CORNEAL PRESERVATION AT 4°C WITH CHONDROITIN SULFATE CONTAINING MEDIUM*

BY *Richard L. Lindstrom,* MD (BY INVITATION),
Donald J. Doughman, MD,
Debra L. Skelnik, BS (BY INVITATION),
AND (BY INVITATION) *Elizabeth A. Mindrup,* BS

INTRODUCTION

THE FUNCTIONAL STATUS OF THE ENDOTHELIUM AND SUSTAINED MAINTENANCE OF corneal deturgescence after corneal storage at 4°C are of great clinical importance. Limited short-term storage of corneas (4 days or less)[1,2] at 4°C has been provided by McCarey-Kaufman (M-K) medium and whole globe storage in moist chamber.[4,5] Chondroitin sulfate, a negatively charged glycosaminoglycan, has been utilized in Japan to extend the storage and preservation of whole globes for up to 3 days.[6] In addition, chondroitin sulfate containing preservation medium has significantly enhanced the quality of donor tissue and prolonged the length of storage at both 34°C and 4°C in the Minnesota corneal preservation system.[7-9]

In a recent clinical study, 83 donor corneas were transplanted after 1 to 7 days storage at 4°C utilizing a commercially prepared corneal preservation medium formulation, CSM (Aurora Biologicals, Ltd), containing 1.35% chondroitin sulfate. Excellent endothelial cell preservation with up to 12 months postkeratoplasty follow-up was observed with no primary donor failures.[10] Clinical studies with K-Sol[11] (Cooper-Cilco, Inc), a 4°C corneal storage medium containing 2.5% chondroitin sulfate have also demonstrated successful transplantation after up to 14 days storage.[12] However, in a study by Bourne,[13] significantly greater endothelial cell

*From the Department of Ophthalmology, University of Minnesota, Minneapolis, Minnesota. Supported in part by an unrestricted grant from Research to Prevent Blindness, Inc.

loss was found in patients whose corneas were stored in K-Sol at 4°C for more than 7 days with two primary donor failures reported.

Corneal storage media are a composite of balanced salts, amino acids, energy sources, antioxidants, buffering agents, cell membrane stabilizers, deturgescents, and antibiotics. The lower temperature of the 4°C storage method reduces the metabolic rate of the cornea, but the storage medium must still support the basal requirements of the cornea. Temperature reduction changes membrane lipids, proteins, and water structures, each of which could alter barriers hindering the ease of passive diffusion, carrier-substrate interaction and energy coupling relationships of the active transport mechanism.[14] Thus, disturbances in membrane function as well as morphological and biochemical alterations may be of greater consequence at this temperature as a direct result of the lower metabolic rate. Therefore, critical evaluation of physiologic parameters such as ionic and amino acid composition, bicarbonate equilibrium, available energy sources, dissolved oxygen levels, osmolality and pH should be observed with respect to each preservation medium. Parameters for extended 4°C storage should be defined as to the reversibility of cell damage incurred during storage.

We, therefore, designed a study to assess these two commercial 4°C storage media, CSM and K-Sol, analyzing length of storage time and ability to support corneal endothelial cell viability.

MATERIALS AND METHODS

Sixteen pairs of human research corneas were obtained from the Lions Eye Bank of Washington and Northern Idaho for this comparative study of 4°C media. Paired human donor eyes, unsuitable for transplantation because of donor age or disease, were removed within 8 hours of death. Whole globes were decontaminated by a vigorous irrigation with sterile balanced salt solution. Excess conjunctival tissue was trimmed and the sclera was scored with a number 11 Bard-Parker blade 2 to 3 mm from the limbal area (360 degrees). The cornea was excised by making an initial incision onto the sclera to the suprachoroidal space, with the remainder of the corneal rim being excised with a corneal scissors. Corneoscleral rims were removed and placed endothelial side up in 20 ml of commercial CSM or K-Sol medium in a random fashion, and transported by automobile and commercial airlines surrounded by ice to the Minnesota Lions Eye Bank research laboratory. Four paired corneas were stored in each medium at 4°C for four different time intervals: 7, 10, 14, and 22 days. After 4°C storage, corneas were equilibrated for 2 hours at 34°C in closed

bottles of test medium and then evaluated. The corneas were evaluated in a masked fashion following removal from unlabeled storage bottles.

Corneal thickness measurements were made using a Leitz upright microscope fitted with a micrometer. The micrometer dial indicator was attached to the microscope stand above the stage with the set screw placement through the stage, directly under the foot of the dial indicator. The corneal thickness measurement involved focusing on the endothelium, setting the set screw to bring the dial micrometer to "zero", raising the stage to bring the epithelium into focus, and recording the dial indicator reading. Three thickness readings were made at two different central corneal sites to determine the mean corneal thickness.

Endothelial cell viability was determined after staining the corneas with 0.1% trypan blue, whereas cell membrane integrity and areas of denuded endothelium were assessed after staining with alizarin red S. Areas of damaged or denuded endothelium were quantitated by computer assisted digitization and analysis. Central endothelial cell counts were determined from high power magnification (250×) photographs. One way analysis of variance and the Newman-Keuls multiple range test were used to evaluate statistical significance ($P < 0.05$) between the two test groups.

Evaluations of the medium components were conducted on sealed bottles of media as received from the manufacturers. Electrolyte analysis of Na^+, K^+, Cl^-, Ca^{2+}, glucose determinations, HCO_3^-, pCO_2, pO_2, and pH values were conducted by the Department of Laboratory Medicine and Pathology at the University of Minnesota under standard laboratory conditions. HCO_3^-, pCO_2, pO_2, and pH values were determined at 34°C. The osmolality of the media was determined using a Precision Systems freezing point depression osmometer. Gentamycin concentrations were quantitated by the Department of Toxicology at the University of Minnesota, employing a fluorescence polarization immunoassay.

RESULTS

CORNEAL THICKNESS

The mean (± SD) corneal thickness for corneas stored in CSM and K-Sol for 7, 10, and 14 days is presented in Fig 1. No statistical difference in corneal thickness ($P < 0.05$) was seen at 7, 10, or 14 days storage between CSM and K-Sol. K-Sol corneas at 7 days were thinner (463 ± 27 μm) than the CSM stored corneas (501 ± 42 μm) at the same time period. K-Sol stored corneas increased in corneal thickness with time at 10 days (513 ±

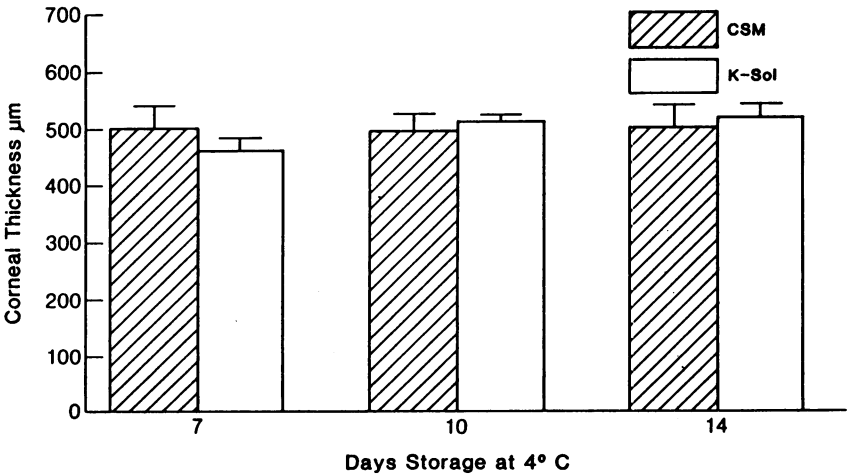

FIGURE 1
Mean corneal thickness of corneas stored in CSM or K-Sol at 4°C for 1, 10, and 14 days.

14 µm) and 14 days (520 ± 26 µm) storage. Corneas stored in CSM maintained similar corneal thickness throughout the 14 day storage time (10 days, 498 ± 28 µm; 14 days, 500 ± 36 µm).

ENDOTHELIAL CELL DAMAGE

Computerized digitization of the damaged endothelial areas allowed direct comparison of the two test media, where damaged areas were expressed as percent of total endothelial area. Endothelial cell damage was noted in the periphery of both the CSM and K-Sol corneas. Trypan blue and alizarin red S staining were present in lines radiating inwardly from the limbal edge, and are probably the result of folds or trauma to the cornea during preparation (Fig 2). The percent mean (± SD) area of endothelial cell damage for corneas stored in CSM and K-Sol is presented in Fig 3. Although greater endothelial cell damage was consistently observed in the K-Sol stored corneas (7 days, 9.5% ± 11.9%; 10 days, 9.8% ± 7.4%; 14 days, 7.3% ± 6.6%) as compared to the CSM stored corneas (7 days, 2.9% ± 2.3%; 10 days, 4.9% ± 4.1%; 14 days, 2.8% ± 1.9%) this difference was not statistically significant ($P < 0.05$) in this small series. The relative mean loss in both test groups was consistent, independent of storage time, up to 14 days.

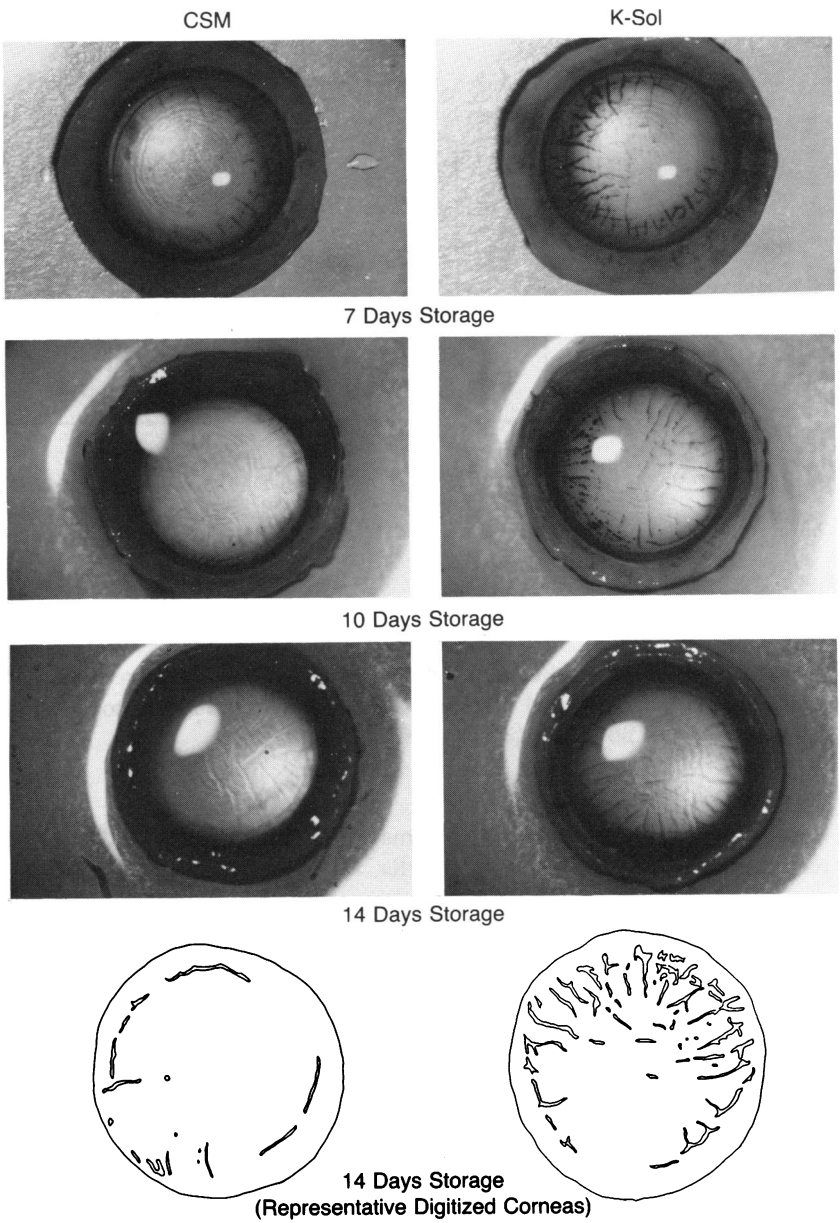

FIGURE 2
Trypan blue staining of corneas stored in CSM or K-Sol at 4°C for 7, 10, and 14 days.

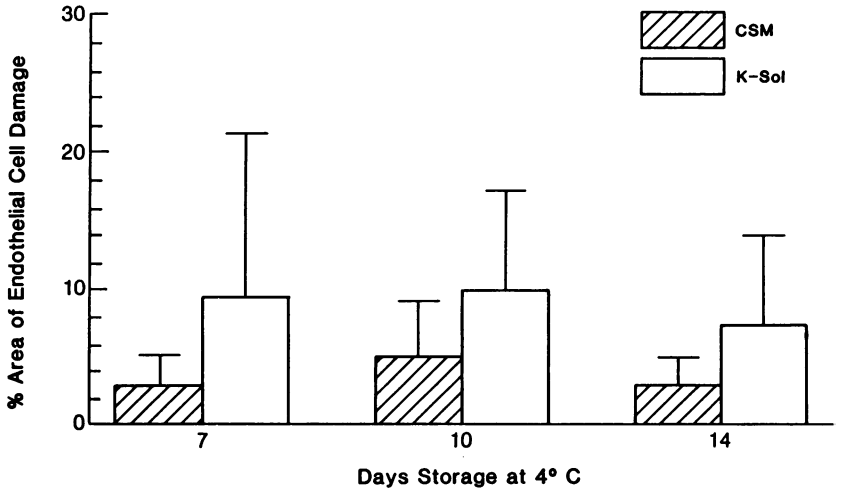

FIGURE 3
Computer digitization of area of endothelial cell damage of corneas stored in CSM or K-Sol at 4°C for 7, 10, and 14 days.

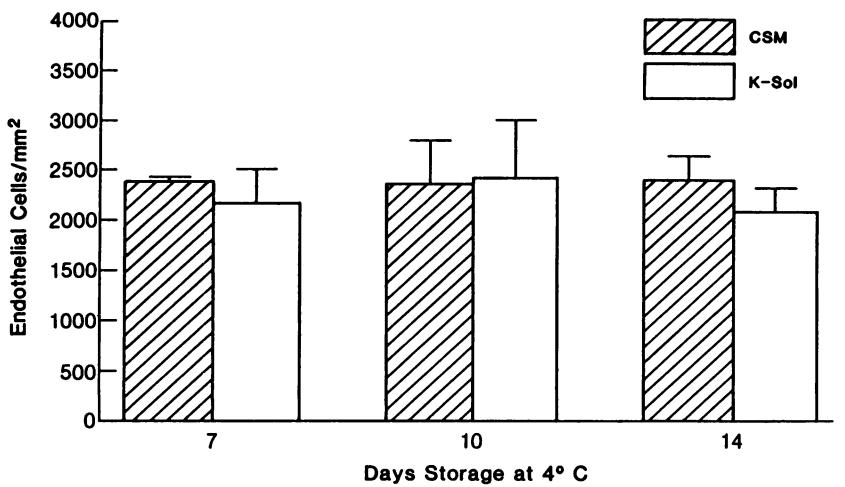

FIGURE 4
Mean central endothelial cell counts for corneas stored in CSM or K-Sol at 4°C for 7, 10, and 14 days.

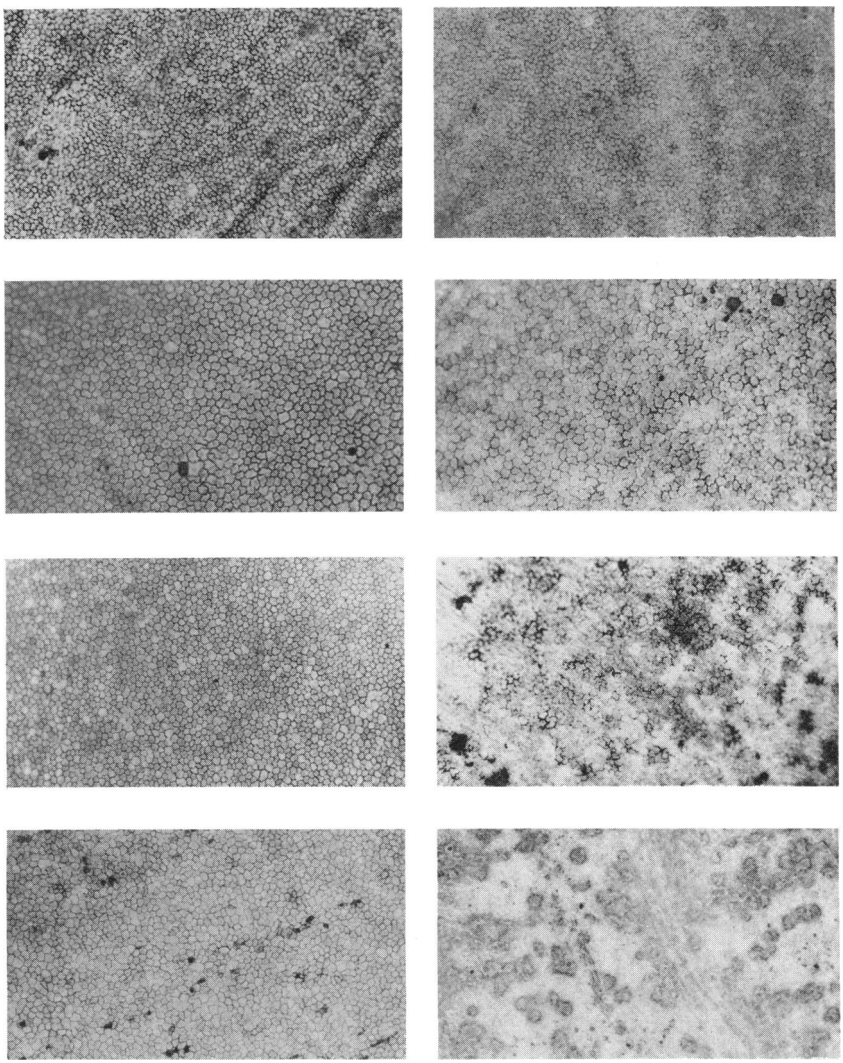

FIGURE 5
Trypan blue and alizarin red S staining of endothelium of corneas stored in CSM or K-Sol at 4°C for 7, 10, 14, and 21 days. Corneas stored in CSM at 4°C for 7, 10, 14, and 22 days exhibit intact endothelial cell monolayers and maintenance of endothelial cell membrane integrity with distinctly stained borders. Corneas stored in K-Sol at 4°C for 7 and 10 days exhibit intact endothelial cell monolayers. At 14 days progressive reduction in endothelial cell membrane integrity is demonstrated by alternation in alizarin red staining and endothelial cell morphology. At 22 days storage, Descemet's membrane was devoid of cells.

CENTRAL ENDOTHELIAL CELL COUNTS

Due to low temperature storage, and the lack of cell migration at this temperature, loss of endothelial cells in the periphery does not effect the central endothelial cell counts. Mean (± SD) central endothelial cell counts are shown in Fig 4. CSM stored corneas had central endothelial cell counts of 2394 ± 41, 2360 ± 427, and 2390 ± 227 cells/mm^2 at 7, 10, and 14 days, respectively. Similarly K-Sol stored corneas had endothelial cell counts of 2177 ± 329, 2414 ± 582, 2085 ± 235 cells/mm^2 for the same time periods. While the endothelial cell count at 14 days was 13% lower in K-Sol, no statistical differences ($P < 0.05$) in central endothelial cell counts were found at 7, 10, or 14 days storage in CSM and K-Sol. At 22 days storage the corneal endothelial monolayer was preserved in CSM (2140 ± 296 cells/mm^2), whereas all K-Sol corneas demonstrated virtually total cell death.

EPITHELIAL AND ENDOTHELIAL CELL MORPHOLOGY

Epithelial cell layers appeared more edematous in corneas stored in CSM, and sloughing of epithelium was observed at 2 to 5 days. K-Sol stored corneas maintained epithelium more consistently during the first 2 to 3 days storage, but sloughing of epithelium was observed at 4 to 5 days. Microscopic evaluation of the epithelium at 7 days for both test media showed rounded, loosely adherent basal epithelium.

In assessing cell morphology trypan blue staining allows a direct measurement of cell viability, while alizarin red S staining allows visualization of cell morphology and the determination of cell adhesion, tightness of intercellular spaces and gross membrane integrity. In the photographs shown in Fig 5, corneas stored in CSM at 4°C for 7, 10, 14, and 22 days exhibit intact endothelial cell monolayers and maintenance of endothelial cell membrane integrity with distinctly stained borders. Corneas stored in K-Sol at 4°C for 7 and 10 days exhibit intact endothelial cell monolayers. Cells in both media demonstrate slight alterations in the classic hexagonal pattern seen *in vivo*, appearing mildly dehydrated and exhibiting greater intercellular digitation. Wider and more irregular staining of intercellular spaces is seen more frequently with K-Sol stored corneas at 7 and 10 days storage as compared to CSM stored corneas for the same time periods. At 14 days storage in K-Sol, progressive reduction in endothelial cell membrane integrity is demonstrated by alteration in alizarin red S staining and endothelial cell morphology. At 22 days storage, K-Sol stored corneas exhibited large areas of centrally denuded Descemet's membrane with occasional clusters of intact cells.

EVALUATION OF MEDIUM COMPONENTS

Components of CSM and K-Sol were compared as to their base medium and additional medium supplementation and are presented in Table I. CSM was formulated from experimental work supporting corneal preservation at 34°C for periods of up to 5 weeks.[9] CSM utilizes Eagles Modified Minimal Essential Medium (MEM) with Earle's balanced salt solution and 25 mM HEPES supplemented with 1.35% chondroitin sulfate, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids and an additional antioxidant. All concentrations of the MEM base medium are maintained as formulated. Sodium pyruvate is added as an alternative energy source, provided to enhance preservation of the cornea during 4°C storage.

K-Sol is composed of TC-199 medium, with Hank's balanced salts and supplemented with 2.5% modified chondroitin sulfate and 50 mM HEPES. The base medium has been diluted approximately 26% with a balanced salt solution to maintain tonicity. The amino acid, vitamin, and other base medium component concentrations are similarly reduced by this dilution. This is demonstrated more clearly when we compare the chemical composition of human aqueous humor[21] with CSM and K-Sol (Table II). The greatest differences in the concentration of components in K-Sol as compared to human aqueous humor are seen in reduced calcium, chloride, and bicarbonate concentrations. Most significant of these components is the low bicarbonate levels (< 4 mM/l) of K-Sol as compared to the physiologic concentration of 20.2 mM/l of bicarbonate found in aqueous humor. The concentration of components of CSM are similar to those found in human aqueous humor, with slightly higher concentrations of sodium, potassium, and glucose.

Dissolved oxygen levels were similar for CSM (170 mm Hg) and K-Sol (168 mm Hg). The mean ($\pm$ SD) gentamycin concentrations evaluated for CSM and K-Sol were 67.3 $\pm$ 0.7 mg/l and 69.6 $\pm$ 3.7 mg/l. The osmolality of CSM was 368 $\pm$ 3 mOsm as compared with 322 $\pm$ 4 mOsm for K-Sol. The pH of CSM and K-Sol was measured at 7.09 $\pm$ 0.1 and 7.31 $\pm$ 0.1, respectively.

DISCUSSION

The ability of the cornea to maintain a relatively dehydrated state is essential to the maintenance of corneal transparency. Deturgescence is an energy dependent phenomenon performed primarily by the endothelial cells.[22-24] The lower temperatures of 4°C storage methods reduce the rate of metabolic functions of the cornea, but still require the storage medium

TABLE I: COMPARISON OF 4°C CORNEAL STORAGE MEDIA

MEDIUM COMPONENTS	CSM*	K-Sol
Base medium	MEM	TC-199
Chondroitin sulfate (%)	1.35	2.5 (modified CS)
L-Glutamine (mM)	2.0	0
Sodium pyruvate (mM)	1.0	0
Nonessential amino acids (mM)	0.1	0
HEPES buffer (mM)	25.0	50.0
Gentamycin (mg/l)	67.3	69.6
Osmolality (mOsm)	368	322
pO_2 (mm Hg)	170	168
pCO_2 (mm Hg)	64	7

*Additional antioxidant added.

TABLE II: CHEMICAL COMPOSITION OF HUMAN AQUEOUS, CSM AND K-SOL

CONSTITUENT	HUMAN AQUEOUS HUMOR (mM/l)	CSM (mM/l)	K-SOL (mM/l)
Sodium	163	206	206
Potassium	2.2-3.9	5.3	4.2
Chloride	132	132	108
Glucose	2.7-3.7	5.2	3.9
Bicarbonate	20.2	19	< 4
Calcium	1.8	1.8	< 1.1
pH	7.38	7.09	7.31
Osmolality	332	368	322

to support the basal requirements of the cornea. In order for the cornea to remain viable, various enzymes must carry out energy dependent functions, maintained by high levels of ATP.

Both chondroitin sulfate media, CSM, and K-Sol, provide good endothelial cell preservation for up to 10 days storage at 4°C. No statistical differences in corneal thickness, central endothelial cell counts or percent area of endothelial cell damage were found at 7 and 10 days of 4°C storage between CSM and K-Sol. CSM maintained superior endothelial cell morphology at 14 and 22 days storage at 4°C. At 14 days storage progressive reduction in endothelial cell membrane integrity was demonstrated in K-Sol stored corneas, and at 22 days storage, K-Sol stored corneas exhibited large areas of centrally denuded Descemet's membrane with occasional clusters of intact cells.

Corneal storage media are a composite of deturgescent agents, amino acids, energy sources, balanced salts, buffering agents, and antibiotics. The base medium of CSM and K-Sol are similar in their nutrient composi-

tion, although the K-Sol formulation reduces the concentration of the TC-199 components by approximately 26% to adjust for the tonicity of the solution. The osmolality of CSM is greater than that of K-Sol and should be lowered to reduce tissue rebound swelling during keratoplasty. CSM supplements its base MEM medium with an additional energy source (sodium pyruvate), additional nonessential amino acids, and an antioxidant to enhance corneal preservation at 4°C. The greatest differences in the concentration of components in K-Sol as compared to human aqueous are seen in reduced calcium, chloride, and bicarbonate concentrations. The concentration of components of CSM are similar to those found in human aqueous humor with slightly higher concentrations of sodium, potassium, and glucose. Antibiotic concentrations found in both CSM and K-Sol were reduced from manufacturers specifications, indicating possible loss of the aminoglycoside due to sterile filtration during media preparation.

Tissue stored in chondroitin sulfate containing medium swells to a greater extent than tissue stored in M-K medium. Dextran, an effective osmotic agent in M-K medium, keeps the cornea thin and maintains the barrier function more efficiently than the modified chondroitin sulfate found in K-Sol.[15] However, the limitations of 4°C storage time in M-K initiated the transition to chondroitin sulfate containing medium as an intermediate storage medium. The major differences in the chondroitin sulfate used in K-Sol as compared to CSM is the removal of the low molecular weight fraction (< 10,000 mw) of chondroitin sulfate polymers in K-Sol.[12] While this may enhance corneal deturgescence, *in vitro* studies involving human corneal endothelial cells have shown increased growth rates when low molecular weight chondroitin sulfates are retained.[16] The lower molecular weight fraction of chondroitin sulfate is more easily degraded and subsequently incorporated into the endothelial cells, enhancing cell attachment.[17-20] Clinically, the corneas stored in CSM become transiently hazy at the close of surgery due to the incorporation of low molecular weight chondroitin sulfate polymers, which attract water. This is offset by the beneficial aspects of their membrane stabilizing activity and incorporation into the cell.

Differences in the formulations of the two chondroitin sulfate media may account for the difference in quality of endothelial preservation after prolonged 4°C storage based on the metabolic requirements of this tissue. Bicarbonate is actively transported across the endothelium, from the stromal to aqueous-facing surface,[25-28] and experimental work with dextran-bound carbonic anhydrase inhibitors suggests that the exit of bicarbonate from the cell is influenced by the enzyme carbonic anhydrase.

Carbonic anhydrase, therefore, contributes to the net flux of bicarbonate.[25] Bicarbonate also has a passive tendency to "leak" across the endothelium from the aqueous humor into the stroma.[26] Together with bicarbonate, a net flux of sodium occurs across the endothelium.[14,29,30] Sodium is a required ion for bicarbonate transport and maintenance of endothelial function.[27,31,32,34] The link between fluid movement, thickness control, potential difference across the endothelium, and the presence of bicarbonate and sodium in the bathing medium appears strong.[34] Bicarbonate concentration was shown to be the most important modification of tissue culture media needed to support rabbit corneal deturgescence after short-term 4°C storage.[35]

The reduction of the bicarbonate concentration in K-Sol may have significant effects on endothelial function and survival. HEPES buffer was first added to M-K 4°C storage medium[36] to stabilize and resist rapid changes in pH due to the inadequacy of bicarbonate buffers alone. Prior investigations indicate that HEPES buffer should be used in conjunction with a bicarbonate and phosphate buffer system due to the bicarbonate requirements of most mammalian cells. Prolonged 4°C storage times with chondroitin sulfate containing medium may contribute to cell damage, possibly related to bicarbonate depravation. In a bicarbonate-poor medium (5 mM), the bicarbonate pump activity is severely compromised.[37] This suggests that there is a minimal bicarbonate concentration required for the effective functioning of the pump. Complete removal of bicarbonate has shown rapid, essentially transient depolarization of the plasma membrane in cultured bovine cells.[38] The parameters of bicarbonate concentration for maintenance of the pump and reactivation of the membrane potential after prolonged 4°C storage time periods has not yet been established.

The phosphatic metabolites of paired cat corneas stored *ex vivo*, using ^{31}P MR spectroscopy, following storage in CSM and K-Sol after 2, 7, and 14 days, demonstrated that endothelial function in epithelial denuded corneas was maintained more effectively with CSM than K-Sol. ATP levels significantly declined in the corneas stored in K-Sol while CSM stored corneas maintained ATP levels throughout the 14 day incubation period.[39] This study is consistent with our findings on the maintenance of endothelial cell morphology and membrane integrity of corneas stored in CSM and K-Sol. A progressive loss of endothelial cell integrity with corneas stored for 14 days in K-Sol is consistent with a concomitant loss of ATP, while maintenance of ATP levels with CSM resulted in preservation of endothelial cell morphology over the same time period. Morphological, functional, and biochemical alterations, and disturbances in membrane

function are early features of irreversible cell damage. These findings are supportive of progressive loss of mitochondrial function with extended 4°C storage. A number of structural and functional alterations can be accounted for, in large part, by the inhibition of adenine nucleotide translocation and an increase in the permeability of the inner mitochondrial membrane. There is a correlation between the loss of reversibility of cell damage and the inability to reverse mitochondrial disfunction.

Intermediate corneal storage at 4°C should provide tissue preservation that is capable of maintaining the functional status of the endothelium and sustaining maintenance of corneal deturgescence postkeratoplasty. Chondroitin sulfate containing medium appears to be safe and efficacious for corneal storage at 4°C for at least 10 days. If corneal storage for more than 10 days is required we prefer 34°C organ culture because of improved epithelial, and more consistent endothelial, preservation.

ACKNOWLEDGMENTS

We would like to acknowledge the donation of research tissue and technical support from the Lions Eye Bank of Washington and Northern Idaho and the Minnesota Lions Eye Bank.

REFERENCES

1. Van Horn DL, Schultz RO, DeBruin J: Endothelial survival in corneal tissue stored in M-K medium. *Am J Ophthalmol* 1975; 80:642-647.
2. Aquavella JV, Van Horn DL, Haggerty CJ: Corneal preservation using M-K medium. *Am J Ophthalmol* 1975; 80:791-799.
3. McCarey BE, Kaufman HE: Improved corneal storage. *Invest Ophthalmol Vis Sci* 1974; 13:165-173.
4. Hassard DTR: Selection of donor material for corneal grafting, in JH King, JE McTigue (eds): *The Cornea, World Congress*. London, Butterworth, 1965, pp 370-383.
5. Binder PS: Eye banking and corneal preservation, in *Symposium on Medical and Surgical Disease of the Cornea*. New Orleans Academy of Ophthalmology, St Lous, CV Mosby, 1980, pp 320-354.
6. Mizukawa T, Manabe R: Recent advances in keratoplasty with special reference to the advantage of liquid preservation. *Folia Ophthalmol Jpn* 1968; 19:1310.
7. Lindstrom RL, Skelnik DL, Mindrup EA, et al: Organ culture preservation with chondroitin sulfate. *Invest Ophthalmol Vis Sci* (Suppl) 1984; 25:266.
8. ———: Minnesota system corneal preservation. *Invest Ophthalmol Vis Sci* (Suppl) 1985; 26:266.
9. Lindstrom RL, Doughman DJ, Skelnik DL, et al: Minnesota system corneal preservation. *Br J Ophthalmol* 1986; 70:47-54.
10. Lindstrom RL, Skelnik DL, Mindrup EA, et al: Corneal preservation at 4°C with chondroitin sulfate containing medium. *Invest Ophthalmol Vis Sci* (Suppl) 1987; 28:167.
11. Kaufman HE, Varnell ED, Kaufman S: Chondroitin sulfate in a new cornea preservation medium. *Am J Ophthalmol* 1984; 98:112-113.

12. Kaufman HE, Varnell ED, Kaufman S, et al: K-Sol corneal preservation. *Am J Ophthalmol* 1985; 100:299-304.
13. Bourne WM: Endothelial cell survival on transplant human corneas preserved at 4°C in chondroitin sulfate for one to 13 days. *Am J Ophthalmol* 1986; 102:382-386.
14. Huff JW, Green K: Characterization of bicarbonate, sodium and chloride flues in the rabbit corneal endothelium. *Exp Eye Res* 1983; 36:607-615.
15. Hull DS, Green K, Berdecia R: Rabbit corneal endothelial ionic and non-ionic fluxes following storage in MK medium and K-Sol. *Invest Ophthalmol Vis Sci* (Suppl) 1987; 28:167.
16. Skelnik DL, Novak AF, Gregerson DS, et al: An in vitro assessment of corneal storage medium CSM, MK and K-Sol using human corneal endothelial cells. *Invest Ophthalmol Vis Sci* (Suppl) 1986; 27:126.
17. Gorden PB, Jenkins CS, Hatcher VB: The effect of extracellular matrix on the detachment of human endothelial cells. *J Cell Physiol* 1984; 121:467-475.
18. Gamse G, Fromme HG, Kresse H: Metabolism of sulfated glycosaminoglycans in cultured endothelial cells and smooth muscle cells from bovine aorta. *Biochim Biophys Acta* 1978; 544:514-528.
19. Robinson J, Gospodarowicz D: Glycosaminoglycans synthesized by cultured bovine corneal endothelial cells. *J Cell Physiol* 1983; 117:368-376.
20. Laterra J, Silbert JE, Culp LA: Cell surface heparan sulfate mediates some adhesive responses to glycosaminoglycan binding matrices, including fibronectin. *J Cell Biol* 1983; 96:112-123.
21. Glasser DB, Matsuda M, Ellis JG, et al: Effects of intraocular irrigating solutions on the corneal endothelium after anterior chamber irrigation. *Am J Ophthalmol* 1985; 99:321-328.
22. Davson H: The hydration of the cornea. *Biochem J* 1955; 59:24-48.
23. Harris JE, Norquist LT: The hydration of the cornea. *Am J Ophthalmol* 1955; 40:100-110.
24. Maurice DM: The location of the fluid pump in the cornea. *J Physiol* 1972; 221:43-54.
25. Green K, Lippert B, Berdecia R, et al: Effect of dextran-bound acetazolamide on rabbit corneal endothelial ion fluxes. *Curr Eye Res* 1986; 5:543-546.
26. Hull DS, Green K, Boyd M, et al: Corneal endothelium bicarbonate transport and the effect of carbonic anhydrase inhibitors on endothelial permeability and fluxes and corneal thickness. *Invest Ophthalmol* 1977; 16:883-892.
27. Hodson S, Miller F: The bicarbonate ion pump in the endothelium which regulates the hydration of rabbit cornea. *J Physiol* 1976; 263:563-577.
28. Mayes KR, Hodson S: An in vivo demonstration of the bicarbonate ion pump of rabbit corneal endothelium. *Exp Eye Res* 1979; 28:699-707.
29. Huff J, Green K: Demonstration of an active sodium transport across the isolated rabbit endothelium. *Exp Eye Res* 1981; 28:699-707.
30. Lim JJ: Na© transport across the rabbit corneal endothelium. *Curr Eye Res* 1981; 1:255-258.
31. Fischbarg J, Lim JJ: Role of cations, anions, and carbonic anhydrase in fluid transport across rabbit endothelium. *J Physiol* 1974; 241:647-675.
32. Hodson S: The regulation of corneal hydration by a salt pump requiring the presence of sodium and bicarbonate ions. *J Physiol* 1974; 236:271-302.
33. ———: Evidence for a bicarbonate-dependent sodium pump in corneal endothelium. *Exp Eye Res* 1971; 11:20-29.
34. Green K, Simon S, Kelly GM, et al: Effect of [Na©], [Cl−] carbonic anhydrase, and intracellular pH on corneal endothelial bicarbonate transport. *Invest Ophthalmol Vis Sci* 1981; 21:586-594.
35. Walkenbach RJ, Corwin JG: Corneal deturgescence during organ culture. *Curr Eye Res* 1987; 6:381-389.
36. Waltman SF, Palmberg PF: Human penetrating keratoplasty using modified M-K medium. *Ophthalmic Surg* 1978; 9:48.

37. Kelly G, Green K: Influence of bicarbonate and CO_2 on rabbit transendothelial bicarbonate fluxes. *Exp Eye Res* 1980; 30:641-648.
38. Wiederholt M, Jentsch J, Keller SK: Electrogenic sodium-bicarbonate symport in cultured corneal endothelial cells. *Pfluger Arch* (Suppl 1) 1985; 405:167-171.
39. Lass JH, Medcalf SK, McBride M, et al: Effects of intermediate-term storage on the metabolic status of the cornea: K-Sol vs CSM. *Cornea*. Submitted April 1977.

DISCUSSION

DR JAY H. KRACHMER. I want to congratulate the authors for continuing to make important contributions in the field of cornea tissue preservation and transplantation. Doctor Lindstrom's presentation concerned three topics: the safety and efficacy of the Minnesota chondroitin sulfate corneal preservation solution based on the Minnesota formula (CSM) and another commercially available chondroitin sulfate preservation solution (K-Sol), and a description of the new 3M/Vision Care corneal trephination system along with a comparison of corneal buttons trephined by that method versus the Iowa Press.

Presently there are three types of corneal preservation solutions. McCarey-Kaufman solution is used for short-term storage usually up to and including 3 days. The organ culture preservation system developed at the University of Minnesota can be used for long-term storage up to 1 month. It is a more ellaborate method which is not currently in extensive use. Because of the need for solutions which could safely preserve tissue for up to 10 or 14 days, chondroitin sulfate containing solutions have been developed. These intermediate storage materials allow for scheduling of surgery, the possibility of future tissue matching programs, and more wide spread distribution of tissue nationally and internationally. The Eye Bank Association of America is currently developing a computerized distribution capability which will take advantage of longer travel time possible because of these intermediate preservation solutions.

The present study, along with previous work, establishes the safety and efficacy regarding endothelial viability of the chondroitin sulfate intermediate storage solution developed at the University of Minnesota. We have needed guidelines regarding how long we can safely store corneas in this solution. This study helps provide that information. The authors recommend using the material up to 10 days. I think most of us using CSM and K-Sol do not use it beyond 7 days. Perhaps this new information will provide an extra 3 days of potential storage. The epithelium does not seem to be as well preserved in the intermediate storage solutions as well as it is in M-K solution even for the first few days. It has been my clinical impression, and I would appreciate comments from Doctor Lindstrom, that K-Sol seems to preserve the epithelium better than CSM. A clinical study to compare K-Sol and CSM in patients would help answer such questions.

Several reports at this year's ARVO meeting compared and defined differences between the chondroitin sulfate (intermediate storage) and the dextran (short-term storage) solutions and between the available commercial chondroitin sulfate solutions. Saggau and Bourne (*Invest Ophthalmol Vis Sci* (Suppl) 1987; 28:166)

compared endothelial cell preservation at 14 days between CSM and K-Sol. Two different lots of media were used. One lot of CSM preserved 95% of central cells as compared to 63% for K-Sol, however this was not statistically significant. The other lot of CSM preserved a statistically lower percentage of cells than K-Sol. This finding raises the extremely important point that very careful quality control should be required of companies producing these media. Independent testing of lots by contracted eye bank laboratories should also be considered. Perhaps we could hear Doctor Lindstrom's thoughts concerning quality control.

The corneal preservation and trephination device developed by 3M and the University of Minnesota is well thought out and practical. It minimizes handling of tissue and maximizes consistency. The fact that it cuts a rounder, more predictable button which is not smaller in size than the trephine, should not necessarily be interpreted as providing reduced corneal astigmatism postoperatively. Even though that may be the case, a clinical trial would be required to establish that fact.

I appreciate the opportunity to discuss this paper and congratulate the authors on once again advancing the quality of eye banking and corneal transplantation.

Dr Robert Drews. The problem of clouding and thickening of the corneal button during the actual transplantation on a patient's eye is always frustrating. When M-K solution first became available and now with K-Sol I switched over and tried refilling the anterior chamber during the suturing of the graft with these solutions and not with balanced salts. They make a significant difference, keeping the graft clear throughout the rest of the procedure. I do run the solutions through a millipore filter to be sure they are sterile even though I know I transfer some of them unfiltered with the button.

Dr Daniel Taylor. In Connecticut, we usually do our grafts within the first 24 to 48 hours after death, and have mainly used M-K medium for short-term preservation. This has resulted in zero primary graft failures and an overall clear graft rate of over 95%. The great majority of our grafts have been clear from day 1 with minimal or no folds in Descemet's membrane. With the recent required testing for HIV-1 virus and Hepatitis B virus, we have had to switch to the longer acting media that contain chondroitin sulfate (CSM, K-Sol) because of frequent delays in obtaining test results that may extend well beyond 72 hours. We have frequently had to delay corneal transplantation some 3 to 5 days following death. Under these circumstances, we have noticed a considerable increase in graft stromal edema with haze and numerous folds in Descemet's membrane, and a much higher incidence of initial and even persistent epithelial defects than we have not previously been accustomed to. Perhaps this is an unfair comparison since I have not actually used any corneas preserved in chondroitin sulfate medium within the first 48 hours.

My question is whether the prolonged stromal graft edema and epithelial defects are caused by the chondroitin sulfate, or whether they are simply due to the longer time intervals between death and transplantation that are being forced

upon us by the required testing. So far, all of the grafts preserved in CSM or K-Sol have ultimately cleared, but many have required several weeks, and a few even several months, to clear. This can cause some degree of anxiety in both the patient and physician, and is frankly quite different from our previous experience.

I would appreciate hearing your comments on these questions. I would like to extend my congratulations to Doctors Lindstrom, Doughman and co-workers for this excellent presentation.

DR RICHARD L. LINDSTROM. Thank you, Doctor Krachmer, Doctor Drews and Doctor Taylor for your comments. Our experience is that K-Sol may preserve the epithelium better than CSM, but the epithelium tends to slough in K-Sol by 4 to 6 days whereas with CSM the epithelium may slough at 3 to 5 days. So, perhaps, it is worse in the CSM. We feel that the difference may be related to the osmolarity, which is higher in the CSM. It is fine for the endothelium but we have now worked with the manufacturer to adjust the medium osmolarity lower. The epithelial defect problem is one we need to solve. We have decided that we would teach ourselves and our fellows to treat the epithelium like it was endothelium.

We treat the patients' ocular surface very gingerly, we found we have to do that with organ culture and that improves our results.

Quality control is crucial. We started out trusting the manufacturers. We found out that we have to be careful about that. We test every lot of CSM medium that Aurora Biologicals makes and we test every lot that 3M makes and we have found, for example, that we have had to tell them to reject some lots of chondroitin sulfate. You can't just go buy any supply of chondroitin sulfate from Japan and find out it's okay. Some of it is toxic to the corneal endothelium or epithelium. So we do have to do independent testing, and whether or not we are really independent testing, we feel responsible so we are now testing all of the lots.

A prospective clinical trial is absolutely required on the astigmatism issue, and we have been trying to enlist other corneal surgeons to try and coordinate a clinical trial. I agree it needs to be done.

To Doctor Drews, I think the idea of using the media as a replacement for the aqueous is a good one. The only thing I would be concerned about is that sometimes there are bacteria in the medium and there may be epithelium in the media, but if you use a fresh bottle rather than the bottle that the cornea comes in, that may be a good idea. We have lately been using Viscoat because Viscoat has an osmolarity of about 370 and also has some chondroitin sulfate in it. The cornea seems to thicken less with the chondroitin sulfate containing media when we use Viscoat as our viscoelastic rather than the sodium hyaluronate, containing viscoelastics.

If you do use the tissue within 24 to 48 hours it is much less different than M-K or other dextran containing media than it is if you wait 7 to 8 days. It apparently has to do with the chondroitin sulfate leaking into the stroma. The epithelium looks pretty good at 1 to 2 days and also the cornea stays much thinner if you use it at that time. We have a new medium that we have used in Minnesota now for a little over 1 year that we call CSM-DEX which is a combination of a chondroitin

sulfate containing media with utilization of low or high molecular dextran. This medium has only been used in our department, but we have now done about 60 corneas and we seem to get equal preservation of the endothelium with better epithelium and less of the rebound stromal swelling. I think that may end up being the answer. The evolution, in a sense, needs to continue.