Epithelial Ion Transport in Rabbit Corneas Following Myopic Keratomileusis

Casimir A. Swinger,† Oscar A. Candia,† Sergiu Marcus,† Barbara A. Barker,† and Ernest W. Kornmehl†

In isolated rabbit corneas that had undergone lamellar keratectomy or myopic keratomileusis, the stimulation of chloride transport by $10^{-5}$ M amphotericin B was normal both at 1 week and at 28 days following surgery. At 28 days following surgery, both groups responded to $10^{-7}$ M epinephrine. The response to $10^{-5}$ M amphotericin B was normal both at 1 week and at 28 days following surgery. We conclude that, although the Na-K pump was not affected by the lamellar keratectomy and cryolathing, that either the epithelial β receptors and/or the cAMP pathway were temporarily inhibited for at least 1 week following surgery. A lamellar keratectomy, therefore, can have an adverse effect on the epithelial transport system of the corneal epithelium even though the epithelium may appear normal clinically. Invest Ophthalmol Vis Sci 27:1277–1280, 1986

In myopic keratomileusis, a resected lamellar disc from the central cornea is frozen and thinned to effect a flattening of the cornea and correction of the myopic refractive error. The thickness of stromal tissue moved is dependent on the correction and typically ranges from 0.10–0.20 mm.

Following clinical myopic keratomileusis, the central corneal thickness is frequently greater than that predicted by the carving parameters. As it is certain that a given amount of tissue has been resected, such a finding may be explained by an increase in the state of hydration of the cornea. This may result from a temporary diminution in the physiologic capacity of the corneal endothelium, changes in the ground substance, rupture of cells by the freezing process with subsequent release of cellular contents, and the marked polymorphonuclear influx observed histologically following cryorefractive surgery. Any of these may account for an increase in the water content of the cornea.

It is also known that the corneal epithelium plays both a passive and an active role in helping maintain corneal deturgescence.1–4 This is mediated in large part by an active chloride transport system that has been

bonate3 and sodium fluxes (Table 1) also argue against a strong link between these ions. The data suggest that the movement of sodium may be more strongly influenced by the transendothelial potential (and hence current flows) developed by the net transfer of bicarbonate. This supports the concept that, while the transendothelial net flux of bicarbonate is via a cellular pathway, the net movement of sodium may occur via a paracellular pathway as suggested by one of the models presented by Fischbarg et al.11

Key words: corneal endothelium, sodium fluxes, ambient pH effects

Acknowledgment. We thank Mrs. Sylvia Catravas for her valuable secretarial assistance.

From the Departments of Ophthalmology and Physiology, Medical College of Georgia, Augusta, Georgia. Supported in part by research grants from the National Eye Institute, EY04558 (KG) and EY04479 (DSH), a departmental award from Research to Prevent Blindness, Inc., and the Joseph B. Hall Foundation. Submitted for publication: July 19, 1985. Reprint requests: Keith Green, PhD, DSc, Department of Ophthalmology, Medical College of Georgia, MCG Box 3059, Augusta, GA 30912-0300.

References

shown to be capable of stimulation and inhibition by a number of endogenous compounds and drugs. Past studies of corneal refractive surgery have been limited to anatomic and optical studies, with little attention to the effects of such procedures on the physiology of the cornea. Because of an apparent increase in the hydration of the cornea following myopic keratomileusis and the lack of physiological studies of this procedure, we investigated the effects of myopic keratomileusis upon epithelial ion transport in the rabbit cornea.

Materials and Methods. Surgery: Myopic keratomileusis was performed on ten adult New Zealand albino rabbits (2–3 kg) under general anesthesia. The procedures used in this study conform to the Arvo Resolution on the Use of Animals in Research. In seven cases, one eye underwent myopic keratomileusis, and the other eye, which served as a control, underwent only lamellar keratectomy. In the other three rabbits, keratomileusis was performed bilaterally. Surgery was carried out as previously described. In six rabbits, the bilateral lamellar keratectomies were performed with the Barraquer microkeratome, and in four rabbits they were performed by manual dissection. In all instances, the diameters of the keratectomies were approximately 8 mm, and the depths were approximately 0.25 mm.

In the keratectomized eyes, the resected tissue was immediately replaced on the bed and aligned with a previously made reference mark. The tissue was fixated with four interrupted 10-0 nylon sutures and a running, 8-bite, anti-torque suture. In the experimental eyes, the resected discs were first placed into KM 26 solution (8% glycerol, 4% dimethylsulfoxide, and 0.25% kiton green dye in 0.1 M phosphate buffer, pH 7.4) for 1 min and then placed onto the Barraquer cryolathe. The discs were then frozen and carved for a myopic correction of 6 D utilizing the Barraquer computer program 3 MKM. The length of time the tissue was frozen was 3 min in all cases. The carved discs (lenticules) were then rapidly thawed in normal saline, replaced on the bed, and sutured as described for the control eye.

The animals were frequently evaluated with a slit lamp to ensure that the clinical appearance of the corneas was normal and that there was no scarring or vascularization. Re-epithelialization was complete in 4 days or less. No medications were used postoperatively, and the nylon sutures were removed at 10 days following surgery. Two corneas became cloudy and were excluded from the study. Two additional rabbits were anesthetized and sacrificed, and four corneas were dissected for immediate electrophysiologic measurements without surgery.

Electrophysiology: Animals that underwent keratomileusis were sacrificed with an intravenous overdose of sodium pentobarbital 1 week or 4–7 weeks after surgery. Corneas were atraumatically dissected from the globe and mounted in a double-sided Lucite chamber as previously described. They were bathed in a Ring-er's solution described by KLye at 37°C and bubbled with air for a pH of 7.9.

Conventional agar-Ringer's bridges connected to calomel cells were used for measuring the potential difference. Current was sent through another pair of agar bridges to maintain the tissue short-circuited by means of a voltage clamp apparatus. As parameters of epithelial transport function, we chose the short-circuit current (SCC) and its response to both epinephrine and amphotericin B. The responses for the two groups were compared to normal, unoperated rabbit corneas.

Results. Previous studies have demonstrated that maximal stimulation of the SCC in the rabbit cornea is achieved with $10^{-6}$ M epinephrine. Figure 1 demonstrates the typical SCC response to epinephrine in the normal, freshly dissected rabbit cornea, in isolated corneas 8 and 28 days after keratectomy, and in isolated corneas 7 and 28 days after keratomileusis.
Table 1. Increase in SCC (μA/cm²) produced by epinephrine and 10⁻⁵ M amphotericin B at various postoperative times.

<table>
<thead>
<tr>
<th>EXP</th>
<th>1 Week</th>
<th>4-5 Weeks</th>
<th>7 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epi</td>
<td>AMP-B</td>
<td>Epi</td>
</tr>
<tr>
<td>C1</td>
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<td>3.0</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>0</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>0</td>
<td>14.0</td>
<td></td>
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<tr>
<td>C6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C7</td>
<td>5.0</td>
<td>10.6</td>
<td>1.6</td>
</tr>
<tr>
<td>C8</td>
<td>1.4</td>
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</tr>
<tr>
<td>C11</td>
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<tr>
<td>Mean ± SE</td>
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<td>8.0 ± 3.2</td>
<td>2.7 ± 1.1</td>
</tr>
<tr>
<td>MKM 1</td>
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</tr>
<tr>
<td>MKM 2</td>
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<td>7.4</td>
<td></td>
</tr>
<tr>
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</tr>
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<td>MKM 4A</td>
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<td></td>
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<td>MKM 8</td>
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<td>MKM 10A</td>
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<tr>
<td>MKM 10B</td>
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<td></td>
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<tr>
<td>MKM 11</td>
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<td></td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0</td>
<td>6.3 ± 1.4</td>
<td>1.6 ± 0.4</td>
</tr>
</tbody>
</table>

C = lamellar keratectomy.
MKM = myopic keratomileusis.

Epinephrine was tested at 10⁻⁷ M. In the 1 week experiments, 10⁻⁵ M did not elicit a response. Normal response to epinephrine is 2.6 ± 0.5 (n = 25) μA/cm² and to amphotericin B is 14.2 ± 3.2 (n = 16) μA/cm² from previous experiments in our laboratory.

Discussion. This is the first investigation of the effects of corneal surgery on epithelial physiology. In myopic keratomileusis, a lamellar keratectomy is performed, and the tissue is placed into a cryoprotectant, frozen, and carved. Frequently, there is an epithelial defect for 1 or 2 days following the surgical procedure and a mild superficial punctate keratitis that can last for up to 1 week. Because of the severe trauma inflicted upon the corneal epithelium, we were not surprised to find an alteration of corneal epithelial physiology. Less expected, however, was the adverse effect on epithelial transport provided by lamellar keratectomy alone. Following a lamellar keratectomy, the corneal epithelium usually appears normal immediately following surgery and remains so.

The corneal epithelium is a Cl-secreting system that, as most epithelia, has the Na-K pump at the basolateral membrane. Also at the basolateral membrane there is a Na-Cl cotransport system. The apical membrane (tear side) is selectively permeable to Cl. Thus, the trans-epithelial active Cl transport, although originated by the Na-Cl carrier, is dependent on the apical side Cl permeability. This permeability is modulated by cellular levels of cAMP which can be influenced by β-
ardenergic stimulation. Epinephrine stimulates the \( \text{Cl} \)-originated SCC for which the integrity of the entire cAMP pathway is necessary. This pathway is directly involved in the epithelial fluid secretion. Amphotericin B renders the apical membrane permeable to most ions exposing the properties of the Na-K pump. Thus, amphotericin B induces a Na-originated SCC only if the Na-K pump is functional.

The SCC response to epinephrine stimulation was completely inhibited in both the keratectomy control and experimental groups at 1 week following surgery. However, the responses were at normal levels by 1 month following surgery. These findings indicate that a defect exists in the early postoperative period in the pathway responsible for active chloride transport, which is mediated by the \( \beta \)-adrenergic receptors. The location of the deficiency may be at the level of the receptors themselves, the cAMP pathway, or due to an inhibition of the adrenergic nerves, which are known to innervate the anterior corneal tissue. In the keratectomized corneas, either trauma to the epithelium during surgery or a complete severing of the sensory nerves to the cornea may account for the physiological deficit. It is possible that a hypersensitivity response might exist in the first 1 or 2 days following surgery, but this was not investigated. Because the response was similar in both the control and experimental groups, we may conclude that the addition of the tissue preservation, freezing, and lathing procedures inflict little or no more damage upon the corneal epithelial transport system than simple keratectomy alone.

Our results indicate that the functional operation of the Na-K pump is not affected at 1 week by either the lamellar keratectomy or myopic keratomileusis. Certainly, these functions and the integrity of the epithelium itself may be adversely affected in the very early postoperative phase, although we made no attempt to measure them.

It cannot be concluded on the basis of this study that a greater than expected corneal thickness following myopic keratomileusis (C. Swinger, unpublished observations) can be accounted for on the basis of a decreased \( \beta \)-adrenergic system alone. As previously mentioned, increased interfibrillar dimensions between collagen fibrils may result from cellular destruction and alteration of the ground substance.

Our study documents, for the first time, that corneal surgery can adversely affect the physiology of the corneal epithelium. Further studies need to be performed to determine the exact nature of the defect and the types of surgical procedures producing such deficits.

**Key words:** cornea, keratectomy, keratomileusis, Cl transport, Na transport, epinephrine

From the Departments of *Ophthalmology and †Physiology & Biophysics of the Mount Sinai School of Medicine of The City University of New York, and the ‡Beth Israel Medical Center, New York, New York. Supported in part by the National Eye Institute, Grants EY03346, EY00160, and EY01867. Submitted for publication: August 7, 1985. Reprint requests: Casimir A. Swinger, MD, Beth Israel Medical Center, 10 Nathan D. Perlman Place, New York, NY 10003.

**References**


**Localization of Lectin Binding Sites in Human, Cat, and Rabbit Corneas**

Noorjahan Panjwani,*† Paul Moulton,* Joseph Alroy,‡ and Jules Baum*

Paraffin sections of human, cat, and rabbit corneas were stained with nine lectins, using an avidin-biotin-complex procedure to study glycoconjugates of the epithelium, keratocytes, and stromal matrix. Wheat germ agglutinin (WGA) stained plasma membranes of all epithelial cell layers of cat and human and superficial and wing cells of rabbit. Plasma membranes of superficial and wing cells of cat epithelium also stained with peanut agglutinin (PNA) and *Ricinus communis* agglutinin I (RCA-I). Human and cat keratocytes stained with WGA and RCA-I. Stromal matrices of all three species were...