The Balance between Corneal Transparency and Edema
The Proctor Lecture

Henry F. Edelhauser

The corneas of all animal species remain transparent because of the epithelial barrier, the stromal latticelike arrangement of the collagen fibrils, and the endothelial cells. Depending on the species, the corneal epithelium, the corneal endothelium, or both have a major role in regulating corneal hydration. The ultimate goal is for the cornea to remain transparent and to provide the major refractive function of the eye. The one exception is that of the aquatic fishes, since their lenses serve in the major refractive role.

The comparative optical characteristics of the vertebrate eye have been eloquently summarized in Gordon Walls’ text, *The Vertebrate Eye and Its Adaptive Radiations*. The purpose of this review is to discuss the various factors that regulate corneal hydration and transparency and to illustrate some clinical situations in which the function of the corneal endothelium becomes compromised.

CORNEAL STRUCTURAL ADAPTATIONS

The animal cornea consists of a basic structure of two covering layers—the epithelium and endothelium—and a central collagen stroma (Fig. 1). Variations in the thickness of the corneal epithelium are a structural adaptation that occurs in fishes and allows them to adapt to a wide variety of environmental conditions. The fish cornea, as well as that of all other animals, remains transparent, because it transmits greater than 90% of the incident visible light. Besides structural adaptations, corneal transparency is preserved by cellular active transport mechanisms, which keep the hydrophilic corneal stroma less hydrated than it can become. The ideal physiological corneal hydration is approximately 78% in humans. An increase or decrease from this ideal level can result in corneal opacity. Several studies have established that the limiting layers of the cornea are the epithelium and endothelium, which are sites of active ion transport that regulate the hydration of the hydrophilic stroma. Further studies have shown in mammals that a reduction of corneal metabolism (i.e., anaerobiosis, or lowered temperature) adversely affects the barrier properties and transport functions of both the epithelium and endothelium, resulting in an increase in corneal thickness and a loss of transparency.

The elasmobranch (sharks, skates, and rays) cornea resists swelling because of unique structural adaptations in the stroma (i.e., sutural fibers). Smelser has elegantly shown that the shark cornea does not swell or become edematous from a loss of epithelial barrier function. The corneas of more advanced teleosts (salt and freshwater bony fish) swell and become edematous and opaque when the epithelial barrier is compromised.

Various structural and metabolic adaptations develop in the cornea that enable aquatic animals to maintain transparency. For example, elasmobranchs have a relatively thin cornea (160 μm) of which the epithelium makes up approximately one third of the thickness. The corneal stroma of elasmobranchs resists swelling when placed in deionized water or when held at 4°C for 12 hours. This is also a characteristic of the spectacle of the sea lamprey, which is the most primitive fish in the evolutionary tree (Fig. 1). Stromal swelling is limited in these animals, because sutural fiber complexes serve to hold the collagen lamellae together by interconnecting adjacent collagen bundles (Fig. 1). The sutural fibers have been found to be composed of type I collagen fibrils. The impermeable nature of the elasmobranch corneal epithelium, as in most other fishes studied, is another major factor that contributes to the maintenance of corneal transparency. In contrast, the corneal endothelium of the elasmobranch is primitive and has a minor physiological role in corneal hydration. It can best be observed histologically or with specular microscopy.

The more advanced marine teleosts (e.g., sculpin) differ from the elasmobranchs in that the teleost cornea is composed of two distinct layers. It can be mechanically or histologically separated into an outer stroma and an inner stroma (Fig. 1). This was first described by Fisher and Zadunaisky, who visualized five morphologically distinct zones with the specular microscope. The outer and inner stroma are not substantially attached to one another, but appear to be slightly adherent to one another through a mucoid layer (similar to that found between the primary spectacle and the cornea of the sea lamprey). In contrast to the elasmobranch corneal epithelium, the epithelium of salt water teleosts comprises only 14% of the total corneal thickness. It has been shown to be impermeable to water; however, the stroma becomes edematous and opaque when kept at 2°C for up to 22 hours. Evaluation of swelling characteristics have shown that the outer stroma exhibits more swelling than the inner stroma.

This greater swelling ability is related to the different anatomic arrangements of the collagen fibrils in the two layers. The collagen fibrils of the inner layer are compact and highly interwoven, whereas the collagen fibrils of the outer layer are spread apart and loose (Fig. 1). With the existence of a large osmotic gradient across the cornea, the loose stroma of the outer layer may easily become overhydrated. Thus, an additional barrier controlling corneal hydration in the salt water teleost is found in the endothelium and possibly anterior to Descemet’s membrane. These fish possess a thicker Descemet’s membrane and a more well-developed endothelium than the elasmobranchs. Ultrastructure studies have shown an extensive iridescent lamellar structure anterior to Descemet’s membrane that may assist in removing corneal water and conserving intraocular fluid. This structure has also been speculated to function as a refraction gradient for absorbing various light rays.
The freshwater teleost (e.g., rainbow trout) illustrates the next evolutionary step in the development of the cornea. It maintains ideal hydration and transparency by possessing a thick corneal epithelium that comprises 40% of the total corneal thickness (Fig. 1). This has been described by Smelser for the carp cornea, one species of freshwater teleost. Therefore, both the elasmobranch and the teleost maintain their ideal hydration levels and transparency, primarily by virtue of their epithelia. The freshwater teleost cornea has one continuous stroma that consists of bands of collagen fibrils interspersed with keratocytes. On removal of the corneal epithelium, marked stromal swelling occurs in fresh water.

The central corneal thickness of most mammalian corneas varies from 0.200 mm in the guinea pig to 0.809 mm in the bovine cornea; human corneas typically average 0.540 mm in thickness (Fig. 1). In mammals, corneal transparency is primarily maintained by the corneal endothelium. Similar to less-advanced animals, corneal edema and stromal swelling result in opacification. Both the epithelium and endothelium of the mammalian cornea prevent corneal swelling by functioning as diffusion barriers to the fluid (tears or aqueous humor) and by acting as sites of active ion transport, to induce the osmotic movement of the water out of the stroma.

A high epithelial mitotic index ensures rapid wound repair of the human corneal epithelium and, thus, maintenance of barrier function. By comparison, the human corneal endothelium (composed of 350,000–500,000 cells; 2000–5000 cells/mm²) has a limited capacity for mitosis and serves as a limited diffusion barrier to aqueous humor, yet possesses numerous metabolic pump sites for the active transport of ions and fluid movement. The corneal endothelium serves the major role of maintaining corneal hydration in terrestrial mammals. It is also the primary cellular area of concern in human disease states or when endothelial cells become damaged. When a large number of cells are damaged due to disease, the endothelium loses both its barrier and pump functions, resulting in corneal swelling and opacity.

Clinically, when one evaluates the mammalian cornea with a slit lamp, it appears as one homogenous transparent tissue. However, from a histologic, ultrastructural, and biochemical point of view, there is a difference between the anterior third and the posterior two thirds of the human cornea. The anatomic separation of the corneal stroma into an anterior and posterior region in salt water teleost fishes may help explain from a teleologic viewpoint why this structural and biochemical difference developed in humans.

In the mammalian cornea, the anterior stroma has less water than the posterior stroma (3.04 g H₂O/g dry weight for the anterior and 3.85 g H₂O/g dry weight for the posterior). This difference may be due to atmospheric drying through the corneal epithelium and distribution of the two proteoglycans of the corneal stroma. Previous biochemical studies have shown that the anterior corneal stroma has a higher ratio of dermatan sulfate to keratan sulfate. Similarly, the anterior stroma has less glucose than does the posterior stroma. This latter finding is not unexpected, since corneal glucose is obtained from the aqueous humor and the corneal epithelium utilizes most of the free glucose.

The clinical importance of the proteoglycan ratios in the mammalian corneal stroma relates to stromal hydration and water distribution. Dermatan sulfate has less watersorptive capacity, but greater water-retentive capacity; whereas, the keratan sulfate possesses a greater water-sorptive capacity, but a meager ability to retain the stromal water. Therefore, when corneal edema typically occurs, stromal swelling is predominant in the posterior stroma. In addition, because the posterior stroma has a lower water-retention capacity, due to

---

**Figure 1.** Comparative anatomy of corneas for the sea lamprey, elasmobranchs, salt water, and freshwater teleosts and mammals.
the higher ratio of keratan sulfate to dermatan sulfate, it is relatively easy for the corneal endothelium to remove the water once the barrier and metabolic pump have become re-established.

**CORNEAL ENDOTHELIAL DEVELOPMENT AND CELL DENSITY**

The anatomic and physiological development of the rabbit corneal endothelium has been studied by Stiemke et al., who correlated the morphology, hydration, and Na/K ATPase pump site density from birth to young adult age. They found that the body weight and the diameter and surface area of the cornea increase in the rabbit from birth to 3 months of life. As the cornea enlarges from birth to adulthood, there is a marked decrease in corneal endothelial cell density (ECD; from 10,577 to 4,077 cells/mm²; Fig. 2). In addition to the decrease in ECD, the endothelial cells develop tight junctions, the endothelial permeability decreases, the number of endothelial cell pump sites and density increase, and the total corneal hydration decrease (Fig. 3), resulting in a transparent cornea. Although not as well studied, a similar developmental process presumably occurs in the human cornea, since the central ECD decreases from birth when there are 5000 – 6000 cells/mm² to adulthood when there are 2500 – 3000 cells/mm².

Recently, Amann et al. measured the corneal ECD in the central, paracentral, and peripheral zones of normal human corneas. Their results showed that the human cornea has an increased ECD more peripherally: peripheral > paracentral > central (Fig. 4). The superior peripheral region of the corneal endothelium consistently had the highest ECD. The increase in the peripheral ECD suggests that a reserve storage population of cells resides near Schwalbe’s line, to spread and remodel, if...
corneal endothelial damage occurs. Figure 5 shows the age-dependent human ECD within these three different corneal regions from the second through the seventh decades of life. The ECDs were determined from 75 human eye bank corneas that were stained with alizarin red. Figure 5 shows that the ECD decreases at an annual rate of 0.59% centrally, 0.45% paracentrally, and 0.40% peripherally. Data from this study and others34–37 have shown that the ECD adjacent to Schwalbe’s line is approximately 20% to 30% higher than in the central cornea. It has therefore been suspected that the endothelial cells around Schwalbe’s line may serve as a regenerative zone that is able to provide a slow continuous population of cells to the central cornea. Unfortunately, in the human cornea, this regenerative capacity is quite limited, as opposed to that of the rabbit. It has been documented that a loss of central endothelial cells occurs at a rate of 0.5% to 0.6% per year38 in human corneas, similar to the data shown in Figure 5. This is a small decline and amounts to a loss of only approximately 100 to 500 cells per year from the central corneal endothelium. The knowledge about the increased peripheral ECD in humans, particularly superiorly, is most important for the clinician to understand, since this area can be damaged by common intraocular surgeries, particularly cataract or refractive surgery involving anterior chamber intraocular lenses (IOLs) or phakic IOLs. Increased peripheral ECD appears to be unique to humans, as similar observations have not been made in other mammals (Table 1).

It is interesting to note that, in a comparison of the central ECD from a Japanese population to that in an age-matched U.S. population, the mean central ECD in the Japanese was significantly higher than that of the U.S. cohort.39 The increased central ECD appears to provide Japanese individuals with a greater functional reserve for future endothelial cell damage.

Human corneal endothelial cells have been shown to have a limited ability to divide both in vivo and in tissue culture. This fact has been well-documented for a number of years. Because of progressive corneal endothelial cell loss (i.e., cell death) in humans, coverage of the posterior corneal surface by endothelium with increasing age relies on cellular migration and spreading (i.e., cells sliding from the periphery and enlarging to cover the surface areas of the defect) for repair. Attempts to understand the limited division potential of human central endothelial cells have been limited due to our current knowledge of corneal endothelial cell biology. Joyce et al.40 have suggested that TGF-β may keep human corneal endothelial cells (HCECs) in a nonreplicative state. Paull and Whitehart41 have shown that both p53 and Tap63 are elevated in the normal central human corneal endothelium, which suggests

### Table 1. Endothelial Cell Densities Determined from Alizarin Red-Stained Cornea

<table>
<thead>
<tr>
<th></th>
<th>Central</th>
<th>Paracentral</th>
<th>Peripheral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse (28 g; n = 10)</td>
<td>2845 ± 209</td>
<td>2866 ± 376</td>
<td>2610 ± 320</td>
</tr>
<tr>
<td>Rat (370 g; n = 10)</td>
<td>2601 ± 280</td>
<td>2506 ± 231</td>
<td>2146 ± 390</td>
</tr>
<tr>
<td>Rabbit (4–5 lb; n = 5)</td>
<td>3926 ± 382</td>
<td>3733 ± 504</td>
<td>3612 ± 365</td>
</tr>
<tr>
<td>Rabbit 2.4 y (9.6 lb; n = 3)</td>
<td>2619 ± 169</td>
<td>2593 ± 428</td>
<td>2308 ± 215</td>
</tr>
<tr>
<td>Primate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhesus</td>
<td>3597</td>
<td>—</td>
<td>4049</td>
</tr>
<tr>
<td>Baboon</td>
<td>4082</td>
<td>—</td>
<td>3861</td>
</tr>
<tr>
<td>Macaque 7 y</td>
<td>3747</td>
<td>—</td>
<td>2546</td>
</tr>
<tr>
<td>10 y</td>
<td>3540</td>
<td>—</td>
<td>2490</td>
</tr>
<tr>
<td>16 y</td>
<td>2703</td>
<td>—</td>
<td>1995</td>
</tr>
</tbody>
</table>

Data are expressed as cells per square millimeter ± SD.

---

**Table 1.** Endothelial Cell Densities Determined from Alizarin Red-Stained Cornea

**Figure 5.** Human corneal endothelial cell density in the central, paracentral, and limbal (far peripheral adjacent to Schwalbe’s line) between the second and seventh decades. (n), the number of corneas counted.
that these proteins may also inhibit the endothelial cell cycle by keeping them in the G1 phase. Our current understanding is that HCECs are held in the G1 phase of the cell cycle and cannot readily pass into the S phase, even when stimulated by injury. Because of the recently reported increased peripheral human ECD and studies by Bednarz et al. that showed a zone of endothelial cell regeneration in the peripheral region of the human cornea near Schwalbe’s line, it has been hypothesized that the peripheral endothelial cells may act as an adult stem cell reservoir. Recently, a study by Whikehart et al. supported this view when they found that central human corneal endothelial cells lack telomerase activity, but endothelial cells adjacent to Schwalbe’s line have this enzyme activity. Similarly, these peripheral endothelial cells stained positive with BrdU, which is a marker for dividing cells. Thus, adult endothelial stem cells may exist near Schwalbe’s line to supply new cells for both the corneal endothelium and the trabecular meshwork.

**CORNEAL ENDOTHELIAL BARRIER AND PUMP**

The corneal endothelium forms an anatomic and physiological barrier between the nutrient-rich aqueous humor and the corneal stroma. Tight junctions are an integral component of the endothelial barrier, connecting cells at the apical-most part of the lateral membrane. Tight junctions of the corneal endothelium are focally present around endothelial cells and serve to restrict selectively the extracellular diffusion of some ions and macromolecules. Because the tight junctions of the corneal endothelium are known to be “leaky,” the enhanced permeability is advantageous, because it permits diffusion of most nutrients into the stroma from the aqueous humor. Metabolic pump sites (Na,K-ATPase) are also found on the lateral membranes of corneal endothelial cells, which serve to transport Na\(^{+}\)/H\(^{+}\) ions from the corneal stroma to the aqueous humor. Overall, this dynamic pump–leak system maintains corneal deturgescence and permits sufficient nutrient delivery into the stroma and epithelium. Disruption of the corneal endothelial cells typically results in corneal edema, swelling, and opacity. For the pump–leak system to function properly, the tight junctions must be intact and continually function as an endothelial barrier. The ultrastructure of the corneal endothelial tight junctions has been studied extensively. The specific molecules that comprise the tight junctions are F-actin, ZO-1, and \(\alpha\)- and \(\beta\)-cadherin. We have recently shown that the corneal endothelium also contains the junctional adhesion molecules JAM-1 and AF6 (Fig. 6). We have shown that blocking the JAM-1 protein with anti-JAM-1 antibody when the tight junctions are disrupted with a Ca\(^{2+}\)-free irrigating solution prevents tight junction formation when Ca\(^{2+}\) is added back to the solution. Overall, these studies confirm that the ZO-1, JAM-1, and AF6 junctional proteins are both effective in maintaining the endothelial tight junctions complex and in maintaining the endothelial barrier.

**FIGURE 6.** Transmission electron micrograph of a human corneal endothelial tight junctional complex (A). Localization of ZO-1 and JAM-1 in rabbit corneal endothelial tight junctions. (Rabbit corneas were fixed with 10% acetic acid in ethanol and blocked with 1% BSA). Corneas were immunolabeled with a monoclonal antibody against ZO-1 (B, green) or tight junctional marker JAM-1 (C, green). Nuclei are shown in blue. As shown in the figure JAM-1 localizes in tight junctions of corneal endothelial cells with a staining pattern identical with that of tight junctional marker ZO-1. Confocal cross-sections of corneal endothelium (D) confirms that JAM-1 localizes in the apical part of the endothelial junction. Reprinted with permission from Mandell KJ, Holley GP, Parkos CA, Edelhauser HF. Antibody blockade of junctional adhesion molecule-A in rabbit corneal endothelial tight junctions produces corneal swelling. Invest Ophtalmol Vis Sci. 2006;47:in press. © ARVO.
The importance of tight junctions in controlling endothelial barrier function was confirmed by measuring permeability in rabbit and human corneas after in vitro corneal endothelial perfusion with 5(6)-carboxy fluorescein as a permeability tracer. Removal of the endothelium in the rabbit cornea increased permeability from $3.19 \times 10^{-4}$ to $31.21 \times 10^{-4}$ cm min$^{-1}$. In the human donor cornea, the permeability increased from $2.26 \times 10^{-4}$ to $12.85 \times 10^{-4}$ cm min$^{-1}$. In the healthy normal human cornea, there was no correlation between endothelial permeability, donor age, or ECD. There is, however, an increase in corneal endothelial permeability in human subjects with acute or severe endothelial damage or disease. For example, patients with Fuchs’ endothelial dystrophy have a defect in the corneal endothelial barrier function and an increase in endothelial permeability.

The corneal endothelial pump site density has been quantitated with tritiated ouabain (one Na,K-ATPase site binds one molecule of $^3$H-ouabain) in human and rabbit corneas. The rabbit corneal endothelium was found to have $3.0 \times 10^6$ sites/cell and the human corneal endothelium had $2.1 \times 10^6$ sites/cell. It is interesting to note that in healthy, normal human corneas—where there is a 0.6% decrease per year in central ECD with age—the endothelial pump site density remains constant with age. In contrast, patients with corneal endothelial cell damage or disease have increased endothelial pump site densities as endothelial permeability increases.

Hence, the increased metabolic pump sites are presumably an adaptation to compensate for the increased corneal endothelial permeability to a certain degree.

Corneal endothelial wound repair after damage usually follows a three-stage process. The first stage is characterized by the establishment of an initial coverage of the wound by migration of adjacent endothelial cells, which forms a temporary incomplete barrier with minimal pump sites and incompletely formed tight junctions. In the second stage, the barrier (i.e., tight junctions) and subsequently the pump functions return to normal levels, the endothelial cells form irregular polygons, the corneal thickness typically returns to normal, and transparency is restored. The third stage involves remodeling of the endothelial cells to form more regular hexagons.

Based on several human studies measuring central ECD by specular microscopy, it has been substantiated that the normal decrease in human ECD is ~0.6% per year from age 15 to 85 years. In comparison, after a penetrating keratoplasty (PKP), the central endothelial cell loss of the donor button over the first postoperative year averages 34.1%. Human corneas before PKP are typically stored in Optisol-GS Solution (Chiron Vision, Irvine, CA) for up to 10 to 15 days before surgery. We have shown that human corneas stored less than 5 days in Optisol-GS have $1.1 \pm 0.1$ apoptotic cells/mm$^2$ and corneas stored over 22 days have $4.9 \pm 0.2$ apoptotic cells/mm$^2$, and storage beyond 22 days increases the number of apoptotic cells exponentially. Komuro et al. have also reported slight endothelial apoptosis in Optisol-GS–stored corneas. They also reported that endothelial apoptosis correlates best with TUNEL-positive keratogenic corneas, but least with storage time. Thus, human Optisol-GS storage (which is the preferred storage media in the United States) causes minimal apoptosis and the marked decrease in ECD after penetrating keratoplasty is apparently due to surgical trauma.

In summary, the healthy normal human corneal endothelium maintains corneal transparency because of increased peripheral ECD, tight junctions that form a leaky barrier to diffusion, and a large number of Na,K-ATPase pump sites. Finally,
these studies also have shown that there is minimal apoptosis of healthy normal endothelial cells with age, enabling the monolayer of corneal endothelial cells to remain intact over a normal lifespan (Fig. 9).

**FACTORS LEADING TO CORNEAL EDEMA**

Mishima first described the substantial physiological reserve of the human corneal endothelium. He also showed that chronic corneal endothelial cell decompensation typically occurs when the central ECD declines to 700–400 cells/mm². There is therefore an enormous reserve of extra endothelial cells to withstand intraocular surgery, trauma, and disease. Figure 10 compares the ultrastructure of the normal human corneal endothelium to that of the corneal endothelium with pseudophakic bullous keratopathy (PBK). The latter cornea typically reveals through specular microscopy a decreased central ECD, increased polymegathism (larger than normal endothelial cells), and indistinct endothelial cell details, which correlates to the ultrastructure of a Descemet's membrane that is sparsely covered by endothelial cells and a thin, newly secreted, diffuse banded posterior collagenous layer. In comparison, Fuchs' endothelial dystrophy has a specular microscopic appearance similar to chronic PBK, with the additional finding of having many focal dark spots due to the guttae, which are focal thick deposits of the banded posterior collagenous layer (Fig. 11). Of note, transmission electron microscopy of corneas affected by Fuchs' dystrophy also shows degenerative-appearing corneal endothelial cells covering the guttae.

During the early development of phacoemulsification (1970s), marked corneal edema was frequently noted after surgery. One of the main causes of this postoperative edema...
was the use at that time of an “incomplete” intraocular irrigating solution (e.g., PlasmaLyte 148; Baxter Healthcare, Deerfield, IL).65,66 This incomplete intraocular irrigating solution when perfused onto the corneal endothelium caused a breakdown of the corneal endothelial tight junctions (Fig. 12) resulting in corneal edema.66 Watsky and Edelhauser67 have shown that corneal endothelial permeability is substantially increased when perfused with a Ca\(^{2+}\)/H\(^{+}\)-free medium. Similarly, we reported that when corneal endothelial intracellular reduced glutathione was oxidized by thiol-oxidation with diamide, the endothelial tight junctions also became disrupted (Fig. 13) and barrier function was lost.68 Transmission electron microscopy further revealed that the endothelial cells separated at the apical junctions and that microfilaments in the apical endothelial cytoplasm formed dense contractile bands. It was concluded from this study that the ratio of reduced to oxidized glutathione in the endothelial cells has a role in the maintenance of the endothelial barrier function by protecting the tight junctions from separating. Ultimately, this led to the development of the first commercially available “complete” intraocular irrigating solution, BSS Plus (Alcon Laboratories, Fort Worth, TX) for cataract and retina intraocular surgery. BSS Plus contains five essential ions (Na\(^{+}\), K\(^{+}\), Ca\(^{2+}\), Mg\(^{2+}\), and Cl\(^{−}\)), bicarbonate as a buffer, dextrose as an energy source, and glutathione as an antioxidant.

**PH AND OSMOTIC TOLERANCE OF THE CORNEAL ENDOTHELIUM**

With all the ophthalmic solutions, medications, and other agents currently used during routine phacoemulsification cataract surgery, it is important to know the pH and osmotic tolerance range of the human corneal endothelium. Studies have shown that the corneal endothelium has a pH tolerance between 6.8 and 8.2, which is similar to that of the natural aqueous humor bicarbonate buffer system.69 Moreover, during cataract surgery, the osmolality of the anterior chamber can easily vary because of the variety of drugs and solutions used in irrigation or injection of the eye. This variation can cause the endothelial cells to become swollen, degenerated, apoptotic, or even necrotic. If all the essential ions are present, corneal endothelial cells have been shown to tolerate a wide range of osmolalities from 250 to 350 mOsmoles.70 Therefore, both the

---

**Figure 12.** Scanning electron micrograph of rabbit corneal endothelium perfused for 1.5 hours with Ca\(^{2+}\)-free intraocular irrigating solution. The cells are separating at the tight junctions (A). Transmission electron micrograph of the same cornea showing loss of the apical tight junctional complexes (B).

**Figure 13.** Scanning electron micrograph (SEM) of rabbit corneal endothelium after perfusion with 4 \(\times\) 10\(^{-4}\) M diamide for 15 minutes (A). The endothelial monolayer is disrupted at the tight junctions. After 30 minute perfusion with 4 \(\times\) 10\(^{-4}\) M diamide, the endothelial cells form clusters of flat islands and larger areas of Descemet’s membrane are exposed (B). (A) Reproduced from The Journal of Cell Biology, 1976, Vol. 68, 567–578 by copyright permission of the Rockefeller University Press.
pH and osmolality of the intraocular solution are critical in maintaining the corneal endothelium.

**TOXIC ENDOTHELIAL CELL DESTRUCTION AND TOXIC ANTERIOR SEGMENT SYNDROME**

Toxic endothelial cell destruction (TECD) is a syndrome of corneal endothelial cell decompensation caused by toxic agents that are introduced into the eye, most commonly during cataract surgery. TECD was first described by Breebaart et al. and Nuyts et al. in 1990 as severe postoperative corneal edema—star-shaped Descemet’s folds, a two-fold increase in corneal thickness, and visual acuity at count fingers within 1 to 3 days after cataract surgery. The mechanism of corneal edema is related to the breakdown of the corneal endothelial barrier function and endothelial cell death. This syndrome has commonly been linked to a detergent solution used in ultrasonic bath cleaners for disinfecting ophthalmic surgical instruments. The main accompanying factor causing this syndrome is that reusable cannulas, which are used to re-form the anterior chamber, often retain residual viscoelastics. During the ultrasonic cleaning procedure, the viscoelastic residue, or sometimes the reusable cannula itself, accumulates toxic ultrasonic detergent residue. When the anterior chamber is re-formed with the toxic residue present, the result is TECD. TECD has also been reported from other contaminants such as the heavy metal ions Cu²⁺ and Zn²⁺ (which coat the hubs of surgical instruments and cannulas).

The cases of toxic anterior segment syndrome (TASS) appear similar to TECD, but with additional damage to the iris, trabecular meshwork, and occasionally the lens. TASS presents clinically as acute postoperative corneal edema with hypopyon and fibrin in the anterior chamber. One of the main causes of TASS is retained residues of viscoelastics and detergents on or in reusable ophthalmic instruments used during phacoemulsi-fication. Ophthalmic instruments in the United States are commonly cleaned with an enzymatic detergent containing subtilisin, a proteolytic enzyme produced from the fermentation of Bacillus licheniformis. It appears that residual viscoelastics on instruments and/or cannulas can absorb this enzyme, and, even when autoclaved to 120°C, the enzyme does not become deactivated, because the enzyme requires higher temperatures (≥140°C) for deactivation. In laboratory experiments, when subtilisin is perfused directly onto the rabbit corneal endothelium, corneal swelling occurs, increasing in severity with increasing enzyme dose (Fig. 14), along with structural damage to corneal endothelial cells (Fig. 15). When the same commercial...
cial enzymatic detergent was further evaluated, it was found to have a marked effect on endothelial junctional proteins and endothelial plasma membrane components (Fig. 16) as corneal endothelial permeability increased 3.6-fold ($6.25 \times 10^{-4}$ to $22.74 \times 10^{-4}$ cm/min). It should be noted that when the anterior chamber is re-formed after cataract surgery, agents placed in the anterior chamber generally remain for at least 4.5 hours. Therefore, since the detergent–enzyme contacts the endothelium up to 4.5 hours, there is ample time for serious toxicity to occur.

**CORNEAL SWELLING AND EDEMA**

The predominant osmotic gradient across the corneal endothelium is related to sodium ion activity in the aqueous humor. Normally, the sodium concentration gradient in the anterior chamber results in a net hydrostatic force to draw water osmotically out of the stroma. This effect is somewhat blunted by other ionic gradients, which reduce the net hydrostatic force across the endothelium to 30.4 mm Hg. When the corneal endothelium is damaged, there is loss of both the corneal endothelial barrier and pump function followed by a loss of ionic gradients, ultimately resulting in corneal edema and swelling. Histopathology of a human cornea with marked corneal edema typically shows a loss of artifactual clefting in the stroma, a marked increase in corneal thickness, and intercellular and extracellular edema of the basal epithelial cell layer of the epithelium. Ultrastructural studies of edematous human corneas in the anterior, middle, and posterior stroma (Fig. 17) show that the collagen fibrils are not equally distant from each other after edema occurs, which may partially explain the haze caused by corneal edema. In addition, corneal edema is

**FIGURE 16.** Scanning electron micrograph (A) of rabbit corneal endothelium perfused with 1% Medline Enzymatic Detergent solution, which contains subtilisin, shows swollen intracellular regions and disrupted intracellular junctions. Transmission electron micrographs (B, C) shows increased intracellular vacuolization (arrows), abnormal subcellular organelles, and irregular plasma membrane (original magnification, $x 4350$). Reprinted with permission from Parikh C, Sippy BD, Martin DF, Edelhauser HF. Effects of enzymatic sterilization detergents on the corneal endothelium. *Arch Ophthalmol.* 2002;120:165–172. © 2002, American Medical Association. All rights reserved.

**FIGURE 17.** Transmission electron microscopy of the edematous corneal stroma showing the collagen fibrils in the anterior (A), middle (B), and posterior (C) regions of the stroma. There is a diffuse separation of the fibrils in each region of the stroma, with the largest changes in the middle and posterior regions. Original magnification, $x 95,400$. 

Normally, the sodium concentration gradient in the anterior chambers results in a net hydrostatic force to draw water osmotically out of the stroma. This effect is somewhat blunted by other ionic gradients, which reduce the net hydrostatic force across the endothelium to 30.4 mm Hg. When the corneal endothelium is damaged, there is loss of both the corneal endothelial barrier and pump function followed by a loss of ionic gradients, ultimately resulting in corneal edema and swelling. Histopathology of a human cornea with marked corneal edema typically shows a loss of artifactual clefting in the stroma, a marked increase in corneal thickness, and intercellular and extracellular edema of the basal epithelial cell layer of the epithelium. Ultrastructural studies of edematous human corneas in the anterior, middle, and posterior stroma (Fig. 17) show that the collagen fibrils are not equally distant from each other after edema occurs, which may partially explain the haze caused by corneal edema. In addition, corneal edema is
associated with loss of stromal proteoglycans (keratan sulfate and dermatan sulfate) and hydropic degeneration or cell lysis of keratocytes. Figure 18 summarizes the causes of corneal edema that have been described in this review. If a compromised endothelium already exists, all of these factors can have a more rapid onset.

**Effect of Corneal Endothelial Decompensation in LASIK Corneas**

In human corneas that have been treated by laser in situ keratomileusis (LASIK), the central and paracentral lamellar interface wound between the flap and stromal bed has limited wound strength that averages around 2.4% of normal corneal stroma as measured with a tensiometer. The LASIK scar varies in thickness from 0.4 to 11.4 μm thick (mean thickness, 4.5 μm). It has been found to be hypocellular and is composed predominantly of abnormally large, non–fibril-bound proteoglycans. In cases of endothelial decompensation, the post-LASIK cornea preferentially becomes edematous posteriorly and in the interface wound. This sometimes results in the development of a fluid pocket between the flap and bed at the hypocellular primitive stromal scar (Fig. 19). Recent laboratory studies conducted on post-LASIK human eye bank corneas have shown that a lamellar fluid pocket can develop at the LASIK interface wound within 3 hours, if there is a loss of the corneal endothelial barrier pump function (Fig. 20). This post-LASIK complication emphasizes the importance of having a good functioning endothelium with a high ECD (i.e., good barrier and pump function).

This review describes the teleologic development of the cornea and shows the importance of the corneal epithelium and endothelium in maintaining corneal transparency. In mammalian species, the balance between corneal transparency and edema is controlled predominantly by the corneal endothelial barrier and metabolic pump function. Loss of either the metabolic pump or the barrier function results in edema and loss of transparency.

References


Acknowledgments

My very sincere and deeply appreciative thanks to ARVO, the Awards Selection Committee, The Board of Trustees, and colleagues and members of ARVO for bestowing this great honor on me. It is quite impossible to express fully my appreciation for being selected to receive the Proctor Medal. In accepting an award of this sort no person stands alone; with the awardee are all of the teachers and all of the graduate students, fellows, and coworkers who have contributed to the awardee’s work. I consider The Proctor Medal to be the highest kudo any ophthalmic researcher can receive, because it is awarded by men and woman whom I respect beyond all others. It will be my proudest possession!

A special thanks goes to my wife Barbara and to my family, who have been so supportive over the past years. I would also like to thank George Smeber, PhD, the 1961 Proctor Medal awardee who suggested that I join ARVO. V. Everett Kinsey, PhD (the 1952 Proctor awardee), and Venkat Reddy, PhD (the 1979 Friedenwald awardee), who helped me publish my first paper in IOVS in 1965. Over the past 39 years, I have had the support of two great Ophthalmology Department Chairs, Richard O. Schultz, MD, MS, at the Medical College of Wisconsin, and Thomas M. Aaberg, Sr, MD, MSPH, at Emory University, both of whom have recognized the importance of basic scientific and translational research. I thank them for their unending support!

I would like to thank and give a special tribute to the many graduate students, postdoctoral fellows, administrative assistants, and colleagues who have conducted the research and aided my laboratory over the years and who have made this award possible. A special thanks to Glenn Holley, BS, my long-time laboratory assistant, Nancy L. Hernault, BS, for her skill with electron microscopy, Patrick DeLeon, BA, for his medical illustrations, and Daniel Dawson, MD, for help in the laboratory and manuscript review.


53. Mandell KJ, Holley GP, Parkas CA, Edelhauser HF. Antibody blockade of junctional adhesion molecule-A in rabbit corneal endothelial


64. Waring GW. Posterior collagenous layer of the cornea ultrastructural classification of abnormal collagenous tissue posterior to Descemet’s Membrane in 30 cases. Arch Ophthalmol. 1982;100:122–139.


