Effects of mechanical agitation on endothelial function of preserved corneas.

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Corneas from adult albino New Zealand rabbits were stored in either MK medium or moist chambers at 4° C. for 18 to 24 hours. During that time, half the corneas from each group were agitated for 8 hours to simulate the motion that might occur during long-distance shipment. The corneas were then placed in specular microscopes and perfused for 4 hours with a modified Krebs-Ringer solution at 34° C. Serial measurements of corneal thickness made during temperature reversal showed that agitation had no effect on endothelial viability of corneas stored in either solution. Electron microscopy of selected corneas confirmed this finding.

McCabe and Kaufman have described a simple, practical method for prolonging the storage time of corneal tissue to be used for keratoplasty. This method of preservation is an important advance in the intermediate-term (2 to 5 days) storage of corneal tissue, where an excised human donor cornea can be placed in a mixture of TC-199, 5 per cent dextran, and antibiotics (MK medium) and can remain in excellent condition for at least 4 days. Corneas can easily be transported under controlled conditions of temperature, hydration, and sterility, but gross mechanical agitation and vibration are possibly undesirable factors which cannot be prevented. It is not known how well an MK-stored or moist chamber-stored cornea will tolerate mechanical agitation as might occur during long-distance shipment. The purposes of our experiments were to test, and compare with moist chamber-stored corneas, the tolerance of MK-preserved corneas to agitation simulating the trauma of shipment and to describe any changes in the corneal endothelium.

Materials and methods. Albino New Zealand rabbits (2 to 3.5 kilograms) were killed with an overdose of sodium pentobarbital. The eyes were promptly enucleated with the adjacent lids and conjunctiva and treated as follows:

1. MK medium-stored eyes. Five pairs of corneas were removed with a 4 to 6 mm. scleral rim, as described by Dikstein and Maurice, prior to storage at 4° C. After the lids and conjunctiva were separated from the globe, the cornea was held on a mounting bar at -25 mm. Hg while the lens, iris, and ciliary body were carefully removed. The endothelium was rinsed gently with Ringer's solution and the cornea was removed from the mounting bar and placed into a vial containing 20 ml. of MK medium. Utmost care was taken not to allow folding of the cornea at any time during the preparation, since this adversely affects endothelial function. In all cases one eye of a pair was mechanically agitated while the fellow eye was stored undisturbed.

2. Moist chamber-stored eyes. Five pairs of intact rabbit globes were fixed to a sterile moist chamber rack with a 22-gauge needle through the optic nerve stump. One eye was mechanically agitated and the fellow eye was stored undisturbed. Both moist-chamber eyes and corneas in MK medium were stored for approximately 18 to 24 hours.

Agitation. A total of 10 corneas were subjected to mechanical agitation at 4° C. An agitator (Fig. 1) was designed by the Biomedical Engineering Department of the Medical College of Georgia to simulate the motion and vibrations of a cornea during transportation. Corneas were agitated in their original storage containers at a controlled frequency for a duration of 8 hours of the total 18 to 24 hours' storage, at a calculated maximum acceleration of 0.16 g per oscillation. The agitation phase occurred so that there were about 2 hours of nonagitated time following the shaking period, and prior to temperature reversal studies. A force of 0.16 g applied constantly over a period of 8 hours should be far in excess of actual forces sustained by a cornea during 8 hours of shipment by air or ground transportation. An additional two corneas were agitated at 0.66 g for 8 hours; one in moist chamber and one in MK medium.

Temperature reversal studies. Following storage, all corneas were studied with the specular microscope for temperature reversal (moist-chamber corneas) or maintenance of corneal thickness (MK-stored corneas) at 15 mm. Hg pressure over a period of 4 hours. The moist-chamber corneas were dissected out of the globe in the usual manner, and mounted in the microscope immediately prior to temperature reversal studies. The epithelial surface of the cornea was covered with silicone oil. Perfusion was with a modified Krebs-Ringer solution, with reduced glutathione and adenosine, at 34° C. Serial measurements of thickness (to an accuracy of 2 μm) were made on a total of 18 corneas. Corneal thickness measurements were taken twice every 30 minutes for a period of 4 hours. Immediately following perfusion, selected corneas were fixed in cold glutaraldehyde for electron microscopy.

Results. MK-preserved corneas are initially thin because of the dehydrating effect of 5 per cent dextran. Thus, even during storage at 4° C where there is poor endothelial function, the corneas are maintained artificially in a dehydrated state. Both agitated and nonagitated MK corneas showed very little change in thickness during 4 hours of perfusion (Fig. 2). The initial and
final thicknesses of control and experimental corneas were 405 ± 10 μ and 399 ± 8 μ and 398 ± 9 μ and 409 ± 5 μ, respectively.

In moist-chamber corneas, a characteristic temperature-reversal curve is obtained since the corneas swelled during storage. Both agitated and non-agitated moist chamber-stored corneas showed identical thinning rates (Fig. 2), indicating good endothelial viability irrespective of the corneal history. The results indicate that the endothelia of both MK-preserved corneas and standard moist chamber-preserved corneas are unaffected by the agitation and vibration to which they were subjected. The initial and final thicknesses of control and experimental corneas were 584 ± 17 μ and 448 ± 32 μ, and 616 ± 16 μ and 456 ± 21 μ, respectively.

When the MK-preserved corneas were first observed under the specular microscope, the endothelial cells appeared to be grossly swollen. As MK medium was washed away and physiologic temperature (34° C.) was restored, the cells rapidly assumed a normal appearance. This storage change in the appearance of the MK-preserved endothelial cells in no way affected the ability of the cells to maintain the cornea at a constant thickness during perfusion and temperature reversal.

It can be seen in Fig. 3 that there are no morphologic differences between the endothelial cells from either agitated or nonagitated corneas at the electron microscopic level, showing that a normal endothelial morphology is well maintained in the face of 8 hours of constant mechanical agitation at 0.66 g. The excellent morphologic appearance and physiologic function (since the behavior pattern parallels that at lower g forces) of the corneal endothelium are sustained even after 4 hours of perfusion.

**Discussion.** There is much experimental evidence which indicates that the endothelium is physiologically important in corneal thickness regulation, and that the endothelium is very susceptible to trauma. Endothelial preservation has been shown to be vital if a stored cornea is to be used for penetrating keratoplasty. The history of the globe and particularly of the cornea after death is of greatest importance in determining the suitability of the tissue for transplantation. Fac-
Fig. 3. Electron microscopic (EM) comparison of corneal endothelium following 8 hours of agitation of 0.66 g. A and B, Moist-chamber storage. A, Mitochondria and cytoplasm are more swollen due to temperature reversal effects. (Transmission EM, x17,600.) B, Inter- cellular spaces and endothelial cell membranes are undamaged. (Scanning EM, x1,000.) C and D, MK-medium storage. C, Morphology is unchanged from normal. (Transmission EM, x17,600.) D, Undamaged. (Scanning EM, x1,000.)
tors of importance include elapsed time from death to enucleation, whether or not the eyelids were closed prior to storage, and transportation trauma.

Our results suggest that for short-term storage (less than 24 hours) there is no particular advantage of the MK-preserved rabbit cornea over the conventionally stored moist-chamber rabbit eye. In both cases, the endothelial cells showed excellent viability as determined by temperature reversal studies (Fig. 2). Beyond the 24 hour period of storage, MK-preserved corneas have been shown to be superior for a storage duration of up to 4 days, in part because of good metabolic storage conditions compared to the moist-chamber storage procedure. The work of Van Horn and associates indicates that the MK extends the useful period of human corneal storage from 24 hours up to 4 days. After 4 days both MK medium-preserved corneas and moist chamber-preserved eyes show loss of endothelial viability as determined by staining with trypan blue.

Specular microscopic observation of rabbit corneal endothelium during perfusion in the present experiments shows that 8 hours of constant agitation of either an MK-stored or moist chamber-stored rabbit cornea have little morphologic effect on the in vitro appearance of the endothelial cells. Specular microscope morphology can be a misleading prognostic sign of endothelial viability, however, since it is possible to have a perfectly normal appearance of the endothelial cells as viewed with the specular microscope in a cornea that will subsequently fail to deturgesc due to temperature reversal or gradually swell. Sherrard did, however, note that endothelial cells showed gross vacuolization before irreversible swelling of the cornea occurred. Our experience suggests that temperature reversal is a superior index of corneal viability as compared to morphologic changes viewed under the specular microscope, since corneas deswelled or held constant thickness despite the poor initial appearance of endothelial following storage in MK medium. The electron microscopic evidence, however, does indicate that the endothelia of both agitated and nonagitated corneas were in excellent condition and appeared normal.

MK medium-preserved corneas would appear to be eminently practical for long-distance shipment, if extrapolation from our rabbit studies to human corneas is valid. If good endothelial cells are present at the time of enucleation, it appears that an MK-preserved cornea could be shipped great distances and used with confidence up to a period of 4 days following enucleation. It is, however, critical that the cornea be not grossly distorted during handling. Moist chamber-stored globes should maintain a cornea in good condition for at least 24 hours provided that secure fixation of the globe is maintained during transportation.

It appears, therefore, that 8 hours of agitation (as would be expected on cross-country shipment) of either MK or moist-chamber corneas, occurring soon after tissue removal, are of no consequence to the subsequent behavior of the cornea in terms of its deswelling capability. The type of disturbance met by such corneas during transportation would not seem to adversely affect their use for keratoplasty.

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REFERENCES

Corneal water and electrolyte content following storage in moist chamber and MK medium. DAVID S. HULL, KEITH GREEN, AND KAREN BOWMAN.

Rabbit corneas were subjected to MK-medium or moist-chamber storage for periods up to 7 days. Sodium, potassium, and chloride concentrations and hydration were measured. The hydration of corneas stored in MK medium was less than that of those stored in moist chambers. The sodium concentration of MK corneas remained stable for up to 7 days; the potassium concentration, however, decreased with increasingly longer periods of storage. Chloride concentrations quickly equilibrated with the high chloride level of MK medium. The concentration of sodium and chloride in moist chamber-stored corneas fell with progressively longer storage periods. Potassium showed an initial decrease in concentration followed by an increase in concentration that paralleled, and equilibrated with, the increasing aqueous humor concentration.

Ionic concentration and hydration have been well documented in normal rabbit corneas.1 It was the purpose of this investigation to determine and compare the concentrations of sodium, potassium, and chloride, as well as the hydration, in rabbit corneas stored in MK medium and moist chambers for various periods of time.

Materials and methods. Albino rabbits weighing about 2 kilograms were sacrificed with an overdose of sodium pentobarbital. Eyes were enucleated and either stored, as the whole eye, in a moist chamber at 4° C; or the cornea with a 2 mm. scleral rim was removed from the eye and stored in a 20 ml. vial of Medium 199 with 5 per cent dextran (MK medium) at 4° C. After varying periods of time the corneas were removed, gently blotted with filter paper, and weighed in flasks that had been dried at 100° C, for at least 12 hours and cooled for 30 minutes in a desiccator. After drying at 100° C, for 24 hours, corneas were again weighed after cooling for 30 minutes in a desiccator and the dry weight and hydration calculated. One milliliter of 0.1N nitric acid was added and the corneas shaken for 2 hours for the purpose of electrolyte extraction.1-3 Chloride was determined on the acid extract with a Buchler digital chloridometer. Sodium and potassium determinations were made with a Corning Model 450 flame photometer. Calculations at each time period were based on the data of four rabbits, which is presented as the mean ± standard error of the mean (± S.E.M.).

Results. Hydration. Corneas stored in MK medium showed a gradual increase in hydration until, at 24 hours, a value was achieved which remained constant for up to 7 days (Fig. 1, A).

Moist chamber-stored corneas showed an increased hydration at 24 hours and continued to slightly increase in hydration over the next 6 days (Fig. 2, A). The 24 hour and 7 day hydrations of corneas stored in moist chambers were 84.2 ± 0.2 per cent and 86.7 ± 0.2 per cent, respectively. Corneas stored in MK medium showed hydrations of 79.8 ± 0.2 per cent at 24 hours and 80.5 ± 0.6 per cent at 7 days.

Sodium. Corneas stored in MK medium showed a maintenance of sodium concentration for storage periods of up to 7 days (Fig. 1, A). Moist chamber-stored corneas demonstrated a decreased sodium concentration, compared to normal, fresh corneas at 24 hours and 7 days (Fig. 2, A).

Potassium. Corneas stored in MK medium demonstrated a decrease in potassium after 1 hour, which continued to gradually fall with progressively longer periods of storage (Fig. 1, A).

Corneas stored in moist chambers showed a reduced potassium concentration at 24 hours (Fig. 2, A). This is similar to the concentration of potassium of corneas stored in MK medium for 24 hours. At 7 days, corneas stored in moist chambers showed a potassium concentration which had increased and was significantly greater than that at 24 hours. The increase paralleled that which occurred in the aqueous humor of the enucleated eye (Fig. 2, A and B).

Chloride. Corneas stored in MK medium demonstrated a rapid rise in chloride concentration at 1 hour, and at 6 hours had achieved a value close to that of the bathing solution (Fig. 1, A and B). The concentration remained constant and elevated for up to 7 days' storage.

Moist chamber-stored corneas showed decreasing chloride concentrations with progressively longer storage periods, which reflected the fall in concentration that occurred in the aqueous humor (Fig. 2, A and B).

Discussion. Hydration. The hydration of fresh rabbit corneas (73.97 ± 0.22 per cent) compares favorably with that in previous reports.1 The hydration gradually increased to 79.8 per cent at 24 hours and remained essentially constant for up to 7 days. It is known that dextran enters the corneal stroma and within 24 hours an equilibrium is reached between about 15 per cent of the stromal water and the surrounding MK medium.5 Hence this