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**References**


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**Corneal Storage in MK Medium and K-Sol®**

**Effect on Ionic and Non-Ionic Fluxes**

David S. Hull, Rosa Berdeco, and Keith Green

Rabbit corneas were stored at 4°C for 3, 7 or 14 days in either modified MK medium or K-Sol®. Corneal endothelial permeability to inulin following storage in modified MK was significantly less at each time examined than that found in corneas stored for either 3, 7 or 14 days in K-Sol. Inulin permeability after storage in K-Sol was increased at all times relative to unstored control corneal tissue, but only at 7 and 14 days in MK medium. Dextran permeability was similar following 3 days of storage in either solution, but dextran permeability following storage in modified MK was significantly less than the values found in corneas stored for 7 and 14 days in K-Sol. Dextran permeability was also significantly increased relative to control, at any storage time in MK medium but was increased at 7 and 14 days in K-Sol. Inulin and dextran permeabilities after storage in MK medium were maintained more closely to values found in fresh tissue than corneas stored in K-Sol. Net endothelial sodium fluxes following storage in modified MK medium were markedly less than those found in corneas stored for 3, 7 and 14 days in K-Sol. Net sodium fluxes are maintained better in K-Sol than in MK medium relative to control values. Net bicarbonate fluxes following storage in modified MK medium were significantly less than the 3-day values in K-Sol, but similar to the values after 7 and 14 days of K-Sol storage. All net ion fluxes, except for sodium at 7 days storage in K-Sol, were significantly lower than control values. MK medium appears to preserve endothelial barrier function better than K-Sol, but K-Sol preserves net ionic movement better than MK medium. Invest Ophthalmol Vis Sci 28:2088–2091, 1987

MK medium has been used to store corneas for periods of 72–96 hr prior to usage for penetrating keratoplasty. K-Sol® (Cilco, Inc., Huntington, WV) is a new corneal storage medium in which 2.5% chondroitin sulfate replaces the dextran in tissue culture medium. It is thought that the chondroitin sulfate in this solution enhances the storage characteristics of tissue culture medium, thereby allowing for longer storage periods. It was the purpose of this investigation to evaluate and compare corneal endothelial sodium ion, bicarbonate ion, inulin, and dextran fluxes following storage for 3, 7, and 14 days in...
Table 1. Corneal endothelial non-ionic fluxes after different storage times

<table>
<thead>
<tr>
<th>Time</th>
<th>Modified MK medium</th>
<th>K-Sol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inulin</td>
<td>Dextran</td>
</tr>
<tr>
<td>Control</td>
<td>1.55 ± 0.04</td>
<td>1.69 ± 0.04</td>
</tr>
<tr>
<td>3 days</td>
<td>1.70 ± 0.12</td>
<td>1.22 ± 0.09†</td>
</tr>
<tr>
<td>7 days</td>
<td>2.78 ± 0.11†</td>
<td>1.59 ± 0.07</td>
</tr>
<tr>
<td>14 days</td>
<td>2.39 ± 0.11†</td>
<td>1.59 ± 0.05</td>
</tr>
</tbody>
</table>

Values are X 10^-6 cm/sec, and are the mean ± SEM of six corneas at each time and solution except for control corneas where n is 4. These values apply to both storage solutions since they were measured on fresh, unstored corneas in bicarbonate Ringer solution identical to that used for measurements on stored corneas.

Values that do not increase beyond 7 days.

* Significantly greater (P < 0.01) than paired corneas in MK medium.
† Significantly different (P < 0.02) from control values.
‡ Significantly different (P < 0.05) from control values.

**Table 2.** For bicarbonate, Jnet markedly decreased from control values following 3 days of storage and remained low following 7 and 14 days of storage in MK medium. Following 3 days of storage in K-Sol, Jnet and Jsr,endo were significantly greater than in the corresponding paired corneas stored for 3 days in MK medium. The change in Jnet for bicarbonate was caused by an approximately 20% fall in Jst,endo and an approximately 30% increase in Jsr,endo relative to control.

The endothelial permeability was calculated as before with dimensions of cm/sec. Sodium and bicarbonate fluxes were determined as previously described.

**Materials and Methods.** Adult albino rabbits weighing approximately 3 kg were killed with an intravenous overdose of sodium pentobarbital. Corneas were de-epithelialized, the eyes removed, and the corneas mounted on Teflon rings. These techniques conformed to the ARVO Resolution on the Use of Animals in Research. One cornea of each pair was placed in a 20 ml bottle of modified McCarey-Kaufman corneal storage medium (Aurora Biologicals, Ltd., Williamsville, NY) with 100 μg/ml gentamicin, and HEPES buffer. The contralateral cornea was placed in a 20 ml bottle of K-Sol corneal storage medium with 100 μg/ml gentamicin, and HEPES buffer. The corneas were then stored at 4°C for periods of 3, 7, or 14 days. A total of six corneas were stored in each medium for each time period.

After storage, corneas were removed and mounted in water-jacketed chambers at 37°C as previously described. The chambers were filled with Krebs-bicarbonate Ringer, with 0.3 mM reduced glutathione and 0.5 mM adenosine, at pH 7.3 and 307 ± 2 mOsm that was bubbled with a 97% O2-3% CO2 mixture during preparation. The solution facing the de-epithelialized corneal surface contained a Teflon stirring bar that was stirred at approximately 400 rpm using an externally mounted horseshoe magnet attached to a small motor. Ringer, dual-labeled with 1.12 μCi/ml dextran (carboxyl14, mol wt 60,000-90,000; New England Nuclear, Boston, MA) and 1.07 μCi/ml inulin (14C, mol wt 5000; Amersham, Arlington Heights, IL), was used to replace the Ringer bathing the endothelial surfaces of corneas. Tissues were then allowed to equilibrate for 1 hr. Normal sampling procedures were then employed with 5 ml flushes of the least radioactive chamber with fresh non-radioactive Ringer every 30 min. Samples were collected, weighed, sampled and counted. Counts were corrected for the 10.5% 14C spillover into the 3H channel and the 0.5% 3H spillover into the 14C channel.

The endothelial permeability was calculated as before with dimensions of cm/sec. Sodium and bicarbonate fluxes were determined as previously described. Paired corneas were used, one of each pair for storage in K-Sol and the other of each pair in MK Medium. Either the stroma to endothelial flux or the endothelial to stroma flux was determined on at least five paired corneas. Unidirectional fluxes from stroma to endothelium (Jendo) and from endothelium to stroma (Jendo) were computed 5-7 and net flux (Jnet) was expressed as the algebraic sum of the two. In all cases, the mean of the data at 30 min intervals was computed and the standard error of the mean determined. A comparison of experimentals and controls was made with the t-test: paired corneas were compared using the student t-test and comparison to controls was made with the two-tailed t-test. A P level of 0.01 was used to determine statistical significance for values following storage in MK medium versus storage in K-Sol, and for comparison of stored corneas versus control corneas.

**Results.** The corneal endothelial permeabilities to inulin (effective molecular radius, 14 Angstroms) and dextran (effective molecular radius, 38 Angstroms) are shown in Table 1. The permeabilities for both solutes after storage in either solution are similar after 3 days. After 7 and 14 days of storage the permeability to inulin and dextran is significantly greater following storage in K-Sol than it is following storage in modified MK medium. The solute permeability increases with time of storage in both media, reaching values that do not increase beyond 7 days.

The corneal endothelial ionic fluxes are shown in Table 2. For bicarbonate, Jnet markedly decreased from control values following 3 days of storage and remained low following 7 and 14 days of storage in MK medium. Following 3 days of storage in K-Sol, Jnet and Jsr,endo were significantly greater than in the corresponding paired corneas stored for 3 days in MK medium. The change in Jnet for bicarbonate was caused by an approximately 20% fall in Jst,endo and an approximately 30% increase in Jsr,endo relative to control.
Corneal endothelial ionic fluxes after different storage times

Table 2. Corneal endothelial ionic fluxes after different storage times

<table>
<thead>
<tr>
<th></th>
<th>Modified MK medium</th>
<th>K-Sol</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$J_{in}$, $J_{endo}$, $J_{net}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>r</td>
<td></td>
</tr>
<tr>
<td>Bicarbonate:</td>
<td>Control: 3.99 ± 0.11</td>
<td>3.99 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>3 days: 3.28 ± 0.11†</td>
<td>3.91 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>7 days: 3.360.11†</td>
<td>3.05 ± 0.17†</td>
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<tr>
<td></td>
<td>14 days: 3.34 ± 0.10†</td>
<td>3.08 ± 0.11†</td>
</tr>
<tr>
<td>Sodium:</td>
<td>Control: 12.56 ± 0.36</td>
<td>12.56 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>3 days: 9.79 ± 0.29†</td>
<td>11.50 ± 0.33*</td>
</tr>
<tr>
<td></td>
<td>7 days: 10.76 ± 0.35†</td>
<td>16.51 ± 0.94†</td>
</tr>
<tr>
<td></td>
<td>14 days: 9.63 ± 0.30†</td>
<td>15.69 ± 0.77†</td>
</tr>
</tbody>
</table>

Values are in μeq/cm²/hr and are the mean ± SEM of at least five corneas at each time and solution.

* $P < 0.01$ compared to paired corneas.
† Significantly different ($P < 0.01$) from control values.

Control values ($n = 10$) apply to both solutions since they were measured on fresh corneas bathed in bicarbonate Ringer solution identical to that used for flux measurements on stored corneas.

trol values. Net sodium flux also decreased rapidly following storage in MK medium, but was better maintained in K-Sol. The $J_{net}$ values for sodium are significantly greater for K-Sol-stored corneas than MK-stored corneas following 3 and 14 days of storage, and nearly achieved significance ($P = 0.04$) following 7 days of storage. The reduction in $J_{net}$ for sodium following storage in MK medium is the result of an approximately 20% decrease in $J_{endo}$ relative to control values. Following storage in K-Sol, $J_{endo}$ is maintained at 3 days and increased by approximately 25% at 7 and 14 days. $J_{net}$ was similar to control values following 3 days of storage in either solution, but at 7 and 14 days was better maintained by MK.

Discussion. The slight increase in passive non-electrolyte permeability with time seen in the present experiments with endothelia stored in modified MK medium (Table 1) resembles previous data under similar conditions using original MK medium. The original MK medium used a bicarbonate buffer system which was inherently unstable and allowed the solution pH to fluctuate. Modified MK medium has all of the constituents of the original MK medium and in addition contains HEPES buffer, gentamicin and phenol red. No change was found in dextran permeability with 10 days storage in original MK medium. The present comparison of MK medium and K-Sol indicates that modified MK medium preserves the barrier function to non-electrolytes better than does K-Sol for storage times greater than 3 days. Corneal endothelial permeability to both inulin and dextran after 7 and 14 days of K-Sol storage is significantly increased relative to those values found following storage in modified MK medium. The endothelial permeability in K-Sol is approximately 50% greater than that in modified MK medium for both solutes after 7 and 14 days of storage (Table 1), indicating greater stability of the endothelial barrier following storage in modified MK medium. This may in part explain the somewhat prolonged stromal edema that is noted clinically following transplantation of corneas stored in K-Sol.6

Corneas stored in MK medium become thicker during storage compared to the more constant thickness in K-Sol,9 and the relative permeability found in the present studies could reflect these differences in thickness. At the time of measurement, however, two factors make this possibility most unlikely. First, the permeability measurements are made after at least 1 hr equilibration of the de-epithelialized corneas in Ringer following removal from the storage media and prior to permeability or flux determinations, and since stroma swells rapidly,10 no differences should exist in corneal thickness regardless of the corneal storage history. Second, previous studies11 have shown that inulin permeability of endothelial/stromal preparations are the same regardless of the stromal hydration.

Previous studies12 have shown that bicarbonate fluxes of endothelia stored in original MK medium showed a large decrease in $J_{endo}$ coupled with a small increase in $J_{net}$ relative to fresh corneas. The present data with modified MK medium confirm this finding and indicate that the effects are more pronounced following storage in modified MK medium. Jnet for bicarbonate is obliterated following storage in modified MK (Table 2). Jnet for bicarbonate is 50% of the control value after 3 days of storage in K-Sol and drops to near zero thereafter. The decrease in Jnet for bicarbonate following storage in both storage media is caused by an increase in $J_{endo}$ and a decrease in $J_{net}$ compared to control values.

Sodium fluxes are maintained better in K-Sol than in modified MK medium for up to 14 days. The
decrease in Jnet for sodium following storage in modified MK medium is caused by an increase in Jstr and a decrease in Jnrd. In K-Sol, however, despite an increase in Jstr, there is a substantial compensatory increase in Jnrd that causes Jnet to be sustained at about one-half the normal value. The reason for the increase in Jstr with storage in K-Sol at 7 and 14 days is unclear. K-Sol and modified MK medium are identical, except for the difference in colloid osmotic agents: MK medium has dextran and K-Sol has chondroitin sulfate. It is highly unlikely that chondroitin sulfate serves as any metabolic precursor, but it may serve to maintain cellular metabolic integrity better than dextran. Jstr for bicarbonate and sodium is increased following storage in both media, although the increase for sodium following storage in K-Sol is greater than that seen following storage in MK medium. The results imply that the chondroitin sulfate in K-Sol may have a tendency to enhance the passive permeability of the endothelium. All data on sodium and bicarbonate fluxes were obtained under open-circuit conditions; there is thus a possibility that the slower decline in the Jnrd flux in K-Sol may reflect changes in other functions rather than on the pump activity. Nevertheless, the present data correlate with, and offer an explanation for, the clinically observed differences between the behavior of corneas stored in MK medium and K-Sol.

The disparate effects on sodium and bicarbonate fluxes following storage may indicate that sodium and bicarbonate movement across the endothelium occurs by different pathways. Other data obtained by totally different approaches have shown that sodium and bicarbonate movement across the corneal endothelium is probably not linked by a symport mechanism.

In summary, the present data show that modified MK medium preserves the barrier function of the endothelium to large non-electrolytes better than K-Sol does during corneal storage. Storage in K-Sol, however, offers better preservation of ionic movement, particularly that of sodium.

**Key words**: cornea, MK medium, K-Sol, endothelial ion fluxes, non-electrolyte fluxes

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From the Department of *Ophthalmology and the Department of *Physiology and Endocrinology, Medical College of Georgia, Augusta, Georgia. Supported in part by research grants EY-04449 (Dr. Hull), EY-04558 (Dr. Green) and by a Core Grant for Vision Research (EY-04636), from the National Eye Institute, and in part by a Research to Prevent Blindness, Inc., Departmental Research Award. Submitted for publication: January 14, 1987. Reprint requests: David S. Hull, MD, Department of Ophthalmology, Medical College of Georgia, Augusta, GA 30912.

**References**