In order to determine which layers of the corneal stroma bear the stress of the intraocular pressure, 6.0-mm nonpenetrating trephine incisions were made centrally in one eye of each of 16 adult albino rabbits. After epithelial healing, the central corneal thickness was measured over 3 hr at 50 mmHg intraocular pressure in both eyes of the anesthetized rabbits. The animals were then killed and the uniformity and depth of trephine cut determined histologically. The mean differences in swelling rates between the cut and the opposite uncut eyes for trephine incisions of different depths were as follows: 1 ± 2 \mu m/hr for 8–20% depth, 5 ± 2 \mu m/hr for 21–40% depth, and 14 ± 3 \mu m/hr for 41–60% depth (P < 0.01 for all groups). These results indicate that the intraocular tension is probably distributed across all the corneal stromal lamellae rather than being borne primarily by the anterior or posterior layers. Invest Ophthalmol Vis Sci 26:869–872, 1985

Although the epithelial and endothelial cell layers of the cornea offer resistance to the flow of water, they have little structural rigidity to support the tension borne by the cornea. Therefore, the corneal stroma, with more than 200 superimposed lamellae, must bear the force of the hydrostatic pressure gradient that exists across the cornea. Whether this gradient is spread evenly across the full thickness of the cornea or is borne primarily by the anterior or posterior lamellae is unknown and is crucial to an understanding of the effects on corneal curvature of surgical procedures that disrupt the continuity of these lamellae. This study was designed to determine which corneal stromal layers support the force of the intraocular pressure.

When the intraocular pressure is acutely elevated, one would expect increased fluid movement across the hydrostatic pressure gradient in the cornea until a new equilibrium is established. If the force of the intraocular pressure were borne by only a portion of the corneal stromal lamellae, the pressure gradient would be distributed only across these lamellae, and they would be the site of increased fluid movement. Consider three hypotheses (Fig. 1): (1) If the force of the intraocular pressure were borne by the anterior stromal layers, when the intraocular pressure is acutely elevated more fluid would move across the gradient from the central corneal lamellae to the epithelium. Fluid would not be replaced to the central corneal lamellae from the aqueous humor or posterior cornea as rapidly as it is lost anteriorly because the large pressure gradient does not exist posteriorly. The cornea would thin. A partial-thickness anterior trephine cut would shift the tension of the intraocular pressure toward the most anterior stromal lamellae which remain intact, which would then bear the stress. An acute elevation of pressure would force fluid into the anterior (cut) layers where the gradient decreases and fluid flow slows, resulting in swelling of these layers. (2) If all stromal lamellae supported the pressure, an acute elevation of pressure would cause increased fluid movement across the stroma, but the thickness probably would not change substantially. Partial trephination would shift the gradient posteriorly to the remaining uncut layers, and fluid would flow into the anterior (cut) layers, which would swell proportional to the depth of cut. (3) If the pressure were supported by the posterior corneal layers, acutely elevated pressure would move fluid across this posterior gradient into the central corneal stroma more rapidly than it would leave the cornea anteriorly, where no large gradient would exist. The cornea would swell. Cutting the anterior half of the cornea would not affect the gradient because the tension-bearing layers would still be intact; no increased central swelling would result. In each case the peripheral cornea would be expected to respond like the intact cornea because some of the peripheral lamellae running limbus to limbus would remain uncut.

Materials and Methods

All procedures described herein conform to the ARVO Resolution on the Use of Animals in Research. Nonpenetrating central trephine incisions (6.0 mm)
HYPOTHESIS

UNCUT CORNEA

CUT CORNEA

(50% depth)

#1

Anterior Lamellae
Bear Stress

Thinning

Increased Central Swelling

#2

All Lamellae
Bear Stress

Minimal or No Swelling

Slightly Increased Swelling

Proportional to Cut

#3

Posterior Lamellae
Bear Stress

Swelling

No Increased Central Swelling

Fig. 1. Effects on corneal stromal thickness of acutely elevated intraocular pressure: three hypotheses. The hypothetical effects on corneas with partial-thickness trepine cuts is also illustrated.

of various depths were made in one eye of normal albino rabbits weighing 2.4–3.5 kg. Preoperatively, each rabbit received xylazine (5 mg/kg) and ketamine (30 mg/kg) intramuscularly for anesthesia and an aspirin suppository (300 mg). Four to 8 days later, when the wounds had epithelialized, the intraocular pressure was raised to 50 mmHg by 23-gauge needles inserted into the vitreous cavities of both eyes and attached to elevated reservoirs of balanced salt solution; this pressure was constant for the remainder of the experiment. General anesthesia was maintained with sodium pentobarbital intravenously. Each animal’s head was fixed in a frame. Rabbits with wounds that did not epithelialize within 8 days, which included all those with trephine cuts greater than 60% depth, were eliminated from the study.

The central and peripheral (temporal) corneal thicknesses in both eyes were measured ultrasonically every 15 min for 3 hr; each reading was the average of three measurements. The ultrasonic transducer cap was filled with 2% agar. Weighted lid sutures maintained lid closure between measurements. After 3 hr, the rabbits were killed and the eyes were enucleated and fixed in formalin. Histologic sections were made of each quadrant of each corneal wound; the fractional depth of cut in each quadrant was measured with a microscopic reticle and the results averaged for each cornea. Eyes with variations between quadrants of greater than 25% depth were eliminated from the series.

Three rabbits were used to determine the effect of the 23-gauge needle inserted into the vitreous cavity. Neither eye was trephined. Under general anesthesia with sodium pentobarbital, a needle was inserted in one eye of each rabbit, and the pressure was maintained at 20 mmHg in that eye while central corneal thickness measurements were made as above every 15 min on both eyes for 3.5 hr.

The central swelling rate for each cornea during the 3-hr period of elevated intraocular pressure was determined by linear regression analysis. The results were analyzed with the Wilcoxon and Spearman tests; a probability of less than 0.05 was considered to be statistically significant.

Results

Sixteen rabbits completed the study (Table 1). Prior to raising the intraocular pressure, the average difference in central corneal thickness between experimental and control eyes was 11 μm; only two rabbits had differences greater than 20 μm. The control corneas of the deep incision group were significantly thicker than those of the superficial incision group (389 vs 359 μm, respectively; P < 0.001).

The 16 control eyes had a mean swelling rate of 24 μm/hr. The swelling rate for these eyes during the third hour (26 ± 12 μm/hr) was not statistically significantly more than that during the first hour (21 ± 14 μm/hr). There were no significant differences in swelling rates between the three groups of control eyes, although those in the groups with deeper incisions tended to swell more. In the three rabbits used to test the effect of a needle in the vitreous cavity, corneal swelling rates in eyes without needles averaged 1.9 μm/hr and eyes with needles averaged 2.3 μm/hr.

Table 1. Corneal swelling rates at 50 mmHg intraocular pressure*

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Initial corneal thickness (μm)</th>
<th>Corneal swelling rate (μm/hr)</th>
<th>Corrected swelling rate (μm/hr)</th>
<th>Significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial incision</td>
<td>5</td>
<td>Experimental eye 366 ± 22</td>
<td>21 ± 11</td>
<td>1 ± 2</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control eye 359 ± 23</td>
<td>20 ± 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium incision</td>
<td>6</td>
<td>Experimental eye 382 ± 40</td>
<td>28 ± 6</td>
<td>5 ± 2</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control eye 372 ± 45</td>
<td>23 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deep incision</td>
<td>5</td>
<td>Experimental eye 406 ± 27</td>
<td>42 ± 7</td>
<td>14 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control eye 389 ± 9</td>
<td>28 ± 7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± standard deviation.

† Experimental eye minus control eye.
The five eyes with superficial incisions (8–20% stromal depth) showed a mean central swelling rate of 1 μm/hr more than their control eyes (Table 1). The six eyes with medium incisions (21–40% stromal depth) swelled at a mean rate of 5 μm/hr more than their control eyes. The five eyes with deep incisions (41–60% stromal depth) had a mean swelling rate of 14 μm/hr more than their control eyes. These swelling rates were all significantly different from each other and positively correlated with incision depth \( (p = 0.930, P < 0.001) \) (Fig. 2). A significant positive correlation \( (p = 0.700, P < 0.01) \) also was present when the swelling rates were corrected for incision depth (Fig. 3), indicating that the increased swelling with deep cuts was proportionally more than the increase in cut stromal lamellae.

Although central corneal thickness measurements were quite reproducible, peripheral corneal measurements were not. The normal corneas thinned peripherally as much as 20 μm before they thickened again closer to the limbus. Nevertheless, we attempted to measure the peripheral corneas at the same site each time. There was no significant difference in the peripheral swelling rates in the cut (20.7 μm/hr) and uncut (20.5 μm/hr) eyes (paired analysis, \( P > 0.5 \)).

**Discussion**

These results support our basic analysis that when the intraocular pressure is acutely elevated, fluid will move across the corneal stromal lamellae that support the pressure. Thus, in the presence of a deep trephine cut, when only the posterior corneal lamellae are intact and must of necessity support the elevated pressure, the anterior stroma swells. Conversely, when only the anterior lamellae are intact, our analysis dictates that the cornea should thin. Although posterior trephine cuts cannot be performed, a full-thickness trephination closed anteriorly by sutures (penetrating keratoplasty) creates a situation in which the anterior stromal lamellae support the increased intraocular pressure. Thus Olson and Kaufman found that increased intraocular pressure thins the cornea shortly after penetrating keratoplasty in humans.\(^5\) Because in the present study the uncut corneas did not thin and also did not swell like the cut corneas, neither the anterior nor the posterior corneal lamellae alone support the stress of the intraocular pressure. Furthermore, it is likely that in the normal cornea the stress is borne by all lamellae because deeper cuts produced greatly increased swelling (Fig. 2).

These conclusions apply only to corneas of normal thickness. When stromal swelling occurs, the increase in corneal thickness must be in a posterior direction, slackening the posterior lamellae; the anterior lamellae, therefore, must bear the stress of the intraocular pressure when the corneal thickness is increased. This relationship may constitute a compensatory mechanism for the regulation of corneal thickness: As the cornea swells, the stress of the intraocular pressure shifts anteriorly, with the consequent tendency for thinning. This relationship may also explain Moses’ finding that external indentation of the corneas of enucleated human eyes pulls the anterior layers taut.\(^6\)

Our results indicate that corneal thickness increases when the intraocular pressure is increased to 50 mmHg (16 uncut corneas at 50 mmHg swelled an average of 24 μm/hr whereas three uncut corneas at 20 mmHg swelled an average of 2.3 μm/hr). This finding agrees with that of Olsen, who studied human eyes during acute glaucomatous attacks.\(^7\) In contrast, many investigators have found that increased intraocular pressure thins corneas in vitro.\(^8-11\) These in
vitro studies, however, used swollen corneas in which the intraocular pressure must be borne anteriorly, and a decrease in corneal thickness would be expected with increased pressure (see above). Ehlers found an inverse relationship between intraocular pressure and corneal thickness in patients with retinal detachments and with glaucoma. These chronic changes in humans may involve different mechanisms than the acute changes in rabbits that we observed. Ytteborg and Dohlman also studied rabbit eyes in vivo and found no change in corneal thickness for over 2 hr at 70 mmHg intraocular pressure, as predicted by Maurice. We cannot explain the discrepancy between their results and our finding of an increase in corneal thickness of approximately 7% per hour at 50 mmHg intraocular pressure. The theoretical model of Berkley, which takes into consideration inward force vectors resulting from the curvature of the cornea, also predicts an increase in corneal thickness (albeit of less magnitude than we found) under these experimental conditions.

We found a significant difference between the corneal thicknesses of the opposite unoperated eyes in the deep and superficial stromal incision groups. These measurements were taken before elevation of the intraocular pressure on the second experimental day. The rabbits in the two groups were of similar sizes (mean weight = 2.93 kg in deep incision group and 2.97 kg in superficial incision group, P > 0.5). Unfortunately, we did not measure the corneal thicknesses before the experimental eyes were trephined. This increased stromal thickness in the contralateral eyes of the rabbits with deeper corneal incisions may be a spurious finding. If not, it represents an effect on the contralateral eye of a corneal incision in the ipsilateral eye. Such a contralateral effect is not unknown. Maul and Sears have shown disruption of the blood–aqueous barrier in the contralateral eyes of rabbits after stimulation of the ipsilateral trigeminal nerves. The disruption was not prevented by pretreatment with indomethacin.

Key words: corneal stroma, corneal lamellae, corneal stress, corneal trephine, corneal thickness

References