designed to correspond to the human disease as closely as possible.

Though this pilot investigation was in animals, the notion that acute Pseudomonas keratitis is better treated with combined steroid antibiotic therapy cannot be supported by this present study. The therapeutic benefit of addition of steroids to antibiotics on human cases of Pseudomonas corneal ulcer will depend on the results of well-controlled studies in the future.

Our conclusions may be summarized as follows.

1. The use of steroids in combination with gentamicin coverage did not significantly alter the course of keratitis as measured by microbiological assay or clinical study in this experimental protocol.
2. This was true whether steroid therapy was started early or delayed in either higher or lower strengths.
3. There was a correlation between the severity of keratitis and the number of organisms recovered at the time of assay.
4. Clinically inactive eyes can harbor viable Pseudomonas after 1 week of therapy.

From the Department of Ophthalmology, Washington University Medical Center, St. Louis, Mo. This study was supported by research grants from the National Society for the Prevention of Blindness, Inc., and Fausek Ophthalmology Foundation Fund. Read before the Association for Research in Vision and Ophthalmology, Sarasota, Florida, May, 1975. Submitted for publication, May 18, 1976. Reprint requests: George M. Bohigian, M.D., Washington University School of Medicine, Department of Ophthalmology, 660 South Euclid Ave., St. Louis, Mo. 63110.

Key words: Pseudomonas aeruginosa, keratitis, antibiotics, corticosteroids, gentamicin, subconjunctival antibiotics, subconjunctival steroids, cornea.

REFERENCES


Short-term corneal storage at a temperature of 4°C was assessed with human cadaver eyes. Aqueous was replaced by air in one of each of 11 pairs of eyes, stored for periods of up to 96 hours post-mortem. The influence of air on the endothelial layer was measured in terms of corneal thickness, trypan blue staining, and scanning electron microscopy. Air-filled eyes developed less corneal edema over the storage period. No significant differences were found between the endothelial test and control eyes. Degenerative changes in endothelium were documented photographically.

Donor corneae have been shown to remain viable after storage in McCarey-Kaufman medium for 3 to 4 days, after storage in recipient serum for up to 100 hours, and in liquid paraffin at 4°C for 3 weeks. The longest period of successful storage is 422 days, in liquid nitrogen. However, the commonest, simplest technique is the early removal of a donor eye from a cadaver and storage in a sterile container at 4°C.

Some ophthalmologists are critical of leaving the aqueous in contact with the endothelium in the stored eye. McCarey and Kaufman describe how the aqueous contains "products from metabolic wastes and necrosis of tissue." A recent report suggests this is a major reason for storing the cornea separately from the eyeball. In 1963, Klen and associates, after studying cattle eyes stored at 4°C, concluded that corneal transparency reflected corneal viability in their stored corneae and that a number of agents, including air, which they had placed in the anterior chamber, prolonged corneal transparency.

This study tests the hypothesis that intracameral air prolongs the life of corneal endothelium stored at 4°C, and aims to document the endothelial changes occurring, with time, in the stored control eyes.

Methods. Eleven pairs of human eyes which appeared clinically healthy were enucleated as soon after death as practical. A 26-gauge needle on a tuberculin syringe was introduced through the limbal region of the test eye. Aqueous (0.2 mL) was withdrawn and replaced by a similar volume of air. The second eye of each pair served as a control. Each pair of eyes was stored in the same sterile moist chamber, cornea uppermost, at a constant temperature of 4°C. A further cornea was obtained at operation from a 47-year-old woman with keratoconus (Case Y).


Table I

<table>
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<th>Case numbers</th>
<th>1</th>
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<th>4</th>
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<th>6</th>
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<td>72</td>
<td>54</td>
<td>57</td>
<td>72</td>
<td>55</td>
<td>61</td>
<td>54</td>
<td>31</td>
<td>71</td>
<td>47</td>
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<tr>
<td>Death to enucleation (hr.)</td>
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<td>9.5</td>
<td>3.75</td>
<td>6.75</td>
<td>6.5</td>
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<td>5.0</td>
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<td>4.5</td>
<td>3.5</td>
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<td>Total storage (hr.):</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>52</td>
<td>52</td>
<td>48</td>
<td>72</td>
<td>75</td>
<td>48</td>
<td>96</td>
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<td>50</td>
<td>52</td>
<td>52</td>
<td>72</td>
<td>72</td>
<td>75</td>
<td>96</td>
<td>96</td>
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<td>—</td>
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<td>Trypan blue percentages:</td>
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<tr>
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<tr>
<td>Test eye</td>
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<td>30</td>
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<td>8</td>
<td>30</td>
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</table>

The 22 eyes were studied at selected times after storage periods ranging from 28 to 96 hours post-mortem (Table 1). Each cornea was excised with a generous scleral rim. The endothelium was immediately stained with a freshly prepared solution of 0.25 percent trypan blue in 0.9 percent saline for 90 seconds. The stain was gently removed by washing for 90 seconds, and it was then estimated by examining the cells through the entire endothelial layer (Fig. 1, A). Endothelial cells well beyond 48 hours of storage, as shown in Fig. 2, B. In this regard, it will be noted that the donor subject was relatively young and that the eyes were obtained within 4 hours of death. Older cornea stored less well than younger cornea. Added to the knowledge that older cornea contain fewer endothelial cells than the older cornea still retained its mosaic pattern after 96 hours.

When the control cornea alone were compared at the various storage times, a pattern of corneal endothelial change emerged. Fresh cornea, fixed shortly after collection, had a regular hexagonal mosaic of endothelial cells, some variation in cell size, distinct cell boundaries, and little to distinguish nucleus from cytoplasm (Fig. 1, A). After 28 hours of storage, swelling of endothelium produced a fine cobbled appearance across the entire endothelial layer (Fig. 1, B). Endothelium stored for 36 to 75 hours showed shrinkage of cytoplasm and a prominent nucleus, producing a “poached egg” appearance. Fine pitting of the cell surface was apparent. Cell borders were clearly defined (Fig. 1, C). From 75 hours onward, the corneal endothelial surface appearances became more variable. Cases 8, 10, and 11 demonstrated "cellular pucker" in which a large central nucleus was surrounded by cavities in the cytoplasm, with strands trailing from the nucleus. The nuclei remained regularly distributed across the endothelial layer, although cell boundaries were indistinct or absent (Fig. 1, D to F).


development. The introduction of air into the anterior chamber had no clear advantageous or disadvantage in the preservation of the corneal endothelial layer. However, less corneal edema occurred in the air-filled eyes.

It has been possible to record photographically a probable sequence of degenerative changes occurring in corneal endothelium stored over 4 days (Fig. 1). Some cornea can retain healthy endothelial cells well beyond 48 hours of storage, as shown in Fig. 2, B. In this regard, it will be noted that the donor subject was relatively young and that the eyes were obtained within 4 hours of death.

Older cornea stored less well than younger cornea. Added to the knowledge that older cornea contain fewer endothelial cells than
Fig. 1. A, Case Y. Fresh cornea demonstrating the endothelial mosaic, definite cell borders, and smooth surface. (x500.) B, Case 1, 28 hours' storage. Endothelial cell edema with fine surface pitting. (x1100.) C, Case 9, 48 hours' storage. Intact cell boundaries; "poached egg" appearance; pitted cell surface. (x1000.) D, Case 8, 75 hours' storage. The prominent nucleus and trailing cytoplasm appear as "cellular pucker" in areas of endothelial layer. (x600.) E, Case 11, 96 hours' storage. "Cellular pucker" has been produced. (x950.) F, Case 11, 96 hours' storage after aqueous had been replaced by air. Compare with E. (x950.)
Fig. 2. A, Case 5. Cell death was common, but not invariably on the crests of endothelial folds. (×125.) B, Case 7. Endothelium of control eye stored for 72 hours. (×500.)

younger corneas,10 this argues strongly against the use of aged donors for penetrating keratoplasty.

The scanning electron microscopy assistance of Miss Sally Randall, the advice of Professor G. W. Crock and Miss Nanette Carroll, the photography of Mr. J. Scrimgeour, and the typing of Mrs. Beverley Vaudrey are gratefully acknowledged.

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Key words: cornea, preservation, endothelium, viability, scanning electron microscopy, trypan blue, pachymeter, human, storage.

REFERENCES


Laboratory data of a new, highly hydrated contact lens (Weicon 72) indicated that it might be therapeutically superior to the known Weicon 38. The great variability of therapeutic contact lens cases, however, makes a sound clinical evaluation difficult. Therefore the two lenses were tested in a clinical model using patients with traumatic superficial corneal defects. The results were statistically significant, the Weicon 72 proving to be superior to the Weicon 38.

Although considerable progress has been made in the field of therapeutic soft lenses during the past few years,1 therapeutic failures and complications are not uncommon. These may be due