Long-term perfusion of human corneas.

BERND H. SCHIMMELPFENNIG

In twelve isolated human corneas (cadaver time 2 to 16 hr) stromal thickness could be maintained within 0.50 to 0.66 mm up to 20 hr by the use of an endothelial bathing medium consisting of glutathione-bicarbonate Ringer's solution, HEPES-buffer, and 20% human serum. During the first 3 to 5 hr of perfusion, temperature reversal occurred in all corneas. Wound healing processes were observed in nine corneas throughout the experiments after scraping off a 2.5 mm area of central epithelium, which was kept in a moist saline-saturated atmosphere.

The advantages of the perfused isolated cornea as an experimental model for the study of physiological properties have been demonstrated by many investigators. Donn et al. in 1959 measured the active transport of sodium across the epithelium in perfused rabbit corneas, and Maurice demonstrated the ability of the corneal endothelium to dehydrate a swollen stroma. The viability of stored rabbit corneas was shown in perfusion experiments by McCarey and Kaufman in 1974, using the specular microscope which allows in vitro observations of the corneal endothelial layer. However, the reliability and usefulness of perfusion experiments depend upon their duration and the well-being of the limiting corneal cell layers. Klyce and Maurice could maintain the stromal thickness in rabbit corneas within 8% of the minimum value for over 30 hr. In this study, the endothelial bathing solution consisted of a balanced salt solution and a tissue culture medium, and the epithelium was covered with silicone oil. Edelhauser et al. determined that a glutathione-bicarbonate Ringer's solution was the most satisfactory for isolated, short-term-perfused human and monkey corneas.

As yet no data exist on long-term-perfused human corneas which demonstrate dependence on the integrity of epithelium and endothelium in maintaining stromal deturgescence. The purpose of this study has been to find a suitable technique capable of keeping isolated human corneas with functioning epithelium and endothelium for an extended period of time.

Material and methods. Twelve human corneas (cadaver time see Table I) with a 7 mm scleral rim were mounted in a specially designed perfusion chamber (Fig. 1) 25 to 30 min (storage time at room temperature) after the eyes had been removed from donors who died from cardiovascular diseases or accidents. None of them was taking anticancer drugs prior to death. The chamber was connected to a heated water bath by polyethylene tubing and could be placed under the specular microscope or before a slit lamp. The epithelium was kept in a moist chamber; I and O, inlet and outlet tube for endothelial bathing solution; T, thermistor probe; B, back plate with mounting profile and heating system for endothelial bathing solution.
Fig. 2. Sequential endothelial specular photomicrographs taken at the beginning (A), after 4 hr (B), 8 hr (C), and 20 hr (D) of perfusion. The temperature reversed thickness changes are (A to D): 0.47 mm; 0.43 mm; 0.50 mm; 0.58 mm. E and F, Corneal endothelia (Nos. 8 and 10) after 12 and 17 hr perfusion. Arrowheads indicate "leaking points." (Magnification bar = 25 μm.)

epithelium (2.5 mm in diameter), outlined by a trephine, was scraped off by means of a 0.5 mm spatula with a blunt tip. Thickness measurements and specular photomicrographs were taken every 2 hr. Slit-lamp photographs were taken before and after each experiment. A perfusion experiment was considered to be finished if "leaking points" (Fig. 2, D to F) could be observed. Histological sections (5 μm; hematoxylin-eosin stain) were made of each perfused cornea and its control (a central 2.5 mm epithelial lesion was made in an eye immediately after removal from the donor). All tissues were fixed in buffered 10% formalin. After each experiment the perfusion chamber was cleaned with sterile isotonic saline, since in previous experiments sterilization by ethylene oxide resulted in corneas which swelled.

**Results**

**Thickness measurements.** In each experiment stromal thickness decreased within 3 to 5 hr. After
Fig. 3. Slit-lamp photographs of sliding epithelium. A, Initial 2.5 mm central lesion. B, After 20 hr perfusion. (Fluorescein staining; magnification bar = 0.5 mm.)

Table I. Thickness of human corneas after long-term perfusion

<table>
<thead>
<tr>
<th>Cornea</th>
<th>Cadaveric time (hrs)</th>
<th>Duration of perfusion (hr)</th>
<th>Stromal thickness (t)</th>
<th>Δt (mm)</th>
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<td>Initial (mm)</td>
<td>Final (mm)</td>
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<td>1</td>
<td>16</td>
<td>12</td>
<td>0.68</td>
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<td>2</td>
<td>10</td>
<td>11</td>
<td>0.67</td>
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<td>3</td>
<td>10</td>
<td>11</td>
<td>0.54</td>
<td>0.62</td>
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<td>4</td>
<td>5</td>
<td>15</td>
<td>0.60</td>
<td>0.66</td>
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<td>5</td>
<td>4</td>
<td>20</td>
<td>0.47</td>
<td>0.58</td>
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<td>6</td>
<td>2</td>
<td>14</td>
<td>0.52</td>
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<td>7</td>
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this temperature reversal the endothelial cell borders were clearly visible (Fig. 2, B, C). The thickness, ranging from 0.50 to 0.66 mm, could be maintained for 14 hr on the average (Table I).

**Epithelium.** With the exception of three corneas (Nos. 1, 2, 3), the epithelium tended to slide over the denuded area (Fig. 3, A, B). The migrating epithelium was reduced in thickness, having only one to two cell layers at its moving edge (Fig. 4, B).

**Endothelium.** The endothelial changes throughout an experiment had a uniform pattern (Fig. 2, A to C). Narrow cell borders could be observed through a hazy, swollen stroma at the beginning, which became wider and more distinct after the stroma had been dehydrated. Finally, growing dark spots ("leaking points") which were in close relation to cell borders and their common vertices obscured the endothelial cell pattern (Fig. 2, D to F).

**Sterility.** In spite of the use of high concentrations (200 μg/ml) of gentamicin, bacterial growth could not be prevented in all experiments. The most frequent bacterium cultured from the corneas was *Pseudomonas aeruginosa*. Its typical pigment could be observed in culture and on the epithelial surface.

**Discussion.** The results presented here show that isolated human corneas can be perfused with a modified combination of glutathione-bicarbonate...
Fig. 4. Edge of the epithelial lesion. A, Resting epithelium at the border of a lesion (control cornea). B, Migrating epithelium on demuced central stroma from cornea shown in Fig. 3. Note reduced number of cell layers. (Hematoxylin-eosin; ×250.)

Ringer's solution and human serum to considerably extend their lifetime. Cell adhesion factors recently isolated in mammalian and human serum and employed in tissue culture studies may act as a protective protein for the endothelial cell layer. Also, the change of reduced glutathione to its oxidized form, which was shown to enhance transendothelial fluid transport in perfusion experiments, could have been increased by higher oxygen concentrations present in the endothelial bathing solution.

The epithelial repair throughout perfusion of human corneas has not been previously described. It appears to be similar to the first stage of corneal wound healing in an intact eye. The term "leaking point" was chosen to describe
morphological changes which preceded ultimate stromal swelling. Since these dark areas were related to endothelial cell borders, one might presume beginning loss of endothelial transport function or, at least, an enlargement of intercellular spaces. Similar morphological changes were found in donor corneas.\textsuperscript{11} The significance of these alterations for the stromal swelling process is a matter for future experimentation. In contrast, cornea guttata, as seen by specular microscopy, is less related to cell borders and more roundish in shape with some bright dots in the center.\textsuperscript{12} The perfusion technique employed is comparable with an organ culture in that different cell layers continued their functions to maintain corneal transparency. Thus the organ culture model described may offer additional possibilities for studying the behavior of human corneas under experimental long-term conditions, i.e., culturing of viruses in the epithelium, exposure of the endothelium to immune-competent lymphocytes, and epithelial wound repair. A limitation to the experiments is obviously the occurrence of bacterial growth on the cornea. The maintenance of sterile conditions should further extend the corneal lifetime.

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From Univ.-Augenklinik Zurich, Zurich, Switzerland. This work was supported by the A. Bruppacher Foundation of the Zurich Eye Bank. Submitted for publication June 19, 1978. Reprint requests: Bernd Schimmelpfenning, M.D., Department of Surgery, Division of Ophthalmology, Stanford University, Stanford, Calif. 94305.

*Research Fellow of The Swiss Foundation for Medical and Biological Research.

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**REFERENCES**