report for the first time that cultured lens epithelial cell layers and rabbit lenses in vitro transport fluid. Layers of the αTN4 mouse cell line and bovine cell cultures were grown to confluence on permeable membrane inserts. Fluid movement across cultured layers and excised rabbit lenses was determined by volume clamp (37°C). Cultured layers transported fluid from their basal to their apical sides against a pressure head of 3 cmH2O. Rates were (in µl·h-1·cm-2) 3.3 ± 0.3 for αTN4 cells (n = 27) and 4.7 ± 1.0 for bovine layers (n = 6). Quinidine, a blocker of K+ channels, and p-chloromercuribenzenesulfonate and HgCl2, inhibitors of aquaporins, inhibited fluid transport. Rabbit lenses transported fluid from their anterior to their posterior sides against a 2.5-cmH2O pressure head at 10.3 ± 0.62 µl·h-1·lens-1 (n = 5) and along the same pressure head at 12.5 ± 1.1 µl·h-1·lens-1 (n = 6). We calculate that this flow could wash the lens extracellular space by convection about once every 2 h and therefore might contribute to lens homeostasis and transparency.

Cell layers; fluid circulation; lens extracellular space; lens homeostasis; nutrient transport

The lens is an avascular tissue thought to be nourished solely by diffusion. However, simple diffusion of nutrients into the lens has been deemed insufficient to account for its metabolic consumption (18). This suggested to us a need to search for additional mechanisms of nutrient transport into the lens. In this regard, the lens is a structurally and functionally asymmetrical tissue with highly localized transport properties. The fiber cells comprise the vast bulk of the lens, but they have relatively low intrinsic Na-K+ ATPase activity, which makes them unlikely candidates for the additional mechanisms sought. Nutrient uptake by the epithelium followed by transport of nutrients through cell-to-cell gap junctions may be considered for that role, but, although the epithelium communicates with the fiber lens cells via such junctions, it does so mostly in the equatorial region. The lack of gap junctions was demonstrated first for the lens of chicken by Brown et al. (7) followed by a structure-function study (4). Subsequently, a similar lack of gap junctions between epithelial and fiber cells was reported in the lens of the macaque by Kuszak et al. (24). Rae et al. (38) reached a similar conclusion using a dye distribution method. Current evidence, therefore, suggests that such a mechanism does not appear to be sufficient to nourish the entire lens.

A search for explanations would cover hypothetical, still undetected transport mechanisms that would be best sought, prima facie, in the lens epithelium. This layer covers the lens anterior surface, it has functionally different apical and basolateral membranes, and it has been suggested to have a central role in maintaining lens homeostasis and integrity. Asymmetry of transport functions was first demonstrated by Becker and Cotlier (5) and Kinsey and Reddy (23), and more recently a detailed analysis of the transport properties of the lens has been provided in a series of papers by Mathias, Rae, and Eisenberg (28, 30). This subject has been reviewed recently (29).

In other tissues, driving forces for transport of nutrients are usually provided by ionic transport. In this regard, Rae, Mathias, and colleagues (27–29) have proposed that transport of ions by the lens epithelium might lead not only to the observed ionic currents circulating around the lens (41) but also to transepithelial fluid entry into the lens followed by fluid circulation through the lens and equatorial exit. However, fluid movement through the lens has been reported only once, as a short qualitative observation for an excised rabbit lens, and in a direction opposite to what we find (13). Fluid transport across lens epithelium per se apparently had never been measured directly.

From what has been studied so far, all epithelial layers involved in water translocation (and only those) express water channels (10, 11, 32). With this background, since a recent report located the water channel aquaporin-1 in lens epithelium but not in fiber cells (45), we hypothesized that the lens epithelium could transport fluid. We report here that it does, both for cultured lens epithelial cell layers and in vitro rabbit lenses. Implications of this finding for lens homeostasis are considered in our discussion below.

MATERIALS AND METHODS

Cultured mouse lens epithelial cell layers. The simian virus 40 (SV40)-transformed αTN4 mouse cell line was used. These cells are derived from the lens epithelium of a transgenic mouse (26) and carry a hybrid gene of the murine α-crystallin promoter fused to the SV40 large T antigen. These cells maintain epithelial morphology for many passages and do not differentiate into fiber cells. They synthesize some of the protein markers of lens epithelial cells: α-crystallin,
β-crystallin, α-insert-crystallin, and γ-crystallin (this last is present in low concentrations) (42). Sixth-generation cells were subcultured in 25-cm² flasks filled with DMEM with high (4.5 g/l) glucose plus 10% fetal bovine serum (FBS) and antibiotics (penicillin and streptomycin). After confluence, the cells were harvested with a Ca²⁺-free trypsin solution, spun at 1,000 rpm (Sorvall GLC-1 centrifuge, HL4 rotor) for 5 min in DMEM, resuspended in DMEM, and seeded into transparent, permeable, tissue culture-treated inserts (internal diameter 24 mm, no. 3492, Transwell, Costar, Cambridge, MA; cells from 1 flask were split among 4 inserts). Confluence was determined both microscopically and by determining the specific resistance of each insert. Cells reached confluence after 3–4 days, and experiments were performed 1–3 days after confluence.

Cultured bovine lens epithelial cell layers. Bovine eyes were washed with physiological saline containing 50 µg/ml garamycin. The lenses were excised under sterile conditions in a laminar flow hood, and the epithelial cells were gently scraped off. The dislodged cells were aspirated with a pipette and added to 5 ml of DMEM containing 20% FBS and antibiotics. The cell suspension was centrifuged as above, and the pellet was resuspended in 2 ml of the same medium. For primary cultures, the cells were apportioned into 75-cm³ tissue culture flasks (Falcon 3023). After 2 days and every 2–3 days thereafter, the cells were fed with fresh DMEM containing 20% FBS. After the cells reached confluence (in 5–7 days), they were detached and subcultured at a ratio of 1:5 using trypsin plus EDTA. Only cells from passages 1 and 2 were used, since their morphology remains constant during those passages (2). Within 1–3 days after becoming confluent, the cells were harvested with trypsin plus EDTA and plated on Costar inserts at ~10⁶ cells/insert. Cells reached confluence in 5–7 days; they were fed every 2–3 days and examined by microscopy throughout the culture period. Fluid transport experiments were performed 1–7 days after confluence. Confluence was determined by microscopy and was also ascertained by determining the resistance of each insert before a given experiment (see below).

Fluid flow measurements: cultured cell layers. The rate of fluid movement across experimental preparations was determined by the Bourguet-Jard volume-clamp method (6) as modified in our laboratory (12, 31). We used a specially designed chamber having a top section and a bottom section separated by the cell layer attached to the permeable membrane of a Costar insert and connected hydraulically to the recording apparatus (see Fig. 1). Each chamber section had a water jacket for temperature control (37°C). The top section of the chamber enclosed the insert holding the cells. The bottom section of the chamber accommodated a steel mesh disk covered by a nylon net to prevent sagging of the flexible permeable support.

The nanoinjector fluid flow measurement setup was similar to that used earlier by us (31), except for its detector. The photodetector previously used to sense fluid flow was replaced with a glass microelectrode (microfilament glass GCF-100-6, A-M Systems, Toledo, OH; filled with 3 mol/l KCl) that sensed by electrical contact the level of a water meniscus in a connecting capillary tube (Fig. 1). To avoid microelectrode blockage, the voltage applied to it was limited to ~100 mV; an amplifier generated transistor-transistor logic voltages to drive the nanoinjector.

This setup maintained constant volume in the bottom section of the chamber into or out of which fluid was pumped by the experimental preparation. We recorded the rate of reinjection into or out of the bottom section; this rate directly corresponds to the rate of transport across the epithelial layer (Fig. 1). The relative positions of the chamber and the detector were such that the hydrostatic pressure difference applied to the apical side of the cell layer (top section of the chamber) was usually 3.0 cmH₂O. Hence, when the layers

![Fig. 1. Fluid flow apparatus used for our experiments. Cultured cell layer on a permeable substrate (insert) separates 2 sections (upper and lower) of experimental chamber. Chamber walls are jacketed for temperature control; for clarity, jackets are shown on left only. Solution in upper section can be changed as desired. Vertical position of entire chamber can be varied to obtain desired hydrostatic pressure difference (ΔP) between it and microelectrode detector for fluid level. In most experiments, this ΔP was 3 cmH₂O. Inset: holder for an excised rabbit lens, which can be clamped in chamber in place of cultured cell layer insert.](http://ajpcell.physiology.org/)

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transported fluid actively, they moved fluid from the bottom chamber to the top one against a hydrostatic pressure difference.

The analog output voltage from the nanoinjector was proportional to the flow rate and was fed to a paper chart recorder and a computer via an analog-to-digital interface (DI-120, DATAQ Instruments, Akron, OH; data were analyzed with the Windaq program from DATAQ). Both sections of the chamber were filled with identical solution (usually DMEM), except when inhibitors of fluid transport were used. Once the preparations were mounted, only the upper chamber was accessible for solution exchanges or gassing. Hence, to maintain pH, we added 25 mmol/l HEPES to the standard DMEM solutions employed.

Fluid flow measurements: in vitro rabbit lenses. Fluid transport measurements were done in the same apparatus employed for flow measurements across cultured cell layers, except that the lenses were mounted in a special insert designed to fit the existing chambers. This required a spherical cavity inside an insert and several gaskets placed so as to separate the top and bottom compartments while avoiding fluid leaks. A schematic diagram of the chamber and lens insert appears in Fig. 1. Three screws join the halves of the holder and effectively contribute to clamp the lens.

Adult male or female albino rabbits (~2 kg) were euthanized with 100 mg/kg of pentobarbital sodium solution (Butler, Columbus, OH) injected into the marginal ear vein. Their eyes were excised and placed in a holder; the lenses were excised and were gently transferred to the experimental holder and effectively contribute to clamp the lens.

Measurements of specific electrical resistance of cultured layers. Electrical resistance of epithelial layers was determined using an Endohm chamber and a resistance meter (both from WPI, Sarasota, FL); the resistance determined using an Endohm chamber and a resistance meter was accessible for solution exchanges or gassing. Hence, to maintain pH, we added 25 mmol/l HEPES to the standard DMEM solutions employed.

RESULTS

Fluid transport by cultured cell layers. We examined the behavior of 68 cultured layers. In 35 of them, fluid leaked through the layer, which we attribute to either the trauma of mounting in the chamber or lack of complete confluence. However, the other 33 preparations transported fluid from their basal to their apical sides against a head of pressure; in vivo, that direction corresponds to transport from the anterior chamber of the eye to the interior of the lens. A number of experiments were conducted with varying pressure heads applied to the preparations. Preparations could transport against pressure heads as large as 10 cmH2O without apparent change in the rate of transport. However, because such high pressure differences are unlikely across an in vivo layer, we chose to standardize on a pressure head of 3 cmH2O. Our experimental device requires some pressure head to keep the tissue and its support relatively immobile, so lower pressure heads were deemed inappropriate for this reason.

The preparations transported fluid spontaneously and continuously for several hours (Fig. 2); the longest time monitored was 6.5 h (not shown). Afterwards, a leak ensued, driven by the pressure head (Fig. 2). For the bovine cell layers, the average rate of fluid transport was 4.69 ± 1.0 µl·h⁻¹·cm⁻² (n = 6); for the αTN4
mouse cell line layers, that rate was $3.31 \pm 0.31 \mu l \cdot h^{-1} \cdot cm^{-2}$ ($n = 27$).

Because the experimental setup is based on keeping the volume of the bottom compartment constant, this compartment is inaccessible during the experiment. For this reason, pharmacological agents could only be added to the top compartment (in contact with the apical cell membranes). Quinidine sulfate, a blocker of K$^+$ channels, inhibited fluid transport in a dose-dependent manner in four experiments. After addition of this inhibitor (50 µmol/l), a slight decrease of fluid transport was observed. After increase of the inhibitor concentration to 150 µmol/l, a further decrease of fluid pumping was observed, which decreased to almost zero within 60 min. Increasing the inhibitor further (to 300 µmol/l) resulted in fluid leak, as fluid movement reversed, driven by the existing pressure head. Figure 3A illustrates a typical experiment.

PCMBs is an unspecific but potent blocker of Hg-sensitive aquaporins. This inhibitor was used at a concentration of 1 mmol/l in seven experiments. After a 30-min delay, fluid pumping began to decrease slowly, and it stopped 50 min later. The direction of fluid flow reversed, indicating a leaky preparation (Fig. 3B). Similar results were obtained with 0.1 mmol/l HgCl$_2$ (Fig. 3C). In another series of experiments, we attempted to determine whether the effect of mercurials can be attributed to aquaporin blockade or to a nonspecific effect by measuring the electrical resistance across the cell layer during application of PCMBs or substitution of a Ca$^{2+}$- and Mg$^{2+}$-free solution with 2 mM EDTA. As shown in Fig. 3B, inset, after exposure to PCMBs and after a delay, both electrical resistance and fluid transport decreased; the decrease in fluid transport seems to precede slightly the decrease in resistance. The replacement of ambient Ca$^{2+}$ and Mg$^{2+}$ (Fig. 3B, inset), as may be expected, resulted in a precipitous and nearly instantaneous drop in resistance. The effect of HgCl$_2$, although fast, appears more gradual by comparison.

Last, we found no effects from addition of ouabain (1 mmol/l), Prozac (1 mmol/l), 5-nitro-2-(3-phenylpropylamino)benzoic acid, or amiloride to the apical side. This inhibitor was used at a concentration of 1 mmol/l in seven experiments. After a 30-min delay, fluid pumping began to decrease slowly, and it stopped 50 min later. The direction of fluid flow reversed, driven by the existing pressure head. Figure 3A illustrates a typical experiment.

Fluid transport across in vitro rabbit lens. We detected a spontaneous movement of fluid across lenses; the direction of such movement was consistent with that observed in cultured lens cell layers.

1) Lenses were mounted "inverted," that is, with their posterior pole facing the top compartment, and with a pressure head of 3 cmH$_2$O applied to the posterior side of the lens. In this case, fluid moved from the bottom to the top compartment, against the pressure head (fluid transport), at a rate of $10.3 \pm 0.62 \mu l \cdot h^{-1} \cdot lens^{-1}$ ($n = 5$). One of these experiments is shown in Fig. 4A. Fluid transport was generally observed for some 3 h after mounting, during which time it decreased slowly and continuously. Given the inaccessibility of the epithelium (facing the bottom chamber, cf. Figs. 1 and 4A), no inhibitors were used in this case.

2) Lenses were mounted "upright," that is, with their anterior pole facing the top chamber, and with a pressure head of 3 cmH$_2$O applied to the anterior side of the lens. In this case, fluid moved from the top to the bottom chamber (from the anterior side to the posterior side of the lens, following the pressure head) at a rate of $12.5 \pm 1.1 \mu l \cdot h^{-1} \cdot lens^{-1}$ ($n = 6$) and for 1–3 h after mounting. As in the inverted preparation above, the fluid movement decreased continuously and slowly after mounting. A representative experiment is shown in Fig. 4B. In four experiments, after ouabain (1 mmol/l) was added to the top chamber, fluid movement ceased (not shown). However, given the relatively short duration of fluid movements in control experiments, no reversal of the inhibition was explored.
Because the fluid movements took place along the pressure head, these flows could therefore reflect transport across the epithelium or a passive leak driven by the pressure head. However, the magnitude of the flow was very similar to that of the fluid transport (against a pressure head) observed in the section above and was much lower than the rate of leak through decapsulated lenses (see below), so such movements are consistent with fluid transport by the lens epithelium. In addition, the slow decrease with time of the fluid movements in both cases is consistent with an active process that decays after mounting rather than with a passive leak or an artifactual phenomenon.

So far, we have expressed flows as “per lens” because it is somewhat uncertain which area of lens epithelium would be traversed by these flows. When the epithelium faced upwards, a gasket pressed against it, and, when it faced downwards, the lens capsule rested on a plastic surface with perforations. Hence, in both cases, the epithelial area giving rise to the fluid movements detected could have been less than the total epithelial area. Still, the flow is approximately the same for both cases, so perhaps these uncertain factors played a minor role. Using published dimensions for a rabbit lens epithelium (37), namely, equatorial diameter of 10 mm and anteroposterior length (lens thickness) of 7.0 mm, we calculate an epithelial area of 1.2 cm² using an equation to compute the segment of a sphere. With this correction, the in vivo lens epithelium would be transporting fluid at rates of 8.8–10.4 μl·h⁻¹·cm⁻².

Experimental hydraulic conductance of the lens. The results above suggested that the epithelium was transporting fluid across itself and into the lens. This posed the question of whether the lens mass could exhibit a hydraulic conductance large enough to admit such flow, by 10.220.33.2 on June 13, 2017 http://ajpcell.physiology.org/ Downloaded from
Fig. 6. Schematic cross section of a lens depicting fiber cells and interfiber paracellular clefts on an arbitrary scale. Labels signify dimensions of fiber cells and paracellular clefts (dashed lines); lcnf, lens fiber cell narrow face; lcw, lens fiber cell width; lct, lens fiber cell thickness; hws, half-width of intercellular spaces. Solid lines and arrows highlight paracellular diffusional pathways.

since otherwise the fluid transported would be either leaking back through intercellular junctions or be driven out of the lens via some other paracellular route. To explore this matter, after excising a lens, we dissected off a circular piece consisting of most of its anterior capsule with the epithelium attached. In four experiments, we verified that a hydraulic conductance across the lens exists. Figure 5 exemplifies such data; Fig. 5A shows a representative record of the time course of flow vs. pressure difference, and Fig. 5B shows the data points collected from all the experiments. The slope of the fitted line (24.1 ± 3.0 µl·h⁻¹·lens⁻¹·cmH₂O⁻¹) represents the measured hydraulic conductance of the lens (Lpm). Using the cross-sectional area of the chamber (0.9 cm²), Lpm was

$$L_{pm} = \frac{120 \mu l}{h \cdot \text{lens} \cdot 0.9 \text{ cm}^2 \cdot 5 \text{ cmH}_2\text{O}} = 26.7 \frac{\mu l}{h \cdot \text{cm}^2 \cdot \text{cmH}_2\text{O}} = 7.6 \times 10^{-9} \frac{\text{cm}^2 \cdot \text{s}}{\text{g}}$$

$$= 0.76 \frac{\text{nm}}{\text{s} \cdot \text{Pa}}$$

(1)

Theoretical calculation of lens hydraulic conductance. Given this experimentally determined lens hydraulic conductance, we asked ourselves whether the geometry of the lens was consistent with a paracellular hydraulic conductance of such magnitude. For the ratio of lens extracellular volume to total volume, the literature describes values of 5–12% determined with cell-impermeant radiolabels (8, 33, 46) and 0.5% as determined from electron microscopic images (33). Further work will be required to reconcile this discrepancy; here we chose a mean estimate of 7%, representing the work with impermeant tracers. From this value, given the histology of lens fibers, the intercellular distances can be calculated. We assumed the simple geometry exemplified in Fig. 6, with cell dimensions comparable to those reported for rabbit lens (34). Values and formulas were

$$l\text{cw} = \text{lens fiber cell width} = 10 \mu \text{m}$$
$$l\text{ct} = \text{lens fiber cell thickness} = 3.52 \mu \text{m}$$
$$l\text{cbf} = \text{lens fiber cell broad face} = l\text{cw} - l\text{ct} = 6.48 \mu \text{m}$$
$$l\text{cnf} = \text{lens fiber cell narrow face} = \sqrt{2 \cdot l\text{ct}/2} = 2.49 \mu \text{m}$$
$$c\text{p} = \text{lens fiber cell perimeter}$$
$$= 2 l\text{cbf} + 4 l\text{cnf} = 22.9 \mu \text{m}$$
$$A_c = \text{cross-sectional area of cell} = \frac{l\text{ct}/2(l\text{cbf} + l\text{cw})}{29 \mu \text{m}^2}$$

To simplify, we neglect the z-axis, and we assume that the proportion of extracellular to total volume will correspond to that of the extracellular to total area in a tangential section. With these assumptions, we can calculate the intercellular distance that will result in a 7% ratio of extracellular area to total area. The extracellular area to be apportioned to each cell (hAs) is the product of the cell perimeter times the half-width of the intercellular spaces (hws)

$$h\text{As} = c\text{p} \cdot \text{hws}$$

Because hAs/(hAs + A_c) = 0.07, it follows that hws = 950 Å. Further parameters required are

$$N_c = \text{number of cells per cm}^2$$
$$= 1 \text{ cm}^2/(h\text{As} + A_c) = 3.21 \times 10^6$$
$$\text{tcp} = \text{total cell perimeter}, \text{in cm}^2$$
$$= N_c \cdot c\text{p}/1 \text{ cm}^2 = 7.35 \times 10^3 \text{ cm}^{-1}$$

To calculate the lens hydraulic conductance (Lpc), we assumed that the average anteroposterior length of the interfiber clefts (lifc) was 5 mm. Using Poiseuille's equation for parallel plates (n = 0.007 poise at 37°C), Lpc is

$$L_{pc} = (2 \cdot h\text{ws})^3 \cdot \text{tcp}/(12 \cdot \eta \cdot \text{lifc})$$

$$= 1.22 \times 10^{-9} \text{ cm}^2 \cdot \text{s} \cdot \text{g}^{-1}$$

(2)

This value falls within the order of magnitude of the experimentally determined value (Lpm/Lpc = 6.2), which reinforces the validity of our measurements.

The issue of whether transport of fluid by the epithelium might lead to an undesirable subepithelial pressure buildup also deserves some mention. In this regard, lens conductance values of the order found appear physiologically compatible with the observed rates of fluid transport. Given our Lpm of 26.7 µl·h⁻¹·cm⁻²·cmH₂O⁻¹, a pressure difference of only 0.3–0.4 cmH₂O would suffice to induce the translenticular rates of fluid flow we report here (8.8–10.4 µl·h⁻¹·cm⁻²). Thus the epithelial transport mechanism could induce fluid flow through the lens with only minimal subepithelial pressure buildup.
DISCUSSION

These experiments demonstrate for the first time that the lens epithelium actively transports a sizable amount of fluid from its basal to its apical side. In cultured cell layers, the rates of transport observed (3.3 and 4.7 µl·h⁻¹·cm⁻² for the αTN4 cell line and the bovine lens epithelial cells, respectively; Figs. 2 and 3) were of the order of those found in other fluid-transporting layers (e.g., 4–6 µl·h⁻¹·cm⁻² for the rabbit corneal endothelium). The rate of fluid transport across in vivo lens epithelium was somewhat larger, ~11 µl·h⁻¹·lens⁻¹ (Fig. 4) or 8.8–10.4 µl·h⁻¹·cm⁻².

In both cultured layers and in vitro lens, fluid movement took place against a hydrostatic pressure difference, which is characteristic of energy-requiring mechanisms for the transport of fluid. Moreover, in the cultured layers, the random distribution of the experiments (or in response to inhibitors, when transport stopped, the fluid movement reversed its direction and a leak ensued across these preparations, driven by the hydrostatic pressure difference. This observation is important because it confirms that the layers were intact and functional before cessation of transport.

The blocking of fluid transport by quinidine added to the apical side of the cultured layers (Fig. 3) was consistent with its known inhibitory effect on K⁺ channels (1, 40). Although this observation is consistent with quinidine-sensitive K⁺ channels playing a direct role in fluid transport, this cannot be affirmed unambiguously. The blocking of fluid transport by the mercurials PCMBS and HgCl₂ added to the apical side is consistent with the water channels (45) of the lens epithelium being central to fluid transport. The delay for PCMBS to inhibit fluid transport was of the order of the incubation time required for this agent to act in other systems, such as Xenopus laevis oocytes expressing water channels. However, PCMBS administration also resulted in a decrease of the electrical resistance of the cell layer, which progressed with a time course somewhat delayed but otherwise similar to that of the decrease of fluid transport. A Ca²⁺- and Mg²⁺-free solution expected to cause junctional opening practically eliminated electrical resistance almost instantly (Fig. 3B, inset), so the gradual decrease in electrical resistance after PCMBS could be due to changes in cell shape and enlargement of the paracellular space. Still, given such resistance changes, our current experiments with mercurials do not permit a definitive conclusion regarding the path of water movement across the cell layer. It should be noted, however, that recent evidence obtained with aquaporin-1 knock-out mice (44) strongly suggests a transcellular route for transepithelial water transport.

The rate of fluid transport across in vitro lenses is comparatively large, ~10 µl·h⁻¹·cm⁻². For comparison, the rabbit kidney proximal tubule transports ~150 µl·h⁻¹·cm⁻², based on area of luminal epithelial surface excluding microvilli (19, 43); if the area of microvilli is considered, that number decreases to 3.75 µl·h⁻¹·cm⁻². Fluid transport through the lenticular surface thus would be larger than that through the kidney tubule. However, if one considers that the basolateral area of the lens epithelium is several times larger than the lenticular surface, given the infoldings of its membrane (17), the transcellular flow per unit area falls to the same order of magnitude as that seen in the kidney. The surface of the apical membrane of the lens epithelium is relatively smooth; however, the apicolateral membranes show infoldings (24) that may contribute to an enlargement of the apical membrane area. In addition, the apical membrane is in close proximity to the underlying cell fiber membranes [a 0.1-µm separation can be estimated from electron micrographs (17)], which might lead to more efficient solute-solvent coupling and rates of fluid transport in excess of those seen in other epithelia with more conventional geometry.

Possible physiological significance of lens epithelial fluid transport. From the evidence in this paper, the direction of fluid transport in the lens would be from the anterior surface to the interior of the lens, which may result in fluid circulation within the lens. Given that the lens is an avascular tissue, such circulation might greatly aid in making nutrients available to the lens fibers and removing their waste products, possibly playing an important role in maintaining lens homeostasis and transparency.

In this regard, nutrients could reach the interior of the lens via two conceivable pathways. Some electron microscopic evidence has led to the proposal that, after the uptake of glucose and other nutrients by the epithelial cells, diffusion of nutrients could take place via gap junctions between the epithelial and fiber cells (16). However, more recent studies place this possibility in doubt. Brown et al. (7), Bassnett et al. (4), and Kuszak et al. (24) reported a virtual absence of gap junctions between epithelial and fiber cells in the central area of lens epithelium of chicken and macaque. They found junctions, but rather sparse ones and located only toward the equatorial region of the epithelium. In addition, from dye-transfer studies (38), only 10% of the epithelial cells would be coupled with fiber cells.

The second pathway would be similar to that existing in other tissues, namely, nutrients reaching cells via diffusion along the extracellular space. For the lens, several authors have discussed their evidence in those terms (9, 35). Such a view is apparently consistent with the ubiquitous presence of facilitative glucose transporters in lens fiber membranes (22, 25). Metabolites would leave the lens by the same path. However, the extracellular space of the lens is tortuous, and the effective diffusion coefficient for cell-impermeant solutes in the radial direction is only some 8.5% of that in free solution (34). This poses the question of whether diffusion alone can really provide an adequate supply of nutrients to the lens and clear its waste products.

Faced with this enigma, we performed calculations to explore it. If glucose diffuses in and is being consumed, consumption might outstrip diffusion, depending on their relative rates. In that case, glucose concentration...
could fall to zero, and some regions of the tissue would go unnourished. A similar question gave rise to a classical treatment of diffusion-consumption in muscle by Hill (20), extended to spherical geometry by Gerard (14). Gerard’s equation is

$$C_l = C_e - \left( \frac{U}{6D} \right) (A^2 - r^2)$$

where \( r \) is the radial distance from the center of the lens, \( C_l \) is the glucose concentration in the extracellular space of the lens, \( C_e \) is the glucose concentration in the external medium surrounding the lens, \( U \) is the glucose consumption in mg·s⁻¹·g lens tissue⁻¹, \( D \) is a diffusion coefficient, and \( A \) is the lens radius.

The limiting radial distance at which glucose concentration would become zero \( (r_{GO}) \) is

$$r_{GO} = \left( A^2 - \frac{6 C_e D}{U} \right)^{1/2}$$

We have calculated limiting distances for the lenses of rat, rabbit, and calf. Table 1 shows the results of our calculations, along with values obtained from the literature (3, 15, 21, 47, 48) for the geometry and glucose consumption of lenses of known weight. D for glucose was 8.5% of that in free solution, or \( 7.7 \times 10^{-7} \) cm²/s (34). As Table 1 shows, regardless of species, size, or rate of consumption, extracellular diffusion seems clearly insufficient to meet the metabolic demand, as glucose would penetrate only into the outer 8–10% of the lens radius before being completely depleted. From this, extracellular glucose would be found only in the superficial 25% of the lens mass; in a rabbit lens, this outer layer would correspond to approximately the 400-µm thickness of the layer of differentiating cells and the anterior epithelium (29). The remaining 75% of lens mass would remain without glucose; similar conclusions would apply to other nutrients.

Against this background, we describe a mechanism of fluid transport by the epithelium and postulate a resulting circulation of fluid through the lens extracellular space that would exchange the contents of that space by convection about once every 2 h. Fluid circulation of such magnitude could be very important for lens homeostasis. In the rest of the body, a cell cannot survive unless it has a blood capillary within some 20–100 μm (39). Lens fiber cells exist at distances from aqueous humor many times larger than that physiological limit; clearly some special mechanisms must be involved in their homeostasis. In fact, it has been noted that an active process is required to bring glucose into the lens (18). Apparently no follow-up studies to explore this issue have been conducted in the intervening time, but this isolated observation fits very well with the ideas we present here.

Last, several authors (27–29, 41, 49) have described a circulation of electrical current flow around the lens, originating in Na⁺ extrusion by the epithelial cells of the equatorial region and reentering the lens passively at the anterior and posterior poles. The current loop would then be completed by Na⁺ entry into fiber cells and diffusion back toward the equatorial epithelial cells. It has been postulated that such current flow might be the driving force for a circulatory flow of fluid along the same circular paths through the lens (27–29, 49). Such hypothetical fluid movement would require fluid to 1) be transported out of the equatorial epithelial cells in the apical-to-basolateral direction and 2) passively enter the lens through the epithelium at the anterior pole and the paracellular spaces of the posterior pole. However, contrary to requirement 1, we observe that cultured lens epithelial cells transport fluid in the basolateral-to-apical direction, so they would be expected to drive fluid into the lens (rather than out of it) through the equatorial region. Contrary to requirement 2, the fluid transport across the anterior epithelium is active, not passive, and we observe that it exits the lens through the posterior pole, rather than entering through it. Of course, out of necessity, we clamped the lens in such a way that possible fluid movements across part of its equatorial region were excluded from detection (Fig. 1), so equatorial fluid exit cannot be ruled out without further work. It has been reported (36) that the magnitude of the short-circuit current in rabbit lens is \( \sim 22.6 \) µA/cm². If this short-circuit current is due to the recirculating Na⁺ current, and if we assume isosmotic coupling, such a current would result in a fluid flow of \( 5.7 \) µl·h⁻¹·cm⁻², which is about one-half of the rates we measure for the in vitro rabbit lens. Moreover, this short-circuit current is inhibited nearly 40% in HCO₃⁻-free solutions and 20% in Cl⁻-free solutions, suggesting that the current flow is not just due to passive flow of Na⁺ through the anterior pole of the epithelium. Hence, the circulatory model cannot completely account for our observations. The arguments above suggest that our results are due to a mechanism different from and perhaps in parallel with the measured currents and the postulated coupled circulatory fluid flow.

Our results clearly suggest that the epithelium transports fluid into the lens. Taking into account the known

<table>
<thead>
<tr>
<th>Species</th>
<th>A, cm</th>
<th>Weight, mg</th>
<th>Volume, cm³</th>
<th>Glucose Consumption, mg·h⁻¹·g lens⁻¹</th>
<th>( r_{GO} ), cm</th>
<th>( A - r_{GO} ), cm</th>
<th>%Penetration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat (1 mo)</td>
<td>0.184</td>
<td>26</td>
<td>0.026</td>
<td>2.63 (14)</td>
<td>0.170</td>
<td>0.014</td>
<td>7.8</td>
</tr>
<tr>
<td>Rat (10 mo)</td>
<td>0.237</td>
<td>56</td>
<td>0.056</td>
<td>1.34 (14)</td>
<td>0.215</td>
<td>0.022</td>
<td>9.3</td>
</tr>
<tr>
<td>Rabbit</td>
<td>0.400</td>
<td>270</td>
<td>0.268</td>
<td>0.45 (20, 42)</td>
<td>0.361</td>
<td>0.039</td>
<td>9.7</td>
</tr>
<tr>
<td>Calf</td>
<td>0.560</td>
<td>740</td>
<td>0.736</td>
<td>0.36 (3, 43)</td>
<td>0.526</td>
<td>0.034</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Values are means or calculated values. A, lens radius; \( r_{GO} \), limiting radial distance at which glucose concentration would become zero under Eq. 4. %Penetration = 100 (1 - \( r_{GO}/A \)). Numbers to right of glucose consumption data indicate references for such entries.
presence of membrane channels, transporters, and an aquaporin in lens epithelium, we believe that the simplest explanation for our observations is the heretofore unknown presence of a classical epithelial fluid transport mechanism in this layer. The sheer magnitude of the phenomenon we report suggests that it may be of great importance for lens homeostasis.

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REFERENCES

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