Special characteristics of the polar regions of the rat lens: morphology and phosphatase histochemistry

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Ultrastructural analyses of the normal rat lens revealed a number of characteristics present regionally within the anterior and posterior polar regions: (1) elaborate folding of the plasma membrane, including numerous microvillar processes; (2) wide intercellular spaces at basolateral cell borders; (3) concentrations of mitochondria; and (4) ouabain-sensitive Na-K-ATPase activity. These features, confined primarily to the suture systems and the anterior epithelium, suggested a cortical as well as an epithelial involvement in ion-linked fluid transport, supporting recent physiological investigations of the lens. Acid phosphatase localized in aggregations of smooth endoplasmic reticulum at the lens fiber ends suggested that this micro-organelle may be a source of acid hydrolases in the normal lens.

Key words: rat lens, sutures, electron microscopy, endoplasmic reticulum, mitochondria, intercellular spaces, histochemistry, adenosine triphosphatase, acid phosphatase

In spite of the number of studies which have been devoted to demonstration of regional cytological differences within the lens, little reference has been made to the ultrastructural features of the sutureal regions. Although the topography of sutural lens fiber terminations has been mapped by scanning electron microscopy, this method does not allow observation of intracytoplasmic structures or, because of the disruptive nature of the preparation procedure, the normal associations between cells.

Recent investigations of the normal rat lens by transmission electron microscopy have shown the fiber terminations to be unique in their content of RNA-rich "terminal bodies" and aggregations of membranous micro-organelles. The existence of enlarged intercellular spaces (intercellular vacuoles) within the epithelium overlying the anterior suture and within portions of the posterior suture also has been noted.

The lens sutures are the site of early degenerative change in formation of senile cortical cataract in the rat. This knowledge prompted us to devote more attention to the cytology normally present in the polar regions. The suture systems, like the anterior epithelium, contained morphological features characteristic of transporting epithelia. An appropriate histochemical procedure was employed to determine the presence of Na-K-ATPase, an enzyme indicating transepithelial ionic transport, in these regions of the lens.

A preliminary report of the ATPase studies was presented earlier.
Materials and methods

**Morphological studies.** Eyes of 3- to 9-month-old Wistar, Sprague-Dawley, and Long-Evans rats of both sexes, determined to be normal by slit-lamp microscopic examination, were excised after the animals were killed by cervical dislocation. Initial fixation was for 3 hr in 4% glutaraldehyde dissolved in 0.1M sodium phosphate buffer, pH 7.3. Lenses were removed while in this solution, and at the end of fixation, polar caps were sliced off, and the equatorial region cut into meridional strips. These pieces were fixed secondarily in Sorenson's buffered 2% osmium tetroxide solution, pH 7.3, dehydrated in ethanol, and embedded in Epon 812. Thick and ultrathin sections were cut for correlated phase-contrast and transmission electron microscopic evaluation.

**Histochemical studies.** Wistar rats (ranging in age from 3 to 8 months) with clinically normal lenses were used for this part of the study.

**K-dependent NPPase.** Whole lenses were fixed for 5 min at 4°C in 0.5% glutaraldehyde/3% paraformaldehyde in 0.1M sodium cacodylate buffer at pH 7.2 and then transferred to 3% paraformaldehyde in the same buffer for an additional hour at 4°C. The lenses were incubated according to the technique developed by Ernst 6 7 for localizing the K-dependent component of Na-K-ATPase with p-nitrophenyl phosphate (pNPP) used as substrate. Incubation time was 20 min. Controls included tissues incubated in (1) medium lacking pNPP substrate, (2) medium lacking KCl, (3) complete medium containing 10 mM ouabain (an inhibitor of K-dependent NPPase), and (4) medium containing 2 mM levamisole (an inhibitor of alkaline phosphatase).

After incubation, relative transmittance of the various incubation media at 420 nm wavelength was determined following the procedure outlined by Ernst 6. A Beckman model 6/35 spectrophotometer was standardized against appropriate controls. Lenses were blotted briefly after incubation and weighed on an Ainsworth analytical balance Model 10 NT. The spectrophotometric readings were compensated for differing lens weights and baseline readings of the various blanks to obtain relative readings of nitrophenol (a direct measure of the amount of inorganic phosphate liberated).

Incubated lenses were fixed in 4% glutaraldehyde buffered with 0.1M sodium cacodylate, pH 7.2, for 2 to 3 hr at room temperature, cut into segments, and then postfixed in 1% osmium tetroxide in the same buffer for 2 hr.

**Acid phosphatase.** Lenses were prepared ac-

![Fig. 1. Slit-lamp photograph of in vivo rat lens showing faint vacuoles along posterior suture (arrows).](image-url)

Results

**Morphology.** Lenses of the three rat strains showed similar morphological characteristics at all levels of organization. Biomicroscopic examination revealed intermittent fine vacuoles along the posterior suture (Fig. 1). Phase contrast and electron microscopic observation of the polar regions pointed out several additional features unique to the sutural and adjoining portions of the lens.

**Anterior polar region.** The fiber cell segments peripheral to the anterior suture showed a fairly regular dimension and a close cell-to-cell apposition. By comparison, the sutural fiber terminations were extremely variable in organization and dimension. In spite of this apparent disorder, the cell bor-
Fig. 2. For legend see facing page.
Fig. 3. Electron micrograph of region adjoining posterior lens suture. Loosely associated interdigitating cell processes are shown at arrows. (Calibration bar = 1 μm; ×27,500.)

Fig. 2. A, Electron micrograph of anterior suture region and overlying epithelium. Note elaborate cellular processes (arrow) projecting into widened epithelial interspaces. Epithelial cell at left has dense cytoplasm and an abundance of micro-organelles. cap, Capsule. (Calibration bar = 1 μm; ×12,500). B, Enlargement of area in box in A, showing abundant mitochondria (m) and tubular smooth ER (ser) present in cortical fiber cell ends. (×20,500.)
Fig. 4. Electron micrograph of posterior lens suture. Intercellular dilatations (V) and elaborate cellular processes (arrows), some extending into the capsule (cap), are present in this section. (Calibration bar = 1 µm; ×14,500.) Inset, Phase micrograph of same lens. Arrow shows vacuolar area within posterior suture. (×800.)

Epithelial cells bordering the large interspaces sometimes were seen to have an unusually dense cytoplasmic matrix with high concentrations of micro-organelles (Fig. 2, A). The surrounding cells rarely assumed this appearance.

Epithelium were elaborate cellular processes (Fig. 2, A). Although similar spaces existed in other parts of the central epithelium, they were narrower and more infrequent.
Fig. 5. Enlargement of region within box in Fig. 4, showing groups of mitochondria (M) and smooth ER (ser). (Calibration bar = 0.5 μm; ×31,700.)
Wide lateral interspaces also were prevalent in the germinative epithelial zone.

Posterior polar region. In areas peripheral to the posterior suture, the superficial lens cortex showed relatively smooth cellular outlines, with a moderate number of ball-and-socket junctions. The intercellular spaces generally were uniform and narrow. In regions immediately adjoining the suture, a series of small processes occurred at intervals along the cell borders (Fig. 3). Within the suture, the processes were more elaborate.
and numerous and in some sections were associated with wide intercellular spaces (Fig. 4). The larger interspaces generally grouped together and represented vacuoles observed within the corresponding thick sections (Fig. 4, inset). Their size and distribution suggested that they represented the biomicroscopic sutural vacuoles (compare Figs. 1 and 4). Projections of sutural fiber terminations extended also into the capsule (Fig. 4). The projections consisted of processes contributed by one or more fiber cells. They were structurally similar to the intracapsular projections previously noted in the narrow region immediately posterior to the nuclear bow of rat and human lenses.\(^5\)\(^\text{---}\)\(^8\)

The cytoplasm of the fiber cells was mostly devoid of membranous micro-organelles, except at the cellular terminations in the suture and adjoining subcapsular region, where clusters of small mitochondria and profiles of ER were noted (Figs. 4 and 5).

**Histochemistry**

**K-phosphatase localization.** Vacuoles were prevalent throughout the epithelium of all tissues treated by the NPPase method (Figs. 6 to 8). These were regarded as preparation artifacts due to the necessarily brief initial fixation in glutaraldehyde, a fixative which rapidly inactivates K-NPPase.\(^9\)

Within the anterior polar regions of lenses treated histochemically by the NPPase procedure, reaction product was noted intercellularly between the apicolateral epithelial plasma membranes, along the apical epithelial border, and extending into the lens cortex along the fiber cell margins (Fig. 6). The suture region was more reactive than the more peripheral parts of the polar region. Inclusion of ouabain in the incubation medium reduced the overall enzyme activity but had the greatest effect at the margin between epithelium and fibers (Fig. 7). Levamisol greatly reduced the activity along the apicolateral epithelial margins and between the fiber cells but had little or no effect on the area most sensitive to ouabain (Fig. 8). Exclusion of K\(^+\) ions from the incubation medium reduced but did not eliminate the reaction product. Tissues incubated in substrate-free medium showed no lead precipitate along plasma membranes.

The precipitate commonly seen over the nuclear heterochromatin (Fig. 6) was regarded as nonspecific because tissues incubated in substrate-free medium also contained this precipitate.

In the posterior polar region, reaction product was demonstrated along the apposed surfaces of the superficial fiber cells, but not at the borders adjoining the capsule (Fig. 9). Activity was more pronounced within the suture region than elsewhere. The reaction product was substantially reduced both by
ouabain and levamisol (Figs. 10 and 11) as well as by the exclusion of $K^+$ ions from incubating medium and eliminated by omission of the substrate.

The readings obtained from spectrophotometric analysis of the incubation media confirmed the general trend of the histochemical analyses. The levamisol-inhibited reaction showed a 27% reduction in relative activity, whereas inclusion of ouabain resulted in a 32% reduction. Deletion of $K^+$ ions from the incubation medium caused a reduction almost identical to that of the ouabain-inhibited groups.

Acid phosphatase localization. The histochemical demonstration of acid phosphatase reaction product within epithelial and cortical dense bodies and Golgi apparatus of young normal rat lenses has been reported previously. The profiles of smooth ER present in the anterior cortex usually were reactive (Fig. 12), whereas only intermittent activity was noted in the posterior cortical tubules and vesicles (Fig. 13).

In some lenses, small amounts of reaction product were noted at intercellular sites in epithelium and anterior cortex, although never in the proportions apparent during senile cataractogenesis.

Tissues incubated in substrate-free medium were devoid of lead precipitate. Inclusion of 50 mM NaF in the incubation medium eliminated all enzyme activity except for a small amount localized in the smooth ER.

Discussion

The lens is an epithelial organ which uses an active ionic exchange mechanism to maintain the low water content necessary for its transparency. Physiological data indicate the anterior epithelial layer to be the primary site of the sodium pump, catalyzed by...
Fig. 9. Electron micrograph of posterior suture region. NPPase procedure, unaltered medium. Reaction product is distributed along the apposed fiber cell surfaces but is absent from plasma membranes which abut on the capsule (cap). CX, Cortex. (Calibration bar = 0.5 μm; ×31,200.)

Fig. 10. Electron micrograph, posterior suture region. NPPase procedure, ouabain inhibition. Although the amount of reaction product is reduced with this inhibitor, its distribution is similar to that appearing in noninhibited lenses. cap, Capsule. (×21,100.)

Fig. 11. Posterior suture region. NPPase procedure, levamisol inhibition. Reaction product is greatly reduced. cap, Capsule. (×32,500.)
sodium-potassium-activated ATPase, the activity of which results in the flow of water out of the lens. This concept is supported by the histochemical investigations of Palva and Palkama, who demonstrated that the lateral epithelial membranes contain ouabain-sensitive Na-K-ATPase. They pointed out, furthermore, that the structural similarity between the lateral lens epithelial interspaces and those present in gall bladder suggest the involvement of these channels in transport of fluid from the lens.
Our studies revealed that ion-linked fluid transport activity may be concentrated not only in the epithelium but additionally in the cortical suture systems. Such a mechanism was suggested by the following characteristics of these regions: (1) elaborate folding of the cell membranes; (2) wide interspaces between basolateral cell borders, both in the posterior suture and in the epithelium overlying the anterior suture; (3) concentrations of mitochondria within the sutural ends of fiber cells; and (4) ouabain-sensitive ATPase activity along the borders of the sutural fiber terminations as well as along the lateral and apical epithelial cell borders (Fig. 14).

A primary morphological characteristic of cells involved in fluid transport is the presence of extensive folding of the cell surface lining intercellular spaces or other compartments. This folding, which takes the form of invaginations, plicae, microvilli, etc., is thought to amplify the surface areas available for diffusion and for active transport of solutes. In the lens, the margins of the sutural fiber terminations as well as the lateral epithelial borders present a pattern of highly convoluted plasma membranes. We observed the cell surface in these regions to be additionally amplified by elaborate microvillar processes. The posterior intracapsular projections are extensions of the cell surfaces and the intercellular spaces to the outer layers of the capsule. Their proximity to the outer capsule surface may facilitate the

Fig. 13. Electron micrograph, posterior suture region. Gomori procedure, unaltered medium. Acid phosphatase reaction product is localized within some tubules and vesicles of smooth ER (ser). Note the abundant interdigitating cellular processes. tb, terminal body; cap, Capsule. (Calibration bar = 1 μm; ×16,500.)
Fig. 14. Diagrams to show ultrastructure in anterior (A) and posterior (B) suture regions of normal rat lens. Features suggesting ion-linked fluid transport are regional folding of plasma membrane (convolutions and microvilli), dilated intercellular spaces (V); clusters of mitochondria (M); and K-NPPase activity (heavy cell boundaries). RER, Rough ER; SER, smooth ER; CAP, lens capsule; NUC, epithelial cell nucleus.

movement of materials to and from the lens.

The presence of intercellular dilations in living epithelial tissue has been questioned and it has been suggested that these dilations are due to cellular shrinkage during sample preparation.4–14 These spaces, however, have been observed in microscopic in vitro studies of living epithelial tissues.4–15

In a recent investigation of gall bladder epithelium, a direct correlation was found between size of the intercellular spaces and the rate of fluid transport.15

In samples subjected to the NPPase procedure, we found large intercellular vacuoles to be present throughout the superficial regions of the lens cortex and epithelium. These were interpreted as artifact caused by osmotic damage in the incompletely fixed tissues. In well-fixed normal rat lenses, however, there was a population of intercellular dilations localized in the suture regions. Additionally, in vivo biomicroscopic examination revealed a pattern of vacuoles in the normal lens, primarily in the posterior su-
ture. This evidence indicates that intercellular dilations are normally present in non-cataractous lenses of living rats, at least within the posterior suture.

Mitochondria typically are abundant in cells in which ionic transport activity is highly developed.\(^4\) Organelles of this type concentrated in the lens sutural regions were of diminutive size, a feature which also typifies most lens epithelial mitochondria. Although oxidative phosphorylative activity in the lens reportedly is low, it is possible that mitochondria, strategically located in the epithelial and sutural regions, provide energy for the cation pump(s) which carry out the fluid transport. The greater cytoplasmic density in some epithelial cells at the anterior suture may have represented a degree of cellular dehydration caused by their greater water-removing activity.

In the histochemical portion of this investigation, the method developed by Ernst\(^7\) was used to localize the K-dependent, ouabain-sensitive component of Na-K-ATPase in the normal rat lens. This component, which catalyzes the second (dephosphorylation) step of transcellular ionic transport, has been referred to as K-NPPase because of its ability to use pNPP as a substrate (see Firth\(^16\)). Enzyme activity, which was partially reduced by incorporating ouabain in the incubation medium, was demonstrated along the lateral and apical membranes of the lens epithelial cells as well as on the borders of fibers within the sutural portions of the anterior and posterior cortex. Addition of levamisol or removal of K\(^+\) ions partially blocked the reaction. Although a strict quantitative measure of the enzyme activity for each group was not possible in the present study, both K-NPPase (ouabain-sensitive, levamisol-insensitive, K-dependent) and nonspecific alkaline phosphatase (ouabain-insensitive, levamisol-sensitive, K-independent) were indicated within reactive areas of the lens. The reduction in activity of the control tissues was verified by postincubation spectrophotometric analyses of the various incubation media. A combination of K-NPPase and nonspecific alkaline phosphatase, both hydrolyzing NPP, has been demonstrated within the rat kidney cortex by Ernst,\(^17\) who used l-cysteine instead of levamisol as the alkaline phosphatase inhibitor.

Our results are somewhat at variance with those of Palva et al.,\(^18\) who found K-NPPase reaction to be confined to the adjoining cell membranes of the lens epithelium and absent from the cortex and cortical/epithelial border. Their studies showed the epithelial activity to be (incompletely) sensitive to ouabain but totally resistant to levamisol, indicating the absence of an alkaline phosphatase component. The differences in results obtained in our laboratories might be explained, at least in part, by the dissimilarities of sampling methods. Palva's investigations appear to have included only the equatorial and adjoining anterolateral lens regions. The anterior polar distribution of K-NPPase demonstrated in our laboratory has been confirmed by Unakar and Tsui.\(^19\)

The histochemical demonstration of K-NPPase within the lens polar cortical regions is of special interest in view of the physiological investigations of rabbit lenses recently described by Neville et al.\(^20\) These workers reported one third of the ouabain-sensitive lens Na-K-ATPase activity to be present in the anterior as well as the posterior cortex, the remainder residing within the lens epithelium. Use of \(^{86}\)Rb in conjunction with ouabain indicated that both epithelial and anterior cortical enzyme contributes to cationic transport in the lens, whereas the ATPase present in the posterior cortex apparently is inactive.\(^20\) However, it is possible that cationic transport may occur at a localized region such as the posterior lens suture without being readily detected by physiological methods.

The possible importance of the anterior and posterior cortical smooth ER as a source of acid phosphatase secreted during senile cataractogenesis has been discussed previously.\(^3\) The demonstration of small amounts of intercellular reaction product in normal rat lenses suggests a physiological role of acid hydrolases in the turnover of the plasma membrane.

**REFERENCES**

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Erratum

In Fig. 1, B, of "Anomalous pigment epithelial photoreceptor relationships and receptor orientation" by Fitzgerald, Enoch, Birch, Benedetto, Temme, and Dawson (p. 956, August issue) point one should have been located immediately centered on the inferotemporal pigment epithelial defect. Point zero should have been centered at the center of the fovea, the point of fixation. Point two would then lie on a line to the left of point zero. Added tests establish that the central fovea was the point of fixation. The relationship between the center of the fovea and the inferotemporal lesion is best seen in Fig. 1, A. Thus determinations made at point one were centered on the lesion.