In Vivo Assessment of Mechanisms Controlling Corneal Hydration

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The endothelial pump and evaporation components of corneal recovery were studied in the in vivo human cornea by inducing corneal swelling with the use of hypoxia and monitoring the subsequent decrease in corneal thickness. Corneal recovery follows a nonlinear time course with the rate of recovery decreasing as the cornea thins. Following 60 μm of induced edema, recovery with the eyes open required an average of 2.5 hr to reach baseline corneal thickness, while recovery with the eyes closed took an average of 4.0 hr to reach the normal physiologic corneal swelling (17 μm). Our analysis indicates that for open eye recovery from 60 μm of swelling, the endothelial pump provides 20%, while the osmotic thinning caused by tear evaporation contributes 80% of recovery. During recovery, the rate of water evaporation from the anterior corneal surface remained relatively steady at 2.5 μl/cm² × hr. Comparison of measured vs calculated recovery rates during recovery with the eyes closed suggests that the endothelial pump functions at one speed and that the “pump-leak” theory of corneal hydration control is applicable for the human cornea. Invest Ophthalmol Vis Sci 26:849–856, 1985

Control of corneal hydration is essential for the maintenance of normal transparency.1 A compromise in the control mechanisms, due to either a disease process or intervention, can lead to corneal decompensation and loss of vision.2,3

Both passive and active mechanisms function in control of corneal hydration. The active control of corneal hydration was first demonstrated by Davson4 and Harris and Norquist5 in the “temperature reversal” experiments in which excised rabbit cornea hydration increased with cooling and returned to normal upon rewarming. Further studies established the dependence of this phenomenon on metabolism;6 and isolated the fluid movement mechanism mainly in the endothelium,7 the epithelium having only a negligible pumping mechanism.8 The endothelial mechanism appears to function by the active transport of bicarbonate,9 sodium, and hydrogen ions.10,11 The pump is able to move a significant amount of fluid, 6.7 μl/cm² × hr, and appears to function at one speed.12 A description of the model for the endothelial pump mechanism appears in the literature.13,14

Passive factors that oppose the tendency of the stroma to swell15 and help control corneal thickness were demonstrated by Mishima and Maurice16 who showed that fluid evaporation from the anterior corneal surface can have a considerable thinning effect on corneal thickness. This evaporation increases tear osmolarity and contributes to the maintenance of normal hydration.17,18 Later work by Mishima and Hedbys19 measured the permeability of rabbit corneal epithelium and endothelium to water, showing that these membranes offered some passive resistance to the movement of water into the cornea caused by the stromal swelling pressure.

These active and passive mechanisms provide the basis for the “pump-leak” corneal hydration control theory of Maurice,20 which states that the fluid leaking into the cornea due to the negative stromal pressure is pumped back out by the active endothelial pump mechanism to maintain a constant corneal hydration. The passive (hydraulic) leak of fluid into the cornea appears to occur through the intercellular spaces,12,21 while the flow of fluid out (osmotic) is thought to be across the cell membranes.22,23 The flows through the intercellular pathway and across the cells would be equal when the eyes are closed; however, when the eyes are open the flows are unequal, with a transcorneal flow due to normal evaporation at the epithelial surface.24

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* Major, USAF. The views expressed in this article are those of the author and do not reflect the official policy or position of the Department of Defense of the U.S. Government.

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Table 1. Summary of selected ocular parameters for the 10 subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corneal thickness (μm)</td>
<td>509</td>
<td>24</td>
<td>472 to 551</td>
</tr>
<tr>
<td>Sphere ref. error (D)</td>
<td>-0.54</td>
<td>1.86</td>
<td>+0.75 to -6.25</td>
</tr>
<tr>
<td>Cylinder ref. error (D)</td>
<td>-0.36</td>
<td>0.48</td>
<td>0 to -1.75</td>
</tr>
<tr>
<td>Horiz. keratometry (D)</td>
<td>43.26</td>
<td>1.50</td>
<td>39.75 to 46.00</td>
</tr>
<tr>
<td>Corneal toricity (D)</td>
<td>0.60</td>
<td>0.47</td>
<td>0.37 against to 1.50 with</td>
</tr>
</tbody>
</table>

SD: Standard deviation.

Corneal Swelling

To increase corneal hydration, the anterior corneal surface was exposed to hypoxia by having the subjects wear a Bausch & Lomb U4 hydrogel lens (Bausch & Lomb, Inc; Rochester, NY) with an oxygen transmissibility (Dk/L) of 10.5 × 10−9 (cm/sec) (ml O2/ml × mmHg) for 3 hr with the eyes closed. This contact lens reduced the level of oxygen at the corneal surface to approximately 8 mmHg,27 below the minimum oxygen tension needed for normal metabolism.28 Corneal hypoxia results in increased stromal lactate, which creates an osmotic imbalance and causes corneal swelling.29

Procedure

Changes in corneal hydration were monitored by measuring central corneal thickness30 using a Haag-Streit pachometer (Haag-Streit; Waldwick, NJ) adapted to a biomicroscope and connected to an electronic digital pachometer unit. The pachometer design was similar to that described by Holden et al,31 with the angle between the slit beam and ocular set at 75 degrees to increase sensitivity. Each measurement included 10 readings with a standard deviation of ±4.0 microns.

Baseline corneal thickness was measured at least 3 hr after awakening to eliminate any influence sleep may have on thickness.32 The hydrogel lens was worn on the right eye only, with the left eye serving as a control. After removing the lens, recovery of corneal thickness was monitored for 4 hr. To maintain a constant environment, the subjects remained in the test area throughout each session.

Experiment 1: When the eyes are closed (eg, sleep) the corneal thickness increases, which is thought to be due to a shift in tear tonicity.17-33 The level of normal physiologic closed eye corneal swelling for these subjects was measured after 3 and 6 hr of eye closure when no lens was worn.

Experiment 2: Corneal recovery from induced swelling was monitored with the eyes open, after removing the test lens, by measuring corneal thickness every 15 min for the first hour and each one-half hour for the next 3 hr. On a separate day, the same subjects again had corneal edema induced using the test lens; however, in this session recovery after lens removal was monitored with the eyes closed. Corneal thickness measurements were made for 4 hr at 1-hr intervals with the eye open for 30 sec to allow measurement.

Experiment 3: The difference in recovery rates between opening the eye every hour (Experiment 2)

Materials and Methods

Subjects

Ten subjects, (1 woman, 9 men; mean age, 26.7 ± 4.8 yr; range, 23–37 yr) who were free of ocular disease and had no prior contact lens experience (unadapted), participated in the study. Informed consent was obtained from each subject. A summary of relevant ocular parameters is listed in Table 1.
for 3 hr and after 3 hr with the eye remaining closed was assessed. There was no difference for these two conditions, indicating that opening the eye for a brief period (30 sec) every hour did not affect the time course of recovery with the eyes closed.

**Experiment 4:** Corneal recovery with the eyes closed eliminates the effect of evaporation on recovery; however, the decreased level of oxygen \( (PO_2 \ 55 \text{ mmHg}) \) available from the conjunctiva\(^{14}\) during eye closure might affect the rate of recovery. These effects on recovery were evaluated using six additional subjects. Corneal swelling was induced in both eyes and following lens removal, recovery was monitored with one eye closed and the contralateral eye open, while the subject stayed in a room having 100% humidity and normal \( (PO_2 \ 155 \text{ mmHg}) \) oxygen tension.

**Experiment 5:** The effect of hypoxia (and presumably lactate\(^{29}\)) on the rate of recovery was assessed by inducing the same level of corneal swelling (60 \( \mu \text{m} \)) under two different conditions. First, edema was induced with the Bausch & Lomb U4 hydrogel lens worn during eye closure; and secondly, by wearing a thick hydrogel lens (38% H\(_2\)O, 0.65 mm thick, Dk/L = 0.2 \( \times 10^{-3} \) (cm/sec) (ml O\(_2\)/ml \times mmHg) with the eyes open. This paradigm was chosen since it is believed that corneal swelling induced during eye closure includes both a hypoxic and osmotic component,\(^{35}\) while swelling induced with the eyes open is primarily due to hypoxia. Although lactate levels were not directly measured, it may be assumed that more lactate is in the stroma for hypoxic swelling induced with the eyes open than when the same level of swelling is induced with the eyes closed. If recovery rates from these two hypoxic conditions are the same, then presumably hypoxia and the excess stromal lactate does not affect the dehydration mechanism.

Figure 1 shows the decrease in central corneal swelling over time after lens removal (recovery) with the eyes open following edema induced with hypoxia created both with the eyes closed and with the eyes open. There was no difference in the time course of recovery from the same level of edema induced with the eyes closed or open, indicating that inducing edema using hypoxic stress (presumably due to increased stromal lactate) has no effect on the recovery mechanism.

**Recovery Due To Endothelial Pump**

Calculation of the rate of fluid flow out of the cornea due to the endothelial pump was made based upon the calculated stromal swelling pressure and a steady state endothelial pump rate, using the following formula:

\[
\text{Recovery flow} = \frac{\text{IP}_{\text{swell}} - \text{IP}_{\text{clsd}}}{\text{IP}_{\text{clsd}}} \times \text{Pump Rate} \quad (1)
\]

where \( \text{IP}_{\text{swell}} \) and \( \text{IP}_{\text{clsd}} \) are the stromal imbibition pressures at the mean swelled and closed eye corneal thicknesses, respectively; and the endothelial Pump Rate is taken as 6.7 \( \mu\text{l/cm}^2 \times \text{hr} \), as reported in the rabbit by Baum et al\(^{12}\) for the rate of fluid transport at zero applied transtissue hydrostatic pressure.

This formula states that the amount of decrease in the fluid leak into the cornea equals the net fluid flow out due to the pump and can be calculated from the fractional change in imbibition pressure times the endothelial pump rate. This calculation includes the following assumptions:

1. The water leakage rate into the cornea decreases as the cornea swells and this decrease is directly proportional to the reduction in swelling pressure that occurs with greater stromal hydration.\(^{13}\) The difference in this decreased leak and the endothelial pump rate is the rate at which fluid should flow out of the cornea due only to the endothelial pump.
2. The steady state endothelial pump rate is the fluid flow out of the cornea that equals the fluid leakage in due to the stromal imbibition pressure (IP), to maintain the cornea at the closed eye thickness.\(^{24}\) The difference between the closed and open eye corneal thickness is due to osmotic thinning caused by tear evaporation,\(^{17,18}\) with the cornea re-
Fig. 2. Mean decrease in central corneal swelling vs time for 10 subjects during recovery with the eyes open (open circles) and closed (filled circles) following corneal swelling induced with the eyes closed. Error bars equal ±1 SD and lines were fitted by polynomial equation.

Receiving adequate oxygen to prevent hypoxia during eye closure. The imbibition pressure (IP) is the stromal swelling pressure (SP) minus the hydrostatic compressive effect of the intraocular pressure (IOP = 18 mmHg). The swelling pressure was calculated using the relationships of corneal thickness to hydration, and then hydration to stromal swelling pressure, in which:

\[ H = (7.0 \times CT) - 0.64 \]  

and

\[ SP = 1.555e^{-11} \]  

where \( H \) is gram water/gram dry weight and \( CT \) is corneal thickness in millimeters. The swelling pressure equation shown was derived by reploting the data points of Hedbys and Dohlman and Fatt and Hedbys for human stroma onto a semilogarithmic scale to demonstrate the linear relationship (\( r = 0.965 \)). For our group mean physiologic closed eye corneal thickness of 526 \( \mu \)m, the calculated imbibition pressure is IPclsd = 56.2 mmHg.

The rate of water leakage into the cornea is also related to the hydraulic conductivity (Lp) of the membranes and the pressure gradient across them. However, a number of Lp values have been reported for the corneal endothelium, with large differences between the hydraulically, osmotically, and mathematically determined Lp values. Therefore, as suggested by Burns et al., the rate of water leakage into the cornea was not calculated using the membrane hydraulic conductivity. To eliminate this factor from the calculations, the Lp values of all subjects in this study are taken as equal and normal.

Results

Figure 2 compares the decrease in central corneal swelling over time (recovery) with the eyes open from 60.1 ± 6.2 \( \mu \)m to recovery with the eyes closed from 59.1 ± 6.4 \( \mu \)m of induced swelling. Corneal recovery with the eyes either open or closed follows a nonlinear time course with the rate of recovery decreasing as the cornea thins. When the eyes remained closed during recovery, the time to reach the normal physiologic closed eye swelling, 17.2 ± 4.1 \( \mu \)m (dotted line), was approximately 4.0 hr. When recovery was monitored with the eyes open, the time to reach this same level of edema (17 \( \mu \)m) was 1.25 hr, while the baseline corneal thickness was reached in 2.5 hr. During recovery with the eyes open, the cornea became thinner than baseline (overshoot) by approximately 2–3 \( \mu \)m for 30–90 min during the last 1.5 hr of measurement.

The recovery curves were fitted by the method of least squares to third order polynomial equations as follows:

\[ CS = 60.1 - 18.8t + 2.1t^2 - 0.02t^3 \]  

for recovery with the eyes closed (\( n = 50 \), \( r = 0.9704 \)); and

\[ CS = 59.1 - 46.4t + 11.4t^2 - 0.9t^3 \]  

for recovery with the eyes open (\( n = 110 \), \( r = 0.9881 \)); where \( CS \) is the corneal swelling in microns and \( t \) is the time in hours since lens removal (recovery).

The influence of the lower level of oxygen (PO\textsubscript{2} 55 mmHg) on recovery with the eyes closed was assessed on six additional subjects. Figure 3 shows the decrease in corneal swelling for the eye remaining closed and the contralateral eye that was open and exposed to a 100% humidified environment (PO\textsubscript{2} 155 mmHg). Differences in the time course of recovery for these two conditions were small (approximately 1 \( \mu \)m/hr). These data indicate that the slower recovery rate when the eyes are closed, as compared to when the eyes are open, is not influenced by the reduction in oxygen that occurs with eye closure; but, rather, is a result of the difference between recovery in a 100% humidified and in a normal (60%) humidity open eye environment.

The difference in the slower “leak” for the swelled cornea and the endothelial pump rate is the rate at which fluid should move out of the cornea due only to the endothelial pump when evaporation is elimi-
Comparison of the calculated fluid flow out of the cornea due to a steady state pump (see Materials and Methods) and the measured flow, obtained using the first derivative of equation 4 from Figure 2, during recovery with the eyes closed (ie, without evaporation) is shown in Table 2. There is close agreement between the calculated and measured fluid flow out of the cornea throughout the entire recovery phase. The slight difference in flows, 0.2 µl/cm² × hr (2 µm/hr rate of recovery), is within the experimental error of pachometric measurement. This finding suggests that recovery with the eyes closed is due to the endothelial pump and that the pump appears to function at one speed.

This finding also indicates that the rate of recovery due only to the endothelial pump (during eye closure) is directly related to the amount of swelling remaining. Therefore, by comparing the recovery rates for the open and closed eye at corresponding levels of swelling, it is possible to separate the evaporation and pump components of recovery. The rates of recovery with the eyes open or closed were calculated using the first derivative of the equations derived from the data in Figure 2. The recovery rates were calculated at 15-min intervals during recovery with the eyes open, and at corresponding levels of swelling remaining during closed eye recovery, and are shown in the upper and lower curves of Figure 4. For example, after 30 min of recovery with the eyes open, the amount of swelling remaining is 38 µm and the rate of recovery is 36 µm/hr; while for recovery with the eyes closed, the recovery rate is only 12 µm/hr at this same 38-µm level of remaining edema.

This analysis provides a comparison of recovery rates for corneas at the same level of stromal fluid pressure during both recovery conditions. At any level of swelling remaining, the rate of recovery with the eyes open was faster than that with the eyes closed. Subtraction of the rate of recovery with the eyes closed from the recovery rate with the eyes open, provides quantification of the osmotic effect of tear evaporation on recovery at any level of swelling (middle curve in Fig. 4). These calculations show that the evaporation component of recovery remained steady at approximately 25 µm/hr until the normal level of closed eye swelling (17 µm) was reached; at this point, the rate of recovery decreased as the cornea approached baseline thickness.

The recovery rates for the endothelial pump (ie, closed eye) in Figure 4 were used to calculate the decrease in corneal swelling during open eye recovery that is due to the pump, with the remainder due to evaporation. Each data point in Figure 4 corresponds to the rate of recovery at the level of swelling remaining at 15-min intervals during open eye recovery. The average rate of recovery for each interval was used to compute the corneal thickness decrease due to the pump. For example, the initial closed eye recovery rate at 60 µm of swelling was 19 µm/hr; while after 15 min of open eye recovery, the corneal

<table>
<thead>
<tr>
<th>Recovery time (hr)</th>
<th>Corneal swelling (µm)</th>
<th>Calculated imbition pressure (mmHg)</th>
<th>Fractional decrease in IP</th>
<th>Recovery due to PUMP* (net outflow) (µl/cm² × hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>60.1</td>
<td>36.9</td>
<td>0.34</td>
<td>2.2</td>
</tr>
<tr>
<td>1.0</td>
<td>43.4</td>
<td>43.8</td>
<td>0.22</td>
<td>1.5</td>
</tr>
<tr>
<td>2.0</td>
<td>30.7</td>
<td>49.5</td>
<td>0.12</td>
<td>0.8</td>
</tr>
<tr>
<td>3.0</td>
<td>22.1</td>
<td>53.7</td>
<td>0.05</td>
<td>0.4</td>
</tr>
<tr>
<td>4.0</td>
<td>17.2</td>
<td>56.2</td>
<td>0</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* Recovery due to pump = Fractional decrease in imbition pressure (IP) × Pump Rate. Pump Rate = 6.7 µl/cm² × hr.

Fig. 3. Mean decrease in central corneal swelling vs time for 6 subjects during recovery with the eyes closed (filled circles), and with the eyes open in 100% humidity (open triangles) and 60% humidity (open circles) environments. Corneal swelling was induced with the eyes closed. The standard deviation for each data point ranged from 1.1 to 3.7 µm, with mean SD = 2.2 ± 0.7 µm.
Central Corneal Swelling (urn)

Fig. 4. Rate of recovery vs mean central corneal swelling (remaining edema) with the eyes open (open circles) and closed (filled circles). The evaporation component of recovery (open triangles) is the difference between the open and closed eye rates of recovery at corresponding levels of swelling calculated for the amount of swelling remaining at 15-min intervals during open eye recovery.

swelling had decreased to 48 μm, with the corresponding recovery rate due to the pump now 16 μm/hr. This gives an average recovery rate of 17.5 μm/hr and 4.4 μm of recovery due to the pump during the first 15 min of open eye recovery.

Repeating this process for each 15-min period until the normal closed eye corneal swelling is reached (at which point the pump no longer contributes to recovery with the eyes open), gives an additional 3.5, 2.6, 1.8, and 0.7 μm of recovery due to the pump. These calculations indicate that the endothelial pump contributed 13 μm (20%), while evaporation contributed 47 μm (80%) of the total recovery from this (60 μm) level of swelling. A comparison of the relative contribution of the endothelial pump and evaporation to recovery with the eyes open from 60 μm of induced swelling is shown in Figure 5.

Discussion

Corneal recovery from induced edema follows a nonlinear time course, with the rate of recovery decreasing as the cornea thins. The rate of recovery was considerably faster with the eyes open than with the eyes closed. Our analysis of these recovery rates suggest that recovery from edema with the eyes open includes the effect of tear evaporation from the anterior corneal surface and the endothelial pump, and that when the eyes are closed the endothelial pump is primarily responsible for removing the excess fluid.

We found no difference in the rate of recovery with the eyes open following edema induced with the eyes open or closed. This finding indicates that the use of hypoxic stress to induce corneal edema does not affect the subsequent corneal recovery. The time course of recovery was similar for the open eye exposed to a 100% humidity environment (ie, no evaporation, PO2 155 mmHg) compared to the closed eye; which indicates that the normal decrease in oxygen with the eyes closed (PO2 55 mmHg) does not affect recovery. This finding also indicates that the differences in recovery rates under closed and open eye conditions is due to the environment humidities (100% humidity with the eyes closed vs 60% humidity with the eyes open), which would result in different tear film osmolarities.

Our analysis indicates that for open eye recovery from 60 μm of edema, the endothelial pump provided approximately 20% of the recovery, while the remaining 80% was contributed by the osmotic thinning effect of tear evaporation. However, the contribution of each component is related to the initial level of swelling, since recovery from the first 17 μm of swelling is entirely due to evaporation in our analysis. For example, the calculated contribution of the endothelial pump to recovery is 30, 26, and 13% from 90, 75, and 40 μm of initial corneal swelling, respectively. This suggests that for the normal cornea that
undergoes edema, the osmotic thinning effect of tear evaporation is an important and substantial function in returning the cornea to normal hydration.

The difference between the recovery rates (open and closed eye) at corresponding levels of swelling were used to calculate the rate of recovery due to the osmotic effect of tear evaporation. These calculations indicate that the net flow of fluid out of the cornea due to evaporation remained constant at approximately 2.5 μl/cm² × hr until the cornea approached the normal physiologic closed eye swelling (17 μm), at which point the net flow out decreases. This fluid flow value for the effect of evaporation is in agreement with that found by Mishima and Maurice for the rabbit cornea.

We also noted that the cornea became thinner (overshoot) than the baseline corneal thickness during the last 1.5 hr of open eye recovery. There is normally a transcorneal fluid flow due to evaporation at the epithelial surface, and the transient overshoot we observed during recovery might be due to a disparity between the rate of water removal from the anterior stroma due to evaporation and the fluid movement inward thru the endothelium. The cornea might be expected to be thinner than normal until the normal transcorneal flow is reestablished.

To assess changes in endothelial pump rate for our subjects we compared the measured and calculated fluid flow out of the cornea attributable to the pump. This flow is based on the calculated fractional decrease in stromal imbibition pressure that occurs with increased corneal hydration and a reported baseline endothelial pump rate, 6.7 μl/cm² × hr, for the rabbit endothelium at zero transtissue hydrostatic pressure. The baseline pump rate may also be obtained by extrapolation on the data in Figure 2 of Fischbarg and Warshavsky, which yields a value of about 8 μl/cm² × hr and gives calculated recovery rates similar to those we report. The degree of correspondence between the measured and calculated fluid outflows is dependent upon the limitations of our assumptions and the apparent pump rate employed in the calculation; however, within the variance of the pachometer measurement technique, our conclusions appear to be appropriate.

Comparison of the calculated fluid flow out due to a steady state endothelial pump rate to the measured flow during recovery with the eyes closed (ie, when evaporation was completely eliminated) showed good correspondence (within 2 μm/hr recovery rate) throughout the entire recovery phase. This finding suggests that the endothelial pump functions at one speed regardless of the level of corneal hydration, which is in agreement with the results of Baum et al for the rabbit cornea. These recovery data for the in vivo human cornea also fit the rate of recovery predicted by the "pump-leak" theory of corneal hydration control.

Our findings indicate that inducing corneal edema using hypoxic stress and monitoring the subsequent recovery may provide a clinical test to assess endothelial function. Additional data from a larger section of the population, as well as measurements on corneas with endothelial disease, is needed to validate this test. Further studies of this method to provide a clinical test of endothelial function are presently under investigation.

Key words: cornea, corneal thickness, corneal recovery, endothelial pump, evaporation, pump-leak

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References