ORGAN-CULTURED HUMAN CORNEAS*

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To date fresh corneas have remained the major source of corneal material available for transplantation. Dependence on fresh corneas has several disadvantages:

1) The temporal restriction in the usefulness of fresh corneas imposes an inconvenience on the surgeon, the patient, and the staff of the operating room.

2) The waste of donor material when recipients are not available.

3) The scarcity of donor material, which can occur at times when recipients are available.

Cryopreservation overcomes these disadvantages but adds problems of its own, namely, the necessity for highly trained technicians to freeze and later thaw the corneas, and the expense necessary for the equipment and personnel. In addition, cryopreservation causes ultrastructural damage to epithelial and endothelial cells. Organ culture offers a means of storage which is simpler in design and performance than that of cryopreservation.

This study attempts to establish that the human cornea remains structurally intact in organ culture, as demonstrated by light and electron microscopy.

MATERIALS AND METHODS

Human corneas were obtained from the Eye-Bank for Sight Restoration, Inc., in New York City. Only corneas of good clarity and quality were considered. Twelve corneas were used. The ages of donors ranged from 42 to 64 years, with an average age of 56. All corneas were enucleated within seven hours of the death of the donor and were placed in organ culture within 36 hours. Prior to organ culture the corneas were maintained in moist saline chambers at 4° C. Each

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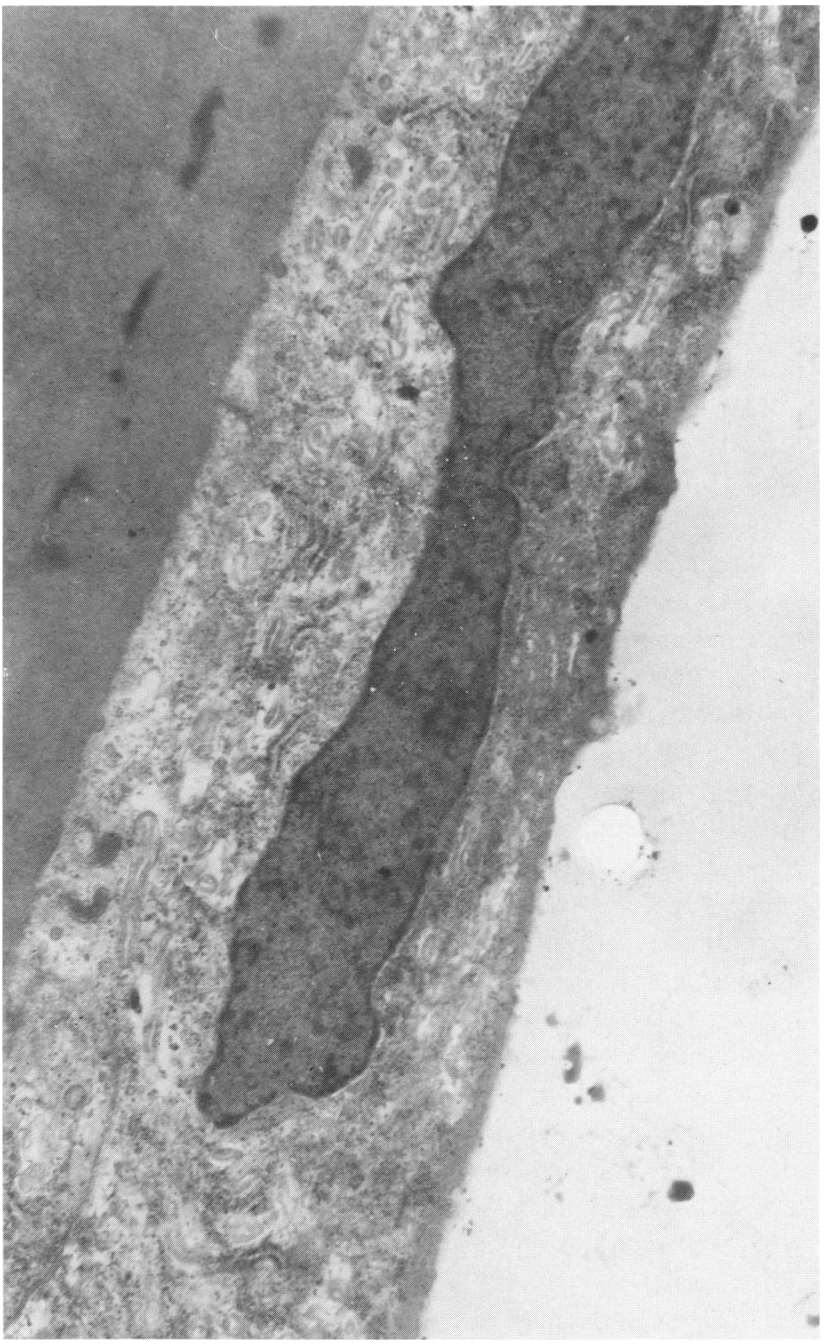

Fig. 1. Endothelium from human cornea stored in organ culture for two weeks. Note intact cell and nuclear membranes. Rough endoplasmic reticulum and mitochondria are preserved. Original magnification X 24,000.

cornea was removed from the eye in such a fashion as to include a circumferential rim of 3 mm. of sclera. The corneas were then rinsed in 0.3% gentamicin sulfate. Sterile technique was employed.

The organ-culture media consisted of the following:

1) Minimal essential medium (Eagles) 1 x with Earle's salt with L-glutamine

2) Calf serum, heat inactivated in a 10% concentration

3) L-glutamine in a 1% final concentration

4) An antibiotic-antimycotic mixture consisting of penicillin, 10,000 units per milliliter, amphotericin, 26 μg. per milliliter, and streptomycin, 10,000 μg. per milliliter, added at a concentration of 1% in the final mixture.[2]

The corneal buttons were placed epithelial side up in Falcon-tissue culture dishes containing sterile organ-culture media. These were stored in a tissue-culture incubator at 37° C. in an atmosphere of 5% CO_2 and 95% air. The culture media were changed twice each week under sterile conditions.

The combination of hematoxylin and eosin stain was employed for light miscroscopy. For electron microscopy the corneas were fixed in 2.5% glutaraldehyde in phosphate buffer, *p*H 7.4 for six hours, and postfixed in 1% osmium tetroxide in phosphate buffer, *p*H 7.4, for one hour in the cold. The fixed tissues were dehydrated through graded concentrations of ethanol. The Epon-embedded materials were cut with a microtomic and stained with saturated uranyl acetate for 10 minutes and with 0.2% lead citrate for 10 minutes. Thin sections were studied with an RCA 36 electron microscope at 80 kV. Thick (1μ.) sections were stained with toluidine blue for orientation.

Results

Throughout their storage in organ culture the human corneas remained clear, although swollen. Twelve corneas were examined by light microscopy. These were taken from organ culture and examined after storage for two weeks, four weeks, eight weeks, and 12 weeks. The following characteristics were noted:

1) Reduction in thickness of epithelial layer from five cells to three or four cells

2) Stromal swelling to approximately twice normal thickness

3) Reduction in the number of stromal cells

Fig. 2. Endothelium from human cornea stored in organ culture for eight weeks. Nuclear and cell membranes as well as subcellular organelles are all well preserved. Original magnification X 24,000.

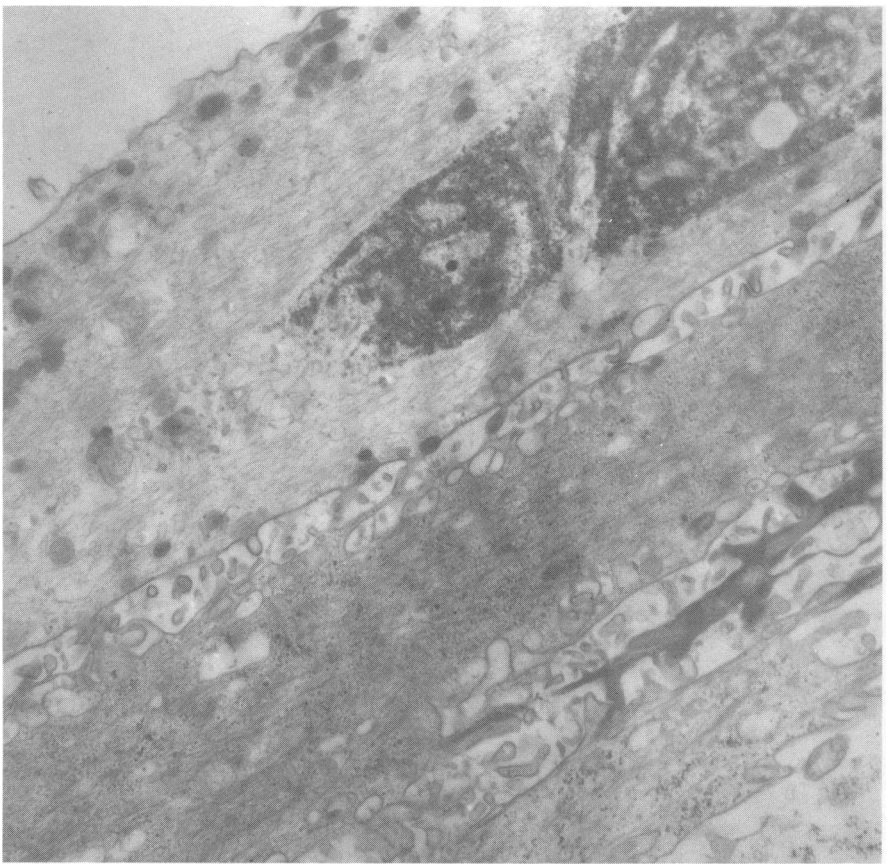

Fig. 3. Epithelium from human cornea preserved for eight weeks in organ culture. Numerous microvillae and intercellular spaces are evident. Original magnification X 24,450

4) Edematous spaces between corneal lamellae
5) Preservation of Bowman's membrane and Descemet's membrane
6) Reduction in the number of endothelial cells

The duration of storage in organ culture did not seem to influence the light microscopic appearance of the corneas. Electron microscopy was performed on three corneas stored in organ culture for two weeks, four weeks, and eight weeks, respectively. In all three corneas the endothelial cell membranes were intact, with lateral interdigitations clearly evident. The nuclear membranes were also intact, and a normal dispersion pattern for chromatin was present. Rough and smooth endo-

plasmic reticulum could be found in the cytoplasm as well as free ribosomes. Mitochondria were present but were reduced in number (see Figures 1 and 2).

In the epithelial cells numerous microvilli were apparent. Large intercellular spaces were present, suggesting epithelial edema. As in the endothelial cells, the cell and nuclear membranes were intact. Mitochondria and rough endoplasmic reticulum could be discerned (see Figure 3).

Summary

Human corneas were studied by light and electron microscopy after storage in organ-culture media for varying periods, ranging from two to eight weeks. The structural integrity of the corneas was demonstrated clearly. Recent research on the ultrastructure of endothelium from organ-cultured human corneas tends to confirm these findings.[3]

Additional work is now in progress on the functional and immunologic aspects of the organ-cultured cornea.

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