Lens Metabolic Cooperation: 
A Study of Mouse Lens Transport and Permeability 
Visualized with Freeze-Substitution 
Autoradiography and Electron Microscopy

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ABSTRACT Transport of metabolites is demonstrated between compartments of the adult mouse lens by freeze-substitution autoradiography. In vivo patterns of lysine incorporation are compared with in vitro patterns of lysine, glucose, uridine, and deoxyglucose incorporation. Intracellular and extracellular distributions of tritiated metabolites are determined by comparison of transported substrates with the nontransported molecules of similar molecular size: mannitol and sucrose. The permeability of the lens intercellular spaces is probed with Procion Yellow at the level of fluorescence microscopy, and with horseradish peroxidase at the electron microscope level. Freeze-fracture electron microscopy reveals gap junctions between epithelial cells, between lens fibers, and between epithelial cells and lens fibers. Zonulae occludentes (tight junctions) are not routinely observed between epithelial cells in the mouse. This latter result is subject to species variation, however, since zonulae occludentes are abundant between chicken epithelial cells. The permeability results suggest that the lens cells are capable of metabolic cooperation, mediated by an extensive gap junction network.

Metabolic cooperation is a phenomenon originally described as occurring between cells in tissue culture (34). Cells in culture which are competent for metabolic cooperation show a contact-dependent exchange of small molecules between adjacent cells' cytoplasms (7, 8, 26, 34). Gilula et al. (12) demonstrated a correlation between metabolic cooperation, microelectrode-measured electrotonic coupling, and gap junctions between cultured cells. The restriction of metabolic cooperation to small metabolites (25) correlates with the 600-700 daltons size limit for intercellular exchange of microinjected fluorescent oligopeptides (11, 33), and with the 2-nm maximum diameter pore size in the gap junction measured by electron microscopy and x-ray diffraction (6, 22).

Metabolic cooperation is a phenomenon thought to occur in tissues and organs in addition to cells in culture. Due to the complexity of the microvasculature, direct demonstration of metabolic cooperation between cells in tissues is technically difficult. The vertebrate eye lens is an avascular organ, however, and therefore lends itself to cooperation studies.

The vertebrate eye lens is a solid mass of prismatic cells, packed tightly into hexagonal arrays, separated by narrow and highly tortuous intercellular spaces (4, 28). The lens is surrounded by the ciliary body, an organ whose epithelium generates the aqueous humor, a fluid that carries nutrients to the lens (30). This paper investigates some of the anatomical pathways which aqueous-borne nutrients follow into the lens interior, and the roles played by intercellular junctions in this process.

Previous studies have defined two general mechanisms that permit the lens selective uptake of low molecular weight compounds from the aqueous humor (19). The first is glycolysis-fueled, ATP-driven energy-transport systems, which are capable of generating concentration gradients of ions and amino acids. The second mechanism is facilitated diffusion systems, which permit sugars, purines, and pyrimidines to gain access to the cytoplasm of the lens cells. It is also accepted that some low molecular weight compounds, such as D-mannitol and disaccharides, as well as higher molecular weight macromolecules, may be denied access to the cytoplasm of the lens cells. It is not clear to what degree these impermeable molecules are also denied access to the lens intercellular spaces by selective permeability barriers.
Because of the cystic nature of the organ, the interior lens cells (fibers) are spatially separated from their food supply by the more cortical cells, and because all the cells in the lens are interconnected by numerous gap junctions (5, 9, 13, 28), only the cortical cells need transport nutrients; internal cells can receive and send small molecules by diffusion through the low resistance pathways. It has been postulated that the lens fiber gap junctions are stable in a low-resistance state (13) unlike gap junctions studied in other tissues that switch readily to a high-resistance state (1–3, 19–21, 31, 32, 35).

In this paper we use a method combining the techniques of freeze-substitution and light autoradiography to study small molecule movement into the anterior compartiments of the lens. The results are correlated both with ultrastructural tracer studies using horseradish peroxidase (HRP) and with freeze-fracture electron microscopy of the lens-epithelial and cortical-fiber intercellular junctions.

MATERIALS AND METHODS

Animals

39-d-old CD-1 white mice were obtained from Charles River Breeding Laboratories, Wilmington, Mass. White fohorn adult chickens were purchased from Mayflower Poultry Co., Cambridge, Mass. Animals were decapitated in the laboratory and the eyes dissected immediately. Only mouse lenses were used in this autoradiography study since the eyes were small enough to give adequate structural preservation routinely with freeze-substitution methods.

Chemicals

Horseradish peroxidase, type II, and diaminobenzidine were purchased from Sigma Chemical Co., St. Louis, Mo. Procion Yellow M-4RF was purchased from Polysciences, Inc., Warrington, Penn. The Procion was prepared as an 0.5% solution in HEPES buffered medium 199, pH 7.4, and stored for several days before use. [3H]lysine (20–40 Ci/mmol sp act), [3H]glucose (5–15 Ci/mmol sp act), [3H]-mannitol (3 Ci/mmol sp act), [3H]sucrose (2–10 Ci/mmol sp act), [3H]deoxy-D-glucose (5–10 Ci/mmol sp act), and [3H]uridine (25 Ci/mmol sp act) were all purchased from New England Nuclear, Boston, Mass. Aqueous acetone was "photrex" reagent grade, J. T. Baker Chemical Co., Phillipsburg, N.J. OsO4 was obtained from Stevens Metallurgical Corp., New York.

Incubation of Lenses

Inconsistent results were obtained when the lenses were removed from the eye. Therefore, following removal of the eye from the animal, a window was cut with a 1–2-mm square opening with fine scissors. This dissection permitted the tracers to gain access to the lens via the anterior chamber without direct manipulation of either the lens or the zonular fibers. The eyes were then placed cornea-up in polyethylene BEEM capsules, cylindrical tip (Catalogue No. 23230, Ladd Research Industries, Inc., Burlington, Vt). The capsules had been previously filled with 400 μl of incubation medium. The medium used in all studies was HEPES buffered medium 199, pH 7.4, with Hanks' salts containing 140 mg/liter Ca++ and 1 mg/liter Mg++. The capsules were then moved to a conventional freezer, held at -20°C for 6–8 h, and then allowed to stand at room temperature for 2 h. The eyes were then rinsed three times with fresh anhydrous acetone, left overnight in a 1:1 mixture of acetone:embedding medium, then embedded in Ladd ultralow viscosity embedding medium (Ladd Chemical Corp., Burlington, Vt). 2-μm sections were collected on glass slides coated with 0.01% chromium potassium sulfate and 0.1% gelatin, and subsequently coated with a 1:1 aqueous dilution of Ilford Nuclear Research emulsion type K5, size A (Ilford Limited, Basildon, Essex).

The emulsion-coated slides were stored in a dark, desiccated environment for 1 mo at 4°C, developed in Kodak D-19 full strength, and the resultant autoradiographs were photographed in "dark field" with either a Zeiss Photomicroscope III or a Zeiss IM35, using the number 3 condenser phase ring in combination with objectives containing either number 1 or 2 objective phase rings.

Fluorescence Microscopy

Fluorescence photographs were taken with the Zeiss Photomicroscope III using epi-illumination from a DC 50W HBO mercury lamp. The Zeiss filter combination 48711 (fluorescein) has a useable overlap with the excitation and emission spectra from Procion Yellow.

Electron Microscopy

Whole mouse eyes with dissected corneas and dissected chicken lenses were incubated in 1 mg/ml horseradish peroxidase (HRP) in medium 199 (as previously described) for 15 min at room temperature. Tissue was fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 1 h, the lenses were cut into 1–2-mm slices, then fixed for an additional 2 h. The HRP reaction was run according to Graham and Karnovsky (14) with the modification of Malmgren and Olsson (23), keeping the reaction mixture at pH 5.1 in 0.1 M cacodylate. Postfixation, dehydration, embedding, and freeze-fracture electron microscopy were performed as described previously (13).

Whole Animal Experiments

To control for possible spurious results from eye removal and dissection of the cornea, a mouse was anaesthetised with 100 μl of Nembutal IP, the body cavity opened, and 10 μCi of [3H]lysine in 0.2 ml saline was injected directly into the inferior vena cava after 15 s. After 1 min, one eye was removed and immediately frozen in Freon 22 and processed for autoradiography as described above; the contralateral eye was similarly removed and frozen after 30 min. In this way, the pattern of movement of lysine into the lens under in vivo conditions could be compared with results obtained with eyes incubated in vitro. To facilitate freeze-substitution and embedment, each orbit was fractured open after freezing at the retinal pole, allowing more direct access of the embedding media to the lens from the vitreous side.

RESULTS

Whole Animal Experiment

Fig. 1 is a dark-field photograph of an autoradiograph of a sagittal section of a whole mouse eye frozen 1 min after intravenous injection of [3H]lysine. The ciliary body is densely labeled, as expected, and label has already moved via the aqueous space to the lens epithelium. The iris and corneal endothelium are also labeled. A gradient of label is seen in the corneal stroma. By 30 min (Fig. 2) the whole cortex of the lens in the contralateral eye has been penetrated by the lysine. The depth of penetration is uniform for most of the circumference of the lens, although there is a higher concentration of lysine in the epithelium. During the freezing, one can observe that the lens frequently cracks and splits (Fig. 2). This structural artifact does not appear to affect the distribution of the radioactivity. The observation that the label shows a time-dependent migration into the tissue demonstrates that the label does not move during processing at this resolution.

In Vitro Experiments

Fig. 3 shows an autoradiograph of the anterior portion of a mouse lens incubated with [3H]lysine in vitro for 15 min. At the resolution of these studies, the pattern of labeling is similar to that of the in vivo experiment, with label concentrated in the epithelium and penetrating some distance into the underlying cortex.

Fig. 4 is a photomicrograph of a mouse lens incubated in...
FIGURE 1 A dark-field photomicrograph of an autoradiograph of a whole eye from a mouse injected intravenously with \[^{3}H\]lysine 1 min before freeze-substitution. Due to the fracturing open of the orbit at the retinal pole before embedding, fragments of retina may be seen near the lens bow. The silver grains are heavily concentrated over the ciliary body and iris (both vascularized). Aqueous-borne \[^{3}H\]lysine has labeled the corneal endothelium and lens epithelium. Differential labeling is also evident in the retinal layers.

FIGURE 2 The eye contralateral to the one shown in Fig. 1 was removed from the mouse at 30 min postinjection, and this dark-field photomicrograph was prepared from the freeze-substitution autoradiograph. Here it can be seen that the label is concentrated over the lens epithelium and is moving centripetally into the lens interior from all surfaces (arrows). Overlapping layers of highly ordered lens fibers produce the moiré patterns evident in the lens center.

Two questions immediately arise regarding these data: (a) Is the \[^{3}H\]glucose still in the form of a small molecule somewhere in the glycolytic pathway or has it been incorporated into macromolecules? (b) Is the \[^{3}H\] intracellular, extracellular, or
FIGURE 3 This dark-field photomicrograph demonstrates that the $[^3]$Hlysine moves into the mouse lens in vitro in a pattern similar to that seen in vivo (Figs. 1 and 2). The label concentrates over the epithelium and moves some distance into the lens interior.

FIGURE 4 Dark-field photomicrograph of autoradiograph of mouse lens incubated in vitro with $[^3]$Hglucose. The label moves into the lens interior similar to lysine, but there is little tendency for the label to concentrate at the epithelium.

both? The following experiments are designed to answer these questions.

To investigate question a, two experiments are presented. In the first, mouse lenses were incubated in $[^3]$Hglucose as before, but instead of being frozen the eyes were immersed for 2 h into glutaraldehyde-containing fixatives, to cross-link any $^3$H incorporated into fixable macromolecules. The eyes were then freeze-substituted as previously described. Fig. 5 shows a phase
photomicrograph of an autoradiograph prepared from this experiment. No label is seen over the lens, indicating that the [3H]glucose and its subsequent metabolites were washed out of the lens during aldehyde fixation. In the second experiment, the eyes were incubated in vitro with [3H]2-deoxy-D-glucose, which should enter the glycolytic pathway and be phosphorylated, but then be trapped in the cytoplasm of the cells in that form. The phosphorylated molecule should neither be utilized in macromolecule synthesis, nor be excreted from the cells. Fig. 6 demonstrates that the pattern of movement of [3H]2-deoxy-D-glucose into the lens is similar to that of [3H]glucose, with the noteworthy exception that the [3H]2-deoxy-D-glucose is concentrated in the epithelium. Taken together, these data suggest that the movement of label into the lens following
[\[^{3}H\]glucose exposure for 15 min is primarily in the form of small metabolites.

The second question seeks to understand whether the glucose is intracellular or extracellular. The [\[^{3}H\]2-deoxy-D-glucose experiment, shown in Fig. 7 and described above, suggests that this label is primarily intracellular. To investigate how much label leaks into the lens via the intercellular spaces, mouse eyes were incubated in vitro with [\[^{3}H\]mannitol and [\[^{3}H\]sucrose, compounds that are similar in size to glucose but that are not transported by the facilitated diffusion mechanisms (18). The

**FIGURE 7** Dark-field photomicrograph of an autoradiograph of a mouse eye incubated in vitro with [\[^{3}H\]mannitol. This nontransported substrate concentrates over the epithelium but fails to label the lens cortex above background.

**FIGURE 8** Dark-field photomicrograph of an autoradiograph of a mouse eye incubated in vitro with [\[^{3}H\]sucrose. The nontransported substrate concentrates over the epithelium but fails to label the lens cortex above background.
mannitol and sucrose, therefore, should have access to the extracellular compartments of the lens similar to glucose, but not the intracellular compartments. The results are shown in Figs. 7 ([3H]mannitol) and 8 ([3H]sucrose). Within the detection limits of autoradiography, the nonmetabolizable substrates appear to concentrate at the epithelium and not to move into the lens interior.

**FIGURE 9** Dark-field photomicrograph of an autoradiograph of a mouse eye incubated in vitro with [3H]uridine and 0.5% Procion Yellow. The uridine is transported by facilitated diffusion systems and shows a grain distribution similar to glucose patterns (Fig. 4).

**FIGURE 10** Fluorescence photomicrograph of the same field seen in Fig. 9. The Procion Yellow stains the lens capsule heavily but, with low numerical aperture objectives (0.4), cannot be detected in the lens interior (but see Fig. 11). The corneal stroma is well stained by the Procion. Note the reciprocal relationship of position of silver grains and Procion Yellow in Figs. 9 and 10, respectively. The uridine tends to be intracellular and the Procion extracellular, except where cell damage has occurred.
the lens cortex. These data demonstrate that the bulk of the label detectable over the lens anterior cortex administered as $[^{3}H]$glucose and lysine must be intracellular, inasmuch as an equivalent dose of nontransported $[^{3}H]$mannitol and sucrose fails to label the lens cortex above background. It is shown below, however, using fluorescent tracers, that the intercellular spaces are indeed penetrated by mannitol and sucrose, although at levels undetectable by autoradiography.

Fluorescent dyes have been used by others to delineate the extracellular compartments in the lens (17, 27, 29). To further investigate the accessibility of the mouse lens intercellular spaces to small molecule penetration, mouse eyes were incu-

**FIGURE 11** Fluorescence photomicrograph of a mouse lens incubated in vitro with 0.5% Procion Yellow. This photograph, taken with the Zeiss Planapo 63/1.4, reveals the lens extracellular spaces stained with Procion. The epithelium appears weakly stained, due to complex interdigitations of the extracellular spaces which are not resolved here, but seen in Fig. 13. Thus, small molecules have rapid access to mouse lens extracellular spaces.

**FIGURE 12** Fluorescence photomicrograph of a mouse lens as in Fig. 11, with the inclusion of 100 mM K$^+$-methanesulfonate and 2 mM EDTA in the incubation medium. Note that damaged epithelial cells (arrow heads) and fibers (black and white arrows) fill to variable degrees with the Procion, revealing that intra- and extracellular dye distribution are distinguishable in the microscope.
bated with [3H]uridine simultaneously with 0.5% Procion Yellow M-4R in the medium as described above. After 15 min, freeze-substitution autoradiographs were prepared, which could also be examined in the fluorescence microscope. Figs. 9–11 show the results of this experiment. The dark-field photograph in Fig. 9 reveals that the [3H]uridine penetrates into the lens interior in a pattern similar to that of glucose penetration. The identical area of the section in Fig. 9 is also seen in Fig. 10, photographed with fluorescence microscopy. At this lower magnification, the Procion Yellow appears to have been excluded from the lens intercellular spaces. Fluorescence photomicrographs taken with a high numerical aperture objective (1.4), however, detect fluorescence in the intercellular spaces (Fig. 11), in agreement with the results of Rae and Stacey (29). After incubation with Procion Yellow in 100 mM K+-methanesulfonate and 2 mM EDTA, the lenses show damaged patterns where Procion is seen to fill some cells (Fig. 12). Autoradiographs of lenses incubated simultaneously with Procion Yel-

![Figure 13](image13.png)

**Figure 13.** An electron micrograph of a thin section of a mouse lens incubated for 15 min with HRP before aldehyde fixation and enzyme histochemistry. The reaction product is seen in the capsule, in the intercellular spaces between the interdigitating epithelial cells, at the interface between the epithelium and fibers (open arrows) and between the lens fibers (closed arrows). No permeability barrier to the passage of HRP into the lens is evident.
low and \[^{3}H\]mannitol appear similar to Fig. 7 (data not shown) demonstrating that Procion does not grossly alter autoradiographic labeling patterns of mannitol compared with controls. The \[^{3}H\]mannitol and sucrose which must have diffused into the lens intercellular spaces in Figs. 7 and 8 do not give a signal on the autoradiographs above background. Hence, although the transported substrates glucose, lysine, and uridine are both intra- and extracellular in the mouse lens, the bulk of the silver grains reflects only the intracellular distribution of the metabolites.

The extracellular leak past the epithelium is not restricted to small molecules. Fig. 13 shows a thin section of mouse lens incubated in vitro for 15 min with HRP. The enzyme has diffused into the cleft between epithelium and fiber (open arrows) and has begun to penetrate into the interfiber spaces (closed arrows).

There are two possible interpretations of these comparative data in the mouse on the interiorization of glucose, deoxyglucose, lysine, and uridine compared to that of mannitol and sucrose. The first would hold that the transportable substrates cross the plasma membrane of the epithelial cells by specific transport systems. The substrates and their metabolic products then move into the lens interior by intracellular diffusion, passing from cell to cell via low-resistance junctions. The second interpretation would hold that all the lens cells, both epithelium and fiber, are capable of transporting their own substrates. The deeply situated lens fibers would transport substrates which first diffuse into the lens via the intercellular spaces. Comparison of the depth of penetration of transported vs. nontransported molecules, however, reveals that a transported molecule (e.g., uridine, Fig. 9) moves over 250 \(\mu m\) into the lens cortex, whereas a nontransported molecule (e.g., Procion Yellow, Fig. 11) moves only 50 \(\mu m\) into the lens cortex. It seems unlikely, then, that the uridine could move 250 \(\mu m\) into the lens cortex via the extracellular spaces, and then be transported into a deep cell's cytoplasm. This point is considered further in the Discussion.

If transported substrates do diffuse from epithelium to underlying fiber cells, then low-resistance junctions must exist between the epithelial cells and the underlying lens fibers, a cytological connection not heretofore demonstrated by ultrastructural methods. To investigate the junctional connections between lens epithelial cells and the lens fibers, freeze-fracture electron microscopy was performed. Both mouse and chicken lenses were used for these studies.

Fig. 14 is an electron micrograph of a freeze-fracture replica of mouse lens. The tissue in Fig. 14 was fractured through three epithelial cells. As reported by Percaccia (24), epithelial cells form gap junctions with one another along their lateral surfaces, and these junctions are characterized by crystalline aggregates of their component connexons (inset B).

At the junction of the lateral surface with the apical surface of the epithelial cell in Fig. 14 (inset A), where zonulae occludentes (tight junctions) occur in other epithelia (10), no evidence could be found for the characteristic webbed pattern of fibrils (P-face) and grooves (E-face), indicating a zonula occludens (permeability barrier) joining the epithelial cells. No evidence of tight junctions was found at this level between the underlying lens fibers, either with one another or with the epithelium, although these junctions may occur in other areas of the lens. At the apical borders of epithelial cells, occasional collections of particles could be detected (inset C) but no continuous fibrils were evident. This finding is consistent with the penetration of Procion Yellow (Fig. 11) and HRP (Fig. 13) into the lens intercellular spaces.

On the apical surface of the epithelial cells, gap junctions are formed with the underlying lens fibers. Fig. 15 shows a montage of the various gap-junctional interactions seen between epithelial cells and fibers. Due to the high density of particles on the mouse lens fiber P-fracture faces, gap junctions on this surface in the mouse are difficult to document, as can be seen in Fig. 15 C. In Fig. 15 A, a gap junction with crystallizing connexons can be seen (arrows) on the E-face of an epithelial cell apical membrane. In other areas, small gap junctions can be found which display a less crystalline morphology (arrows, Fig. 15 B). Occasionally, crystallizing and noncrystallizing gap junctions occur side by side (Fig. 15 D). Although most of these junctions are too small to be detected by thin-section electron microscopy, they occur in clustered domains at relatively high density and are not difficult to find in replicas that show clear E-face pits. Thus, the anatomical pathway for metabolic cooperation between lens epithelial cells and underlying fibers is demonstrable in the mouse.

There is variation between species, however. Fig. 16 is a photograph of a freeze-fracture replica of a chicken lens. Unlike the mouse, the chicken has the branching and anastomosing network of P-face ridges and E-face grooves (solid arrows) characteristic of the zonula occludens in other tissues. On the lateral borders of the epithelial cells, crystallizing gap junctions are found similar to those in the mouse (inset A). The gap junctions connecting the chicken lens fibers and epithelial cells, however, are uniquely of the noncrystallizing type (boxes and insets B and C, Fig. 16).

Comparison of the mouse and chicken lenses thus reveals a variability in the presence of the zonula occludens. In addition, the mouse has both crystallizing and noncrystallizing junctions between epithelium and fiber, whereas the chicken has only noncrystallizing junctions at this site. Both species have only crystallizing junctions between the epithelial cells. Thus, the epithelial cells can simultaneously express more than one junctional phenotype. Since bovine lens fiber noncrystallizing gap junctions are primarily composed of proteins with different electrophoretic mobilities compared to crystallizing gap junctions isolated from mouse liver and heart (13, 15, 16, and footnote 1), it is possible that the two junctional types seen in lens epithelial cells are composed of different polypeptides. Indeed, due to the isolation protocol used by Dunia et al. (9), the 34-kilodalton junctional peptide reported by this group may be a component of the more detergent-stable, crystallizing epithelial gap junctions.

DISCUSSION

The results presented in this study demonstrate that small, transportable metabolites pass into the cytoplasm of the cells in the cortex of the mouse lens whereas nontransported molecules of similar charge and molecular weight are denied access to the cytoplasmic compartments of the lens cortex. The lens intercellular spaces are accessible to small molecules and large proteins. These observations, combined with a lack of any morphologically detectable zonula occludens, demonstrate a lack of a transepithelial permeability barrier in the anterior compartments of the mouse lens. This result is not phylogenetically general, however, in that the morphological counterparts of the mouse lens epithelial cells are different from those in other species. For example, the mouse has noncrystallizing gap junctions between epithelium and fiber, whereas the chicken has only crystallizing gap junctions at this site. Both species have only crystallizing junctions between the epithelial cells. Thus, the epithelial cells can simultaneously express more than one junctional phenotype. Since bovine lens fiber noncrystallizing gap junctions are primarily composed of proteins with different electrophoretic mobilities compared to crystallizing gap junctions isolated from mouse liver and heart (13, 15, 16, and footnote 1), it is possible that the two junctional types seen in lens epithelial cells are composed of different polypeptides. Indeed, due to the isolation protocol used by Dunia et al. (9), the 34-kilodalton junctional peptide reported by this group may be a component of the more detergent-stable, crystallizing epithelial gap junctions.

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of the permeability barrier, the zonula occludens, can be demonstrated in the chicken.

The penetration of the transportable substrates into the lens cortex cytoplasmic compartment either may be due to metabolic cooperation, that is, diffusion of the transported metabolites from the cytoplasm of apical cells to deeper cells via low-resistance junctions, or may be due to the deeper cells transporting substrates themselves from the intercellular spaces. This latter explanation is considered unlikely by the following argument. It can be seen in Figs. 9 and 11, which are photographs of the same lens simultaneously exposed to [3H]uridine and Procion Yellow, that the transported uridine moves to a detectable depth of at least 250 μm (Fig. 9) in 15 min whereas nontransported Procion Yellow moves in the intercellular

FIGURE 14 An electron micrograph of a freeze-fracture replica of mouse lens. The fracture has passed through the apical cytoplasm of three epithelial cells (cells 1, 2, and 3) that interdigitate beneath the apical surface facing the underlying lens fibers. Box A is shown at higher magnification in inset A. In this region, zonulae occludentes are found between cells in other sealing epithelia, and zonulae occludentes are found between lens epithelial cells in the chicken. No zonulae occludentes have been found in between the mouse epithelial cells. Inset B shows a crystallizing gap junction found between adjacent epithelial cells in the mouse. Inset C demonstrates occasional discontinuous particle clusters, found in the apical regions of the lateral epithelial plasma membranes in the mouse, where zonulae occludentes are expected.
spaces only 50 μm into the lens cortex (Fig. 11). The sensitivity of the fluorescent probe is greater than the autoradiography, which tends to minimize the differences in penetration between uridine and Procion Yellow. The experimental concentration of the Procion Yellow is much higher (10 mM) compared with the uridine (10^{-5} mM), giving a concentration gradient six orders of magnitude larger for the Procion. These factors greatly favor a more rapid and deeper penetration of the Procion into the lens cortex. However, the transported metabolites are observed to move more rapidly into the lens than the

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FIGURE 16 An electron micrograph of a replica from freeze-fractured chicken lens. The cytoplasms of two epithelial cells are separated by a narrow fracture face of the epithelial cells' lateral membranes. P-face fibrils and E-face grooves of a zonula occludens (curved arrows) are found on these lateral surfaces. The fracture plane leaves the epithelial plasma membranes and exposes a broad surface of the lens fiber plasma membrane, which directly overlies, and forms junctional interactions with, the apical membrane of the epithelial cells (see boxes and insets, B and C). Inset A demonstrates a crystallizing gap junction found between adjacent lens epithelial cells at their lateral surfaces. Insets B and C are higher magnification photographs of boxes B and C, which show noncrystallizing gap junctions between epithelium and fiber.

Procion, indicating their movement via an additional pathway. The argument that the extracellular spaces offer an unusual barrier to the diffusion of Procion Yellow and not to uridine, glucose, and lysine is believed unlikely due to the observation in Figs. 10 and 11 that the Procion Yellow is free to diffuse many hundreds of micrometers into the dense extracellular stroma of the cornea. This would favor the argument that the relative slowness of the diffusion of Procion Yellow into the lens is a function of the narrowness (20 nm) and tortuosity of the lens intercellular spaces. Therefore, whereas the effects of charge and molecular weight are not quantitatively known, it is reasonable to conclude that the bulk of the uridine, lysine, and glucose seen at a depth of 250 μm in the lens must have arrived there by diffusion through the joined cytoplasms of the
cells, rather than via the extracellular route.

It is important here to enumerate some of the limitations of this study. The conclusions presented are valid only for the anterior surface of the mouse lens; studies on the permeability of the equatorial and posterior surfaces of the lens are in progress. Figs. 1 and 2 do demonstrate, however, that \(^{3}H\)lysine moves into the lens at an equivalent depth from all surfaces, so it cannot be concluded that the epithelium regulates all metabolite movement into the lens.

A second limitation of this study is in the time-course. Because of degenerative changes detected ultrastructurally in mouse lenses maintained in culture after only 30 min (13), data are collected after 15 min of incubation. Therefore, the long-term in vivo permeability properties of the lens may show results significantly different from those reported in this paper.

An additional limitation is provided by the experimental design; because the nonmetabolizable molecules cannot cross the blood-aqueous barrier, the labels have been provided to the lens in vitro, via the anterior chamber. The experiment with \(^{3}H\)lysine is the only in vivo experiment, and provides the only comparison that the in vivo and in vitro patterns of uptake of this label are similar.

Fig. 17 is a summary diagram of the junctional interactions observed between cells in the anterior mouse and chicken lens. Beneath the capsule, adjacent epithelial cells form gap junctions (small, open arrows) with one another. The intraperithelial gap junctions tend to display condensed or crystalline patterns (small, open arrows) with one another. The intraepithelial interface, small gap junctions are found (small, closed arrows) with one another. The intraepithelial-fiber interface indicate epithelium/epithelium gap junctions, the small, closed arrows indicate fiber/fiber gap junctions. The curved arrows near the epithelium/fiber interface indicate epithelium/epithelium zonulae occludentes found in the chicken but not in the mouse.

The junctional interconnections between the cells in the lens are thus appropriately situated to provide low-resistance pathways between the more metabolically active epithelial cells and the deeper fibers. These junctional pathways are probably those used in ion balance in the lens (28); but, in addition, small transported molecules appear free to use these pathways back into the lens interior.

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