Ultrastructure of M-K and refrigerated moist chamber-stored corneas. Bernard E. McCarey, Takashi Sakimoto and Francis Bigar.

McCarey and Kaufman have developed a technique for storing donor corneas by immersing the cornea with its scleral rim in a specific M-K bathing media. By histologic comparison, the M-K media-stored rabbit corneas are superior to the refrigerated moist chamber-stored rabbit corneas. They maintained normal cytoplasmic density but vacuoles and mitochondria swelling were present. In the moist chamber corneas, there was a decrease in cytoplasmic density along with destruction of organelles in these areas. The cell changes in M-K media were slow in progressing during the 14 days, which is in contrast to the faster developing changes in the moist chamber corneas.

Attempts to improve and extend the storage of donor corneas in Eye Banks has been investigated by Capella, Kaufman, and Robbins with cryopreservation, by Stocker with homologous serum, and by Kuwahara and co-workers with a solution of inorganic salts, glucose, mucopolysaccharides, glutamic acid, and ascorbic acid. Cryopreservation is a technically expensive and complex storage procedure which is valuable for specific Eye Banks but not for the average Eye Bank needs. The other techniques have inconvenient storage methods. McCarey and co-workers have introduced another technique in which donor corneas with a scleral rim are immersed in a tissue culture solution with dextran and antibiotics (M-K media). Viable rabbit and human corneas have been stored with this procedure.

The purpose of this paper is to compare the structural alterations of rabbit corneas stored in the M-K media at 4°C, to rabbit corneas stored in the normal Eye Bank moist chamber at 4°C. Transmission and scanning electron microscopy was used to investigate the progressive changes for up to 14 days.

Methods. New Zealand albino rabbits (6 to 8 pounds) were killed with an overdose of sodium pentobarbital intravenously. The eyes were enucleated and flooded with Neosporin for a few minutes. One eye of a pair was stored in a moist chamber under normal Eye Bank conditions. The eye was in a supporting rack within a sterile capped container. The container was stored at 4°C with a few milliliters of saline in it in order to create a humid environment. The mate cornea along with its scleral rim was isolated, then stored at 4°C in a 20 c.c. vial of the M-K media. The media is a solution of tissue culture Medium-199, 5 per cent dextran, and 100 units per milliliter of streptomycin-penicillin mixture. The stored corneas were investigated after 1, 3, 5, 9, and 14 days of storage. There was a total of 16 eyes, with one pair for 1, 3, and 5 days of storage and two pairs for 9 and 14 days of storage.

At the termination of the storage periods, corneas were transferred directly from the M-K media to cold 2.5 per cent glutaraldehyde in Millonig buffer at 4°C. In the case of the moist chamber-stored eyes, the cornea with a scleral rim was carefully excised and immersed in the same fixative. One-half of each cornea was processed for transmission electron microscopy. The tissue was postfixed with 1 per cent osmium tetroxide in Millonig buffer and embedded in Epon-812. The other corneal half was processed for scanning electron microscopy by the freeze-drying method.

Results. Transmission electron microscopy. The endothelial ultrastructure of the stored corneas revealed progressive changes which were characteristic to, but different for, each type of storage technique. As the storage duration was increased these changes became more pronounced.

M-K media-stored corneas. The endothelial cell ultrastructure remained intact during storage in the M-K media, Fig. 1, A, B, and C. The cytoplasmic density, nuclear chromatin, cell junctions, and endoplasmic reticulum was stable during the 14 days of storage in the M-K media. The mitochondria after the first day of storage were normal. As the storage duration increased, there was some gradual disruption of the mitochondria cristae. Small vacuoles appeared on the third day and by the fifth day, more and slightly larger vacuoles could be found in the endothelial cells. They were more common along the posterior membrane of the cells. The number and size of the vacuoles increased further by the fourteenth day. The most unusual alteration of the endothelial cells was the formation of clefts, which were splits or separations in the cytoplasm, Fig. 2, A. The clefts did not always form along a membrane structure, but were always smooth separations of normal density cytoplasm. Very small clefts appeared after one day of storage, and reached a maximum size of about 10 microns by the fourteenth day.

Normal Eye Bank moist chamber-stored corneas. The endothelial ultrastructure had progressive edematous damage as the moist chamber storage...
Fig. 1. The rabbit endothelial cells in A (1 day), B (5 days), and C (9 days) were stored by the McCarey-Kaufman technique and compared to D (1 day), E (3 days), and F (9 days) which were stored by the normal Eye Bank technique. Electron micrographs A, B, and C show fairly stable ultrastructure with increasing vacuolization. A prominent and progressive decrease in cytoplasmic density can be seen in electron micrographs D, E, and F.

(N) nucleus, (IJ) intracellular junction, (ER) endoplasmic reticulum, (M) mitochondria, (V) vacuole, all magnifications, x8,640.
Fig. 2. Electron micrograph A is endothelial cells from a rabbit cornea stored in M-K media for 14 days, x9,200. A cleft (°) can be seen with smooth inner borders. The cell in B was stored for 14 days in a normal Eye Bank moist chamber. Edema had damaged many of its organelles, x9,200.

duration was increased, Fig. 1, D, E, and F. The most prominent alteration was the cell cytoplasm density. It was normal after the first day but afterward, a gradual breakdown became apparent. The cytoplasmic zone anterior to the cell nucleus showed decreased cytoplasmic density and organelle concentration, Fig. 1, F. By the fourteenth day, the changes in this zone were more prominent as well as involvement of the rest of the cell, Fig. 2, B. From the first day of storage, the mitochondria showed signs of swelling and this became more severe as the storage duration increased. Excessive vacuolization was not manifested, even after fourteen days of storage.

Scanning electron microscopy. The endothelial cells of corneas stored in the M-K media had characteristic changes which distinguished them from corneas stored in moist chambers, but these differences were not as pronounced as in the transmission electron microscopy.

M-K media-stored corneas. The endothelium of the one-day-stored corneas appeared normal when viewed with the scanning electron microscope. The 3-, 5-, and 9-day stored corneas had endothelium with flat smooth posterior cell surfaces, but some cells had raised central cell areas as illustrated in Fig. 3, A. On the 14-day-stored corneas, Fig. 3, B, there was an increase in the number of cells with raised central areas.

Refrigerated moist chamber-stored corneas. These endothelial cell surfaces appeared normal even on the ninth day of storage, Fig. 3, C. The cell surfaces ranged from flat to a slight roundness. After 14 days of storage in the moist chambers, the corneal endothelium showed signs of swelling, Fig. 3, D. Some individual cells had central raised areas too, but this did not have the same character as the raised central cell area of the M-K media-stored corneas. There was almost no pitting of the cell surfaces; they were smooth and continuous.

Discussion. The scanning electron microscope
Fig. 3. The endothelial cell scanning electron micrographs A and B are of M-K stored corneas; C and D are of refrigerated moist chamber corneas. A, after 9 days of storage, several endothelial cells developed raised central cell areas (*), x570. B, after 14 days, there was an increase in the number of cells with raised central areas (*), x1,290. C, after 9 days, the endothelium still appeared like normal corneal tissue, x600. D, after 14 days, the tissue was abnormal with inconspicuous cell borders and several swollen cells, x1,350.

enables one to visualize the surface of the tissue, in this study, the endothelial layer. Extensive ultrastructural changes may take place within an endothelial cell before the posterior membrane is altered. The endothelial layer after M-K media storage, Fig. 3, B, differs only slightly from that of the moist chamber-stored cornea in Fig. 3, D, yet the ultrastructure differs considerably in Figs. 1, C and 1, F, respectively. The scanning electron micrograph does not show the cellular edema of the moist chamber eyes which were seen by the transmission electron microscope.

The transmission electron microscopy reveals a fairly consistent endothelial cell ultrastructure throughout the 14 days of storage in the M-K media. This contrasts to the gradual breakdown of the cytoplasm of the moist chamber-stored endothelial cells. The most prominent alteration in the cells of the M-K media-stored corneas is the presence of clefts, Fig. 2, A. These are splits in the normal density cytoplasm and have an uncertain origin, but have been observed in another preservation method. It is possible that they result from the colloidal osmotic force of the dextran drawing water across the endothelial layer, but this is only speculative. They seem to correspond to the raised central cell areas seen in the scanning electron micrographs, Fig. 3, A, but once again this is only reasonable speculation. Other research data have shown the clefts to be reversible after incubating the cornea in vitro in an artificial aqueous solution during temperature reversal testing. The endothelial cells of the moist chamber corneas undergo gradually increasing intracellular
edema and breakdown of the cytoplasm during the 14 days of storage. The rabbit corneas stored in the M-K media have much less ultrastructure alterations and appear more viable than the corneas stored in the standard Eye Bank containers, especially for storage durations greater than nine days.

From the Department of Ophthalmology, College of Medicine, J. Hills Miller Health Center, Gainesville. Supported in part by Grants EY-00446 and EY-00260 from the National Eye Institute and an unrestricted grant from Research to Prevent Blindness, Inc., New York. Submitted for publication May 14, 1974. Reprint requests: Dr. Bernard E. McCarey, Department of Ophthalmology, College of Medicine, Box 733, J. Hillis Miller Health Center, Gainesville, Fla. 32610.

Key words: corneal eye banking, M-K media, transmission electron microscopy, scanning electron microscopy.

REFERENCES
2. Stocker, F. W.: Storage of donor corneas in containers, especially for storage durations greater than nine days.


Sulfur hexafluoride gas (SF₆) has recently been used as an adjunct in the treatment of retinal detachments. In order to determine the change in gas volume and concentration with time following injection of SF₆ into the vitreous cavity, vitreous was aspirated from the rabbit eye and replaced by 100 per cent SF₆. At various time intervals (6, 24, 48, 72, and 96 hours) the gas was aspirated into a syringe, the volume measured, and the sample analyzed with a gas chromatograph for per cent volume concentration of SF₆, N₂, O₂, CO₂. The gas pocket expanded to its maximum volume at 24 hours with a dry concentration of 18.3 per cent SF₆, 71.5 per cent N₂, 4.7 per cent O₂, and 5.5 per cent CO₂. The volume then decreased exponentially reaching the original volume at 96 hours, and the pocket was absorbed in 8 to 10 days. The concentration of O₂ and CO₂ remained stable from 6 to 96 hours, while the concentration of N₂ increased as the concentration of SF₆ decreased. The time for persistence of the gas pocket was related to the initial volume of SF₆ injected.

Sulfur hexafluoride gas (SF₆) has recently been used as an adjunct in the treatment of retinal detachments. A gas pocket injected into the vitreous cavity will tamponade a retinal hole, support the position of the retina, and restore the volume of the globe following surgery. Because of the physical properties of SF₆, a SF₆ gas pocket will expand and persist long enough for a cryosurgical or diathermy adhesion to form between the detached retina and pigment epithelium. Recent studies have shown in rabbits that anterior chamber injection of SF₆ causes proliferation of the corneal endothelial cells and the deposition of a new Descemet’s membrane; a similar effect occurred with anterior chamber injections of air. However histologic, electron microscopic, and electrophysiologic studies of the owl monkey retina following intravitreal injection of SF₆ showed no alteration in retinal structure or function.

Though the dynamic change in a SF₆ gas pocket has been experimentally determined in the peritoneal cavity of the cat, and the subcutaneous gas pocket in the rat, these changes have never been directly measured in the vitreous cavity of the eye. The purpose of this study was to measure the time change in volume and composition of an intravitreal SF₆ gas pocket.

Method. Rabbits (3.5 to 5 kilograms) were anesthetized with pentobarbital, 30 mg. per kilogram intravenously. Preoperative and postoperative intraocular pressure were measured with the Schiøtz tonometer. After mydriasis was induced, the eye was proptosed using sterile procedure by the method of Khodadoust. Hyaluronidase, 150 U. in 0.1 c.c. normal saline, was injected into the vitreous through a 27-gauge needle introduced at the pars plana between the 4 o’clock and 7 o’clock position. A 20-gauge needle was introduced through the previous