The effects of increased hydrostatic pressure upon normal and regenerated rabbit corneal endothelium. WILLIAM H. SCHUT-TEN AND DIANE L. VAN HORN.

The effect of increased hydrostatic pressure upon the ability of normal and regenerated endothelium to deturgescence was studied. Stromal deturgescence occurred as a biphasic response when hydrostatic pressure at the endothelial surface was increased above baseline values. Initially there was a rapid phase of stromal thinning which was dependent upon hydrostatic pressure and endothelial function. This was followed by a slower phase of corneal thinning which was independent of hydrostatic pressure at the endothelial surface for pressures between 15 and 50 mm Hg. The slow phase of thinning represents the steady-state ability of the endothelium to deturgescence the stroma. Regenerated rabbit endothelium functioned similarly to normal endothelium in deturgescing the stroma. In addition, short-term increases in hydrostatic pressure at the endothelial surface did not produce ultrastructural changes in normal or regenerated corneal endothelial cells.

The specular perfusion microscope provides an accurate method for the determination of alterations in corneal thickness due to incomplete perfusion media,\(^1\) drugs,\(^2\) and variations in hydrostatic pressure.\(^3\) Using the specular microscope, Bowman and Green\(^4\) reported that swollen, denuded corneas deturgescence more slowly as hydrostatic pressure at the endothelial surface is increased from 5 to 50 mm Hg. In contrast, Hodge\(^5\) reported that a pressure change from 6 to 37 mm Hg has no effect on the rate of corneal deturgescence.

Elevated intracocular pressure has been shown to produce ultrastructural damage during perfusion of the endothelium in the vervet monkey.\(^5\) However, hydrostatic pressures as high as 60 to 80 mm Hg have been reported to produce no detectable endothelial damage in perfused rabbit corneas.\(^6\)

In light of these conflicting results, the purpose of the present paper is to describe the effects of increased hydrostatic pressure upon the function and ultrastructure of normal and regenerated rabbit corneal endothelium during in vitro perfusion.

**Methods.** New Zealand white rabbits (1.5 to 2.0 kg) without corneal defects were sedated with ketamine HCl (20 mg/kg). Following topical application of 0.4% benoxinate HCl, a 5 mm diameter probe cooled in liquid nitrogen (−196°C) was applied to the central region of the right cornea for 15 sec. This is sufficient to destroy all the endothelial cells in the area of the probe.\(^7\) The left cornea was not frozen. The endothelium of the frozen cornea was considered regenerated when corneal thickness returned to prefreezing levels (about 7 days).

**Preparation of corneas for perfusion.** The corneal epithelium was removed by gently scraping the corneas with a Gill knife. The corneas were then dissected from the globes and mounted in a specular microscope pressure-perfusion apparatus.\(^8\) The endothelial surfaces were perfused with glutathione-bicarbonate-Ringer solution without adenosine at a rate of 0.097 ml/min at 37°C.\(^9\)

**Induction of stromal swelling.** The de-epithelialized surface of the corneas was exposed to 0.9% NaCl for 60 min to induce stromal swelling.\(^10\) During this time, the hydrostatic pressure at the endo-

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**Key words:** lysosomal enzymes, tear fluid, lacrimal gland, acetylsalicylic acid

**REFERENCES**

Results. Exposure of the de-epithelialized surface of the rabbit corneas to 0.9% NaCl for 60 min resulted in approximately a twofold increase in corneal thickness. Stromal swelling did not vary significantly between corneas with normal or regenerated endothelium. When the hydrostatic pressure at the endothelial surface was adjusted to 15, 30, 40, or 50 mm Hg, stromal thinning was found to occur as a biphasic response in corneas with normal or with regenerated endothelium (Figs. 1 and 2). During the first 60 min, a rapid phase of corneal thinning occurred. The rate of stromal thinning during the rapid phase was proportional to the hydrostatic pressure to which the endothelium was exposed. Higher hydrostatic pressures resulted in a significantly greater rate of corneal thinning than lower hydrostatic pressures (Table I).

During the second 2 hr of the perfusion, a slower rate of corneal thinning occurred which was independent of hydrostatic pressure at the endothelial surface. No significant differences (p < 0.05) in slow-phase deswelling rates occurred in normal or regenerated endothelium at any of the pressures tested. There was no significant difference (p < 0.05) in the deswelling rates of corneas with normal endothelium vs rates of corneas with regenerated endothelium during either the rapid
Table I. Deswelling rates ± S.E.M. (µm/hr)

<table>
<thead>
<tr>
<th>Pressure (mm Hg)</th>
<th>Rapid phase (0-1 hr)</th>
<th>Slow phase (1-3 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Regenerated</td>
</tr>
<tr>
<td>15</td>
<td>135 ± 10</td>
<td>156 ± 25</td>
</tr>
<tr>
<td>30</td>
<td>210 ± 14</td>
<td>208 ± 12</td>
</tr>
<tr>
<td>40</td>
<td>236 ± 14</td>
<td>263 ± 28</td>
</tr>
<tr>
<td>50</td>
<td>305 ± 33</td>
<td>272 ± 39</td>
</tr>
</tbody>
</table>

At all pressures tested, there was no significant difference (p < 0.05) in the deswelling rates between normal and regenerated endothelium.

or the slow phase of stromal thinning for pressures between 15 and 50 mm Hg.

SEM demonstrated that endothelial regeneration was complete at the time of enucleation in corneas which had received transcorneal freezing. No evidence of endothelial disruption or vacuolization was present in any of the corneas. TEM confirmed that both normal and regenerated corneal endothelium were ultrastructurally unaffected by the elevated hydrostatic pressures.

A second type of biphasic response was observed when the deswelling rates of de-epithelialized, de-endothelialized corneas were compared to those of de-epithelialized corneas. Although the de-epithelialized corneas exhibited the expected biphasic response described earlier, the de-epithelialized, de-endothelialized corneas had an initial rapid phase of corneal thinning during the first 60 min followed by a phase of stromal swelling during the second 2 hr (Fig. 3). The rate of corneal thinning during the first 60 min was less rapid in de-endothelialized corneas than in corneas with an intact endothelium.

Discussion. The rate of stromal deturgescence in preswollen, de-epithelialized corneas subjected to an elevated hydrostatic pressure at the endothelial surfaces is a biphasic function consisting of an initial rapid phase of stromal thinning lasting approximately 60 min followed by a slower phase of thinning. The rapid phase of stromal thinning is a pressure-dependent response, whereas the slow phase of stromal thinning is independent of hydrostatic pressure for pressures between 15 and 50 mm Hg. There is no significant difference between the slow-phase corneal thinning rates at any of the elevated pressures (30, 40, 50 mm Hg) and the thinning rate of 15 mm Hg. In addition, at each pressure tested, no significant difference is present in the slow-phase rates of thinning in corneas with normal vs. regenerated endothelium.

A functioning endothelium as well as elevated hydrostatic pressure contribute to the rate of stromal thinning during the initial rapid phase. This is evident because de-epithelialized, de-endothelialized corneas do thin during the first 60 min but not as rapidly as de-epithelialized corneas with an intact endothelium. After the initial 60 min, corneas which have been de-epithelialized and de-endothelialized begin to reswell, whereas de-epithelialized corneas with an intact endothelium continue to deturgescce, although at a slower rate. Therefore, during the slow phase, the endothelium alone is responsible for corneal deturgescence.

Bowman and Green3 reported that preswollen, de-epithelialized corneas thin more slowly as the hydrostatic pressure at the endothelial surface is increased. However, this study has demonstrated that once the cornea has adjusted to the new hydrostatic pressure (rapid phase), the rate of corneal thinning is independent of the hydrostatic pressure at the endothelial surface between 15 and 50 mm Hg (slow phase). There are several possible explanations for these differences. Since Bowman and Green did not preswell the cornea to the same extent as was done in this study, minor variations might be expected. More importantly, Bowman and Green used the same cornea for studying multiple pressures by alternating the hydrostatic pressure at the endothelial surface every 60 min. Data obtained during the second 30 min were used to calculate the deswelling rates of the corneas at the various pressures. The results of our study suggest that Bowman and Green probably determined corneal thinning rates while the corneas were still adjusting to the pressure change. By preswelling the corneas to a greater thickness and studying each cornea at only one hydrostatic pressure for the entire 180 min, the slow-phase thinning rates in the present study may more accurately reflect the effect of hydrostatic pressure upon the function of rabbit corneal endothelium.

SEM and TEM demonstrated ultrastructural integrity of the corneal endothelium at all pressures.
tested. This is consistent with results reported by Kaye et al., who found no changes in rabbit endothelium exposed to pressures of 60 to 80 mm Hg.

This study demonstrates that rabbit corneal endothelium functions normally in the presence of short-term hydrostatic pressure increases between 15 to 50 mm Hg. Regenerated rabbit endothelium functions similarly to normal endothelium in the presence of elevated hydrostatic pressure.

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Key words: cornea, corneal endothelium, intraocular pressure, rabbit

REFERENCES


Epithelialization of the corneal endothelium in posterior polymorphous dystrophy. MERYL M. RODRIGUES, TUNG-TIEN SUN, JAY KRACHMER, AND DAVID NEWSOME.

The unusual cell type present on the posterior corneal surface of posterior polymorphous dystrophy patients has been characterized. In addition to microvilli and desmosomes, these cells contain abundant 10 nm filaments which by immunofluorescent staining were shown to consist of keratin proteins, a marker for epithelial cells.

Posterior polymorphous dystrophy (PPMD) of the cornea is a disorder of the corneal endothelium usually associated with autosomal dominant inheritance. Clinically this entity is characterized by bilateral involvement of the posterior cornea. Lesions can range from tiny vesicular areas, thickening, and bands to advanced posterior corneal changes with secondary stromal and epithelial edema, necessitating corneal transplantation. Congenital posterior corneal vesicles have been observed in offspring of families with this condition. Histologically PPMD is associated with irregular multilaminar Descemet's membrane which displays normal anterior banding as well as abnormal cells present in the endothelial layer.

Recently it has been shown that keratin proteins are present in the form of tonofilaments not only in epidermal cells but also in corneal epithelial and a wide variety of other epithelial cells. Of particular interest is the fact that when frozen sections of cornea were stained with antikeratin antisera by indirect immunofluorescent staining, it was found that corneal epithelium was the only cell type that stained; no staining could be detected in the stroma or endothelium. We now report that the unusual epithelial-like cells in the corneal endothelial layer in PPMD can be stained specifically with antikeratin antisera and thus that they contain keratin filaments, a marker for epithelial cells.

Materials and methods. Antibodies prepared against a group of keratins purified from human stratum corneum were used to identify epithelial cells containing keratins by immunofluorescence. The antiserum against the keratin fraction was characterized by immunoelectrophoresis with sodium dodecyl sulfate slab gel and contained antibodies against all the major electrophoretic bands of keratins. For immunofluorescent staining, frozen sections (6 μm thick) were prepared. The air-dried tissue sections were hydrated in phosphate-buffered saline (PBS) and covered with 20 μl of antikeratin antiserum previously diluted 1:48 with PBS. The sections were incubated in a humidified chamber at 37° C for 30 min. They were then washed in three changes of PBS for a total of 30 min, overlaid with fluorescein-conjugated goat anti-rabbit IgG (1:16 diluted; Miles Laboratories, Inc.) and incubated at 37° C for 30 min. After they were rinsed again, these speci-