Effect of 1% Sodium Hyaluronate (Healon®) on a Nonregenerating (Feline) Corneal Endothelium

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A series of experiments were performed to investigate the effect of 1% sodium hyaluronate (Healon®) on the nonregenerating corneal endothelium of the cat. Aqueous humor replacement with 1% sodium hyaluronate resulted in mild, transient elevations of intraocular pressure compared to eyes that were injected with balanced salt solution. Sodium hyaluronate 1% protected the feline endothelium against cell loss incurred by contact with hyaluronate-coated intraocular lenses compared to endothelial contact with lenses that were not coated with sodium hyaluronate. The use of intraoperative 1% sodium hyaluronate, however, did not protect against endothelial cell loss incurred by penetrating keratoplasty or prevent subsequent skin graft-induced corneal homograft rejections. Homograft rejections were milder, however, in some eyes that received grafts coated with 1% sodium hyaluronate. Image analysis of photographs of trypan blue- and alizarin red-stained corneal buttons after trephining, stretching of Descemet's membrane, rubbing against iris-lens preparations, or immediately after penetrating keratoplasty demonstrated that the stretching of the posterior cornea is an important cause of endothelial damage that would not be protected against by a viscoelastic coating. Invest Ophthalmol Vis Sci 27:1485–1494, 1986

Sodium hyaluronate is a viscoelastic glycosaminoglycan that can coat and protect the corneal endothelium against surgical trauma in rabbits and humans. The cat is a more clinically applicable model of the corneal endothelium than the rabbit, however, because the endothelium of the cat, like that of man, heals primarily by hypertrophy and sliding of remaining cells rather than mitosis. The feline species has been useful in the investigation of infant1 and adult2 penetrating keratoplasties, induced corneal transplant rejections,4,5 To investigate the potential protective effect of a viscoelastic glycosaminoglycan on the non-regenerating corneal endothelium of the cat, we performed a series of experiments to investigate 1% sodium hyaluronate (Healon®; Pharmacia, Piscataway, NJ). Specifically, we wished to determine: (1) if aqueous humor replacement with 1% sodium hyaluronate results in toxicity to the corneal endothelium, (2) if 1% sodium hyaluronate protects the corneal endothelium against trauma associated with contact with intraocular lenses, and (3) if 1% sodium hyaluronate protects the endothelium against trauma associated with penetrating keratoplasty and subsequent induced corneal graft rejection. Because 1% sodium hyaluronate did not protect against endothelial cell loss incurred by penetrating keratoplasty, we performed an additional series of experiments to investigate the roles of trephining, corneal rubbing against the iris and lens, and corneal stretching from suturing as causes of endothelial damage during penetrating keratoplasty.

Materials and Methods

The following groups of experiments were performed (specific procedures are detailed subsequently).

Group I: Aqueous Replacement

Nine adult cats received unilateral aqueous humor replacement with 1% sodium hyaluronate (Healon®,...
Pharmacia, Piscataway, NJ). Contralateral control eyes received aqueous humor replacement with balanced salt solution. Intraocular pressures were measured at 1 and 12 hr after the aqueous replacement procedure, and paired experimental and control eyes were subsequently examined clinically each day for 7 days and then weekly until sacrifice. Selected pairs of experimental and control eyes were examined histologically on days 1 (N = two pairs), 2 (N = two pairs), 5 (N = two pairs), and 6 weeks (N = three pairs) after aqueous replacement.

Group 2: Intraocular Lens Induced Endothelial Trauma

Three adult cats received unilateral endothelial trauma by contact with intraocular lenses coated with 1% sodium hyaluronate. Control contralateral eyes were similarly traumatized by lenses, but without sodium hyaluronate. Eyes were examined clinically each day for 7 days and then weekly for 6 weeks.

Group 3: Penetrating Keratoplasties

Thirty-eight adult cats received consecutive bilateral penetrating keratoplasties, performed by three surgeons (from most experienced to least experienced, surgeon 1 = 10 grafts, surgeon 2 = 51 grafts, surgeon 3 = 15 grafts). Half of the eyes (N = 38) received donor corneas with a generous endothelial coating of 1% sodium hyaluronate. The other half received uncoated donor corneas. Categories of transplants included phakic (N = 28) and aphakic (N = 18) rotational autografts, “cultured” homografts that were stored in McCarey-Kaufman (M-K) medium at 7°C for 48 hr prior to surgery. Contralateral eyes received uncultured homografts that were exchanged between pairs of animals. The endothelial surfaces of half of the grafts were coated with 1% sodium hyaluronate during surgery, and the remaining grafts were performed without 1% sodium hyaluronate. Eyes were examined clinically each week for 12 weeks after keratoplasty.

3C. Ten animals received unilateral phakic rotational autografts. Contralateral eyes received “fresh” exchange homografts, performed between pairs of animals. The endothelial surfaces of half of the autografts and half of the exchange homografts were coated with 1% sodium hyaluronate, and the remaining grafts were performed without 1% sodium hyaluronate. Eyes were examined clinically each week for 6 weeks after keratoplasty.

3D. Ten animals each received a unilateral corneal homograft that had been maintained in M-K medium for 48 hr at 7°C prior to surgery. Contralateral eyes received uncultured homografts that were exchanged between pairs of animals. The endothelial surfaces of half of the grafts were coated with 1% sodium hyaluronate during surgery, and the remaining grafts were performed without 1% sodium hyaluronate. Eyes were examined clinically each week for 12 weeks after keratoplasty.

3E. To determine if reduced endothelial cell loss (antigen shedding) or altered antigen processing associated with intraoperative 1% sodium hyaluronate might affect the incidence or clinical manifestations of corneal graft rejection, exchange homografts (of groups 3C and 3D) were induced to reject by performing exchange full thickness skin grafts between matched corneal donor pairs 6 and 12 weeks after keratoplasty. Eyes were examined clinically each week for 8 weeks after skin grafting.

Group 4: Morphological Evaluation of Traumatized Corneas

Coating the endothelium of corneas with 1% sodium hyaluronate did not protect against endothelial cell loss after penetrating keratoplasty (see below). To further evaluate this finding, various types of trauma that a viscoelastic substance might or might not protect against were analyzed. Endothelial damage from rubbing of the cornea against other anterior ocular structures (iris, lens, anterior vitreous), for example, should be reduced by a viscoelastic coating. Endothelial damage from stretching, as might occur during suturing, should not be reduced by a viscoelastic coating. Trephining, rubbing of the endothelial surface across an iris-lens preparation, stretching of Descemet's membrane, and suturing during keratoplasty were each evaluated as sources of trauma that might contribute to endothelial cell loss associated with penetrating keratoplasty. The following specific categories of corneas were studied.

4A. Five freshly excised feline corneas, with a narrow rim of sclera, were trephined in punch fashion from the endothelial surface with an 8.0 mm disposable trephine (Edward Weck & Company, Inc., Research
No. 10  EFFECT OF HEALON® ON CORNEAL ENDOTHELIUM / Bahn et al.

Fig. 1. Methods of producing endothelial rub (A) and stretch (B) injuries. For rub injuries, freshly excised and trephined corneal buttons are rubbed across the moistened iris and lens surface of an eye with the cornea removed. Care must be taken not to grasp Descemet's membrane when studying trauma that results from rubbing. For stretch injuries, freshly excised and trephined corneal buttons are grasped with fine forceps at Descemet's membrane and opposing stroma to gently stretch the membrane. Simply stretching lamellae of the corneal stroma does not damage the endothelium.

Triangle Park, NC), stained and photographed, and tracings of the endothelial surface image analyzed (described below).

4B. Twelve freshly excised and trephined 8.0 mm corneal buttons, and two 8.0 mm corneal buttons trephined after 48 hr storage in McKerey-Kaufman medium, were rubbed 25 times across the moistened iris-lens surface of an eye with the cornea removed (Fig. 1). Also, five freshly excised and five corneas that had been stored for 48 hr in M-K medium at 7°C were trephined and rubbed 100 times across the iris-lens preparation. These corneal buttons were stained and photographed, and tracings were image analyzed.

4C. Five freshly excised, and four corneas that had been stored for 48 hours in M-K medium at 7°C, were trephined with an 8.0 mm trephine. Descemet's membrane and the opposing stroma were grasped with separate pairs of fine-toothed forceps to gently stretch the membrane (Fig. 1). Also, seven freshly excised and trephined 8.0 mm corneal buttons were similarly stretched circumferentially from 2 to 16 times to simulate distortion that results from suturing during keratoplasty. These corneal buttons were stained and photographed, and tracings were image analyzed.

4D. Fourteen 8.0 mm feline corneal buttons that had been transplanted as rotational autografts, and 12, 8.5 mm feline corneal buttons that had been stored in M-K medium for 48 hr at 7°C and transplanted to homologous feline recipients were excised immediately after surgery, stained, and photographed, and tracings were image analyzed.

4E. To correlate our findings in cats with primates, two 8.0 mm feline and two 8.0 mm baboon corneal buttons that had been transplanted as rotational autografts were excised immediately after surgery, processed, and examined by scanning electron microscopy.

Surgical Procedures

Animal housing and maintenance, pre- and postoperative care, and all surgical procedures were performed within guidelines established by the Laboratory Animal Medicine Units at the Uniformed Services University and the University of Michigan. Our methods for ketamine/xylazine anesthesia and pre- and postoperative animal care have been previously described. The provisions of the ARVO Resolution on the Use of Animals in Research were followed.

Aqueous humor replacements were performed on anesthetized animals (experimental Group 1) by grasping the limbal conjunctiva with fine-toothed forceps and entering the eye with a 30-gauge needle on a tuberculin syringe. Aqueous humor (0.6 ml) was gently aspirated, and an equal volume of 1% sodium hyaluronate or balanced salt solution was injected into the eye using a separate syringe. Ophthalmic antibiotic ointment was applied topically at the conclusion of the procedure.

Corneal endothelial trauma was induced with intraocular lenses (IOL) in eyes of anesthetized animals of experimental group 2. The lids were separated with a pediatric wire lid speculum and a 4-0 silk traction suture placed in the superior rectus muscle. Intracocular lenses (IOLAB Model # 61, Covina, CA) were inserted through clear corneal wounds at the superior limbus into the anterior chamber. Lenses with or without 1% sodium hyaluronate, depending upon the experiment, were dragged across the posterior cornea toward the inferior (6 o'clock) limbus and then back out of the wound. The limbal wounds were closed with interrupted 10-0 nylon sutures and the eyes medicated with topical ophthalmic antibiotic ointment.

Lensectomies were performed using an extracapsular technique on anesthetized animals in experimental group 3B. Atropine 1% was applied topically to the eyes the night before surgery to dilate the pupils. At surgery, the lids were separated with a pediatric wire lid speculum and a 4-0 silk traction suture placed in the superior rectus muscle. A fornix based conjunctival flap was followed by a 70° superior limbal incision into the anterior chamber. The anterior lens capsule...
was incised with a bent 25-gauge needle and the nucleus
expressed by pressure at the lip of the superior (12 o’clock) limbal wound and counter pressure at the in-
ferior (6 o’clock) limbus. Residual cortex was irrigated
with balanced salt solution, and the limbal wound
closed with interrupted 10-0 nylon sutures. Postoper-
atively, atropine 1% and antibiotic ophthalmic oint-
ments were applied topicaly.

Penetrating keratoplasties were performed on anesthetized cats of experimental group 3 as previously de-
scribed in detail, except that the endothelial surfaces
of half of the corneas were generously coated with 1% sodium hyaluronate intraoperatively before suturing
into the recipient wound. The keratoplasty procedure
for baboons (experimental group 4E) was the same as
for cats, except that anesthesia was achieved by ketame-
ine (10–20 mg/kg) followed 10 min later by intubation
and inhalational anesthesia with halothane (0.5–2%)
and oxygen 50%/nitrous oxide 50%.

Skin grafts between paired corneal donors (experi-
mental group 3E) were performed 6 (N = 10 animals)
and 12 (N = 10 animals) weeks postoperatively to in-
duce homograft rejections as previously described.

Animal sacrifice was accomplished by an overdose of
intravenous pentobarbital.

Clinical Evaluations

After each surgical procedure, eyes were examined
daily for the first postoperative week with a flashlight
to check the degree of external inflammation, cornea
or graft clarity, and limbal or corneal wound alignment.

Daily intraocular pressures during the first week were
measured using topical anesthesia (Alcaine, Alcon
Laboratories, Fort Worth, TX) and a pneumotono-
meter (Digilab Model 30D, Bio-Rad Laboratories,
Cambridge, MA).* Intraocular pressure in eyes that
underwent aqueous replacement with sodium hyal-
uronate or balanced salt solution was additionally
measured at 1 hr and 12 hr after injection.

Detailed clinical examinations in this series were
performed preoperatively, and every week postopera-
tively on anesthetized animals. The examinations in-
cluded pneumotonomometry, serial slit lamp photogra-
phy, and in vivo specular microscopy of the central

Morphologic Examinations

Central endothelial cell counting, and the prepara-
tion of feline and baboon corneas for light and scanning
electron microscopy, were performed as previously de-
scribed.

Image analysis was performed on tracings of slit-
lamp photographs of trypan blue and alizarin red
stained corneal buttons of experimental group 4. After
each type of trauma (trephining, rubbing, stretching,
and keratoplasty), corneas were stained with trypan
blue, photographed with a photoslit lamp, and then
stained with alizarin red and rephotographed. The
resultant slides were enlarged using a microfiche viewer
(Stromberg Datagraphix Inc, San Diego, CA), the cir-
cumference of the corneal buttons and areas of staining
traced onto clear acetate sheets, and the tracings sub-
ject to computer-assisted image analysis using a Quantimet 720 Image Analyzer (Cambridge/Imanco,
Monsey, NY). The degree of endothelial damage was
estimated as being predominantly linear or circular by
obtaining the mean longest and shortest dimensions
for regions of staining on each cornea. Tracings and
image analysis were performed by a skilled observer
who did not know the source of the photographs.

Results

Group 1: Aqueous Replacement

Among eyes that received aqueous replacement with
1% sodium hyaluronate or balanced salt solution, no
differences were observed in corneal clarity, thickness,
specular microscopy, ocular inflammation, or the his-
tological appearance of the endothelium (Table 1). So-
dium hyaluronate could be identified with the slit lamp
in the anterior chamber for 2–3 days after injection.
Intraocular pressures were elevated for 24–48 hr in eyes
that received aqueous replacement with sodium hy-
aluronate compared to balanced salt injected controls
(Fig. 2).

Group 2: IOL-Induced Endothelial Trauma

Eyes that received endothelial trauma from intra-
ocular lenses with a sodium hyaluronate coating re-
mained thin and clear, while eyes traumatized with
uncoated lenses exhibited transient stromal opacity and
edema for 7–10 days (Fig. 3). These latter eyes also
exhibited a 49% endothelial cell loss 4 weeks postopera-
tively, compared to a 10% endothelial cell loss in
the eyes protected with 1% sodium hyaluronate (mean
cell loss without 1% sodium hyaluronate = 1405 ± 143
and mean cell loss with 1% sodium hyaluronate = 248
± 218, P < 0.001, Student’s t-test).

* In our initial description of keratoplasty using cats, we found
that intraocular pressure measurements with a McKay-Marg style
tonometer were unreliable. The pneumotonometer in this study was
easy to use and provided consistent and reliable results after aqueous
replacement and keratoplasty.
Table 1. Aqueous replacement with balanced salt solution (N = nine eyes) and 1% sodium hyaluronate (N = nine eyes)

<table>
<thead>
<tr>
<th>Postoperative Day</th>
<th>Preoperative</th>
<th>1</th>
<th>2</th>
<th>7</th>
<th>30</th>
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</thead>
<tbody>
<tr>
<td><strong>Corneal thickness (mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balanced salt solution</td>
<td>0.60 ± 0.04</td>
<td>0.66 ± 0.04</td>
<td>0.66 ± 0.02</td>
<td>0.63 ± 0.04</td>
<td>0.63 ± 0.02</td>
</tr>
<tr>
<td>1% Sodium hyaluronate</td>
<td>0.59 ± 0.06</td>
<td>0.64 ± 0.04</td>
<td>0.62 ± 0.03</td>
<td>0.62 ± 0.06</td>
<td>0.62 ± 0.03</td>
</tr>
<tr>
<td><strong>Endothelial cell count (cells/mm²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balanced salt solution</td>
<td>2500 ± 140</td>
<td></td>
<td></td>
<td>2507 ± 103</td>
<td></td>
</tr>
<tr>
<td>1% Sodium hyaluronate</td>
<td>2425 ± 39</td>
<td></td>
<td></td>
<td>2535 ± 103</td>
<td></td>
</tr>
</tbody>
</table>

Group 3: Penetrating Keratoplasties

Among eyes that received penetrating keratoplasties with and without 1% sodium hyaluronate, no differences in intraocular pressure, corneal clarity, corneal thickness, ocular inflammation, or histological appearance of the corneal wound and endothelium were observed. Complications in this consecutive series of transplants were similar to those observed in our previous series of feline keratoplasties and complications that could be attributed specifically to the use of sodium hyaluronate or different surgeons were not observed.† Eyes that underwent extracapsular lensectomies prior to keratoplasty exhibited a 12% endothelial cell loss 16 weeks after the lensectomy procedure (mean preoperative cell count = 2500 ± 155 cells/mm², mean postoperative cell count = 2199 ± 245 cells/mm², P < 0.001, paired student’s t-test).

An analysis of variance was performed on endothelial cell counts performed preoperatively, and at 6 and 12 week postkeratoplasty.† Differences in endothelial cell loss that could be attributed to the use of 1% sodium hyaluronate or different surgeons were not observed (Figs. 4A, B). Two differences by graft type, however, were observed. In the group of grafts that received 1% sodium hyaluronate, an overall effect of graft type was found for cultured homografts compared to exchange homografts, aphakic rotational autografts, and phakic rotational autografts (F = 8.01, P < 0.01, Fig. 4C). Also, in the group of grafts that did not receive 1% sodium hyaluronate, phakic rotational autografts incurred a higher endothelial cell loss than rotational autografts performed on aphakic eyes (F = 3.68, P < 0.05, Fig. 4C).

Homologous corneal transplants routinely rejected after skin grafting with progressive keratic precipitates and subsequent bullous keratopathy as previously described. The eyes of animals that were skin grafted 6 weeks after keratoplasty developed keratic precipitates by 3 weeks after skin grafting and failed with bullous keratopathy (stromal edema and opacity) 3–4 weeks later. Differences in the clinical manifestations of rejection that could be attributed to sodium hyaluronate at the time of keratoplasty were not observed in this group.

Corneal grafts of animals that received skin grafts 12 weeks after keratoplasty rejected later and more gradually than grafts induced to reject 6 weeks after keratoplasty. In this group, corneal homografts that

![Intraocular pressure measurements after aqueous replacement with balanced salt solution (N = 9 eyes) and 1% sodium hyaluronate (N = 9 eyes). Replacement with 1% sodium hyaluronate resulted in a transient elevation of intraocular pressure that was not accompanied by an increased endothelial cell loss (see also Table 1). Error bars are ±SD.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933355/ on 11/25/2018)
were exchanged between pairs of animals rejected more rapidly after the performance of skin grafts than homografts from other (immunologically indifferent) cats that had been maintained in M-K medium (Fig. 5). For both exchange and cultured corneal donor tissues, grafts that did not receive a 1% sodium hyaluronate coating during keratoplasty developed keratic precipitates sooner, and failed sooner, than grafts that received 1% sodium hyaluronate (Fig. 5). Corneal homografts performed without 1% sodium hyaluronate coating also exhibited a trend toward a greater decline in endothelial cell density during rejection than grafts that received 1% sodium hyaluronate (Fig. 5B) (differences in cell density at 15 weeks after keratoplasty are not significantly different [P > 0.20, Student's t-test], although the statistical power is low because of the small sample size).

**Group 4: Morphological Analysis of Traumatized Corneas**

Corneal buttons that were rubbed across an iris-lens surface exhibited patterns of sparse punctate stain with alizarin red (Figs. 6A, B). Corneal buttons that were stretched exhibited prominent linear patterns of staining (Figs. 6C–E). Corneal buttons that were trephined exhibited only an occasional, central line of stain similar to that observed after stretching of Descemet's membrane. Feline corneal buttons that were examined after transplantation exhibited patterns of prominent linear staining that radiated from sutures (Fig. 7). Staining patterns, and the degree of endothelial damage observed were the same with trypan blue and alizarin red.§ Differences in endothelial damage that could be attributed to corneal preservation in M-K media were not observed in these experiments. Ultrastructurally, both feline and baboon corneas exhibited similar linear patterns of endothelial cytolysis after keratoplasty that corresponded to staining patterns observed with trypan blue and alizarin red (Fig. 7). The areas and percentages of endothelial damage observed with each procedure are summarized in Table 2, and a comparison of observed and expected amounts of endothelial damage for each method of endothelial injury are shown in Table 3. Comparison of the longest and shortest dimensions of stained areas of corneas after rubbing, stretching, and keratoplasty injuries are shown in Figure 8.

**Discussion**

Aqueous humor replacement with 1% sodium hyaluronate did not result in toxicity to the endothelium of the cat that could be detected by serial clinical (slit lamp and specular microscopic) and morphological (light microscopic) examinations. This is similar to the findings of previous studies in rabbits' and man. Aqueous replacement with 1% sodium hyaluronate did result in a mild, transient elevation of intraocular pressure that was well tolerated by the feline endothelium. Elevations of intraocular pressure, presumably because of impaired aqueous outflow, have also been observed clinically after the use of 1% sodium hyaluronate in the anterior chamber. Early postoperative elevations of intraocular pressure, however, were not observed after keratoplasty in the cat, and 1% sodium hyaluronate did not alter the clinical or light microscopic appearance of the feline keratoplasty wound.

Although 1% sodium hyaluronate protected against endothelial cell loss incurred by contact with intraocular lenses, differences in endothelial cell loss after penetrating keratoplasty that could be attributed to 1% sodium hyaluronate were not observed at 6 and 12
weeks after keratoplasty. We believe that we would have observed a significant difference in cell counts if 1% sodium hyaluronate conferred a protective effect against endothelial cell loss after keratoplasty in cats. We did, for example, find significant differences when we compared cell loss after contact with intraocular lenses with and without sodium hyaluronate, and before and after extracapsular lensectomies. Although we did not observe a protective effect against endothelial cell loss after keratoplasty with the use of sodium hyaluronate, we did not perform morphometric analysis of the specular micrographs. It is possible that grafts that received intraoperative 1% sodium hyaluronate achieved a more stable endothelium with less cellular pleomorphism compared to grafts that were not protected by 1% sodium hyaluronate.

Two observations relating to endothelial cell loss by category of tissue type require further study. The finding that homografts stored in M-K medium and transplanted with 1% sodium hyaluronate lost more cells than uncultured tissues (exchange homografts, phakic, and aphakic rotational autografts) when transplanted with 1% sodium hyaluronate, suggests that combined M-K medium storage and intraoperative sodium hyaluronate may result in increased endothelial cell loss. The finding that phakic autografts without 1% sodium hyaluronate had a higher endothelial cell loss than aphakic autografts without 1% sodium hyaluronate is similar to the result of a study in man. Why a difference was not observed when autografts were performed with 1% sodium hyaluronate is unclear.

Although the use of 1% sodium hyaluronate did not prevent induced corneal homograft rejections, rejections 12 weeks after keratoplasty were milder in eyes that received 1% sodium hyaluronate compared to eyes that did not receive 1% sodium hyaluronate. This suggests that 1% sodium hyaluronate might confer limited protection against corneal homograft rejection. Such a protective effect does not result from reduced endothelial cell loss (and, by inference, less antigen shedding, as we initially presumed might occur) because endothelial cell loss was the same for grafts with and without 1% sodium hyaluronate. A protective effect against homograft rejection has been observed with hyaluronate-coated tumor cells in mice. Chondroitin sulfate, another glycosaminoglycan, can lessen the se-

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Fig. 4. Comparison of sequential endothelial cell counts before and after penetrating keratoplasties in cats that show the similarities among grafts performed with and without 1% sodium hyaluronate (A), among grafts performed by three different surgeons (B), and among different categories of donor tissue (C). In an analysis of variance, differences that could be attributed to the use of 1% sodium hyaluronate or different surgeons were not observed. Although not evident from this presentation of the data, in the group of grafts that received intraoperative 1% sodium hyaluronate, an overall effect of graft type was shown in that homografts that had been maintained in McCarey-Kaufman medium lost more endothelial cells when compared to corneas transplanted immediately after excision from the donor eye (exchange homografts, phakic rotational autografts, and aphakic rotational autografts) (F = 8.01, P < 0.01). In the group of grafts that did not receive 1% sodium hyaluronate, an overall effect of graft type was shown in that phakic rotational autografts lost more cells than aphakic rotational autografts (F = 3.68, P < 0.05). Error bars are ±SD.
Fig. 5. Sequential corneal thickness measurements (A) and endothelial cell counts (B) of corneal homografts that were induced to reject by performing full thickness skin grafts between corneal donor pairs 12 weeks after the keratoplasty procedure. Corneal homografts between matched corneal donor pairs rejected more rapidly than homografts from indifferent animals that had been maintained in McCarey-Kaufman medium. Homografts maintained in M-K medium, and homografts exchanged between donor pairs that received intraoperative 1% sodium hyaluronate, developed keratic precipitates and developed stromal edema later than grafts without sodium hyaluronate, suggesting a limited protective effect. Homografts without sodium hyaluronate showed a trend toward more rapid endothelial cell loss than grafts with sodium hyaluronate during rejection (cell count differences not significant, \( P > 0.20 \)). Error bars are ±SD.

Fig. 6. Slit-lamp photomicrograph (A) and light photomicrograph (B, X 50) of a cornea stained with trypan blue and alizarin red after McCarey-Kaufman medium storage and 100 rubs across an iris lens preparation demonstrate small, round areas of endothelial damage (arrow). In contrast, a cornea that had been stretched by grasping Descemet's membrane exhibits large linear patterns of staining when similarly stained and photographed (C, D X 50). Tracings of enlarged photographs of corneal buttons (E) can be image analyzed to quantitate the degree and patterns of endothelial damage (Tables 2, 3, Fig. 8).

Fig. 5. Sequential corneal thickness measurements (A) and endothelial cell counts (B) of corneal homografts that were induced to reject by performing full thickness skin grafts between corneal donor pairs 12 weeks after the keratoplasty procedure. Corneal homografts between matched corneal donor pairs rejected more rapidly than homografts from indifferent animals that had been maintained in McCarey-Kaufman medium. Homografts maintained in M-K medium, and homografts exchanged between donor pairs that received intraoperative 1% sodium hyaluronate, developed keratic precipitates and developed stromal edema later than grafts without sodium hyaluronate, suggesting a limited protective effect. Homografts without sodium hyaluronate showed a trend toward more rapid endothelial cell loss than grafts with sodium hyaluronate during rejection (cell count differences not significant, \( P > 0.20 \)). Error bars are ±SD.

Fig. 6. Slit-lamp photomicrograph (A) and light photomicrograph (B, X 50) of a cornea stained with trypan blue and alizarin red after McCarey-Kaufman medium storage and 100 rubs across an iris lens preparation demonstrate small, round areas of endothelial damage (arrow). In contrast, a cornea that had been stretched by grasping Descemet's membrane exhibits large linear patterns of staining when similarly stained and photographed (C, D X 50). Tracings of enlarged photographs of corneal buttons (E) can be image analyzed to quantitate the degree and patterns of endothelial damage (Tables 2, 3, Fig. 8).
matrix into the eye can increase the likelihood of developing systemic suppressor lymphocytes that enhance the "immunological privilege" of the anterior chamber. Additional studies will be required to determine whether the use of 1% sodium hyaluronate has any positive action in reducing the rejection response. Such studies are underway in our laboratories.

The observations that greater endothelial damage results from stretching corneas (Descemet's membrane) compared to rubbing the endothelium of corneas in vitro, and patterns of endothelial damage after keratoplasty more closely approximate stretch injuries than rub injuries (long and narrow as opposed to small and round), indicate that stretching of the posterior cornea is an important cause of endothelial cell damage that is not protected against by a viscoelastic coating. These results reinforce our finding that 1% sodium hyaluronate did not protect against endothelial cell loss in the early postoperative period after keratoplasty in the cat. The finding that staining patterns were similar with trypan blue (a vital stain) and alizarin red reduces the possibility that rub injuries to the posterior cornea produce a population of injured endothelial cells that might be an important cause of continuing cell loss late after the keratoplasty procedure. Although species variation might occur, our finding of similar stretch-like endothelial injuries on baboon corneas after keratoplasty, and the results of a similar study that used human corneas and keratoplasties, demonstrate that our results are probably applicable to primate corneas, including man.

We did not examine varying concentrations of sodium hyaluronate or the effects of other viscoelastic materials, such as chondroitin sulfate or methylcellulose, that might have produced a different result, but stretching the posterior cornea apparently causes large amounts of endothelial damage that would not be lessened by these substances.

Based on the findings of this study, we believe that viscoelastic materials are potentially useful in clinical

### Table 2. Endothelial damage after different methods of injury

<table>
<thead>
<tr>
<th>Procedure</th>
<th>% Of Endothelial Surface Stained With Trypan Blue and Alizarin Red</th>
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<tbody>
<tr>
<td>Trephine Injury (5 Fresh Corneas)*</td>
<td>1.13% (0.96 mm²)</td>
</tr>
<tr>
<td>Rub injury</td>
<td></td>
</tr>
<tr>
<td>×25 (12 fresh corneas)</td>
<td>1.75% (1.18 mm²)</td>
</tr>
<tr>
<td>×25 (2 M-K stored corneas)</td>
<td>1.43% (0.8 mm²)</td>
</tr>
<tr>
<td>×100 (5 fresh corneas)</td>
<td>5.63% (3.12 mm²)</td>
</tr>
<tr>
<td>×100 (5 M-K stored corneas)</td>
<td>3.95% (2.18 mm²)</td>
</tr>
<tr>
<td>Stretch injury</td>
<td></td>
</tr>
<tr>
<td>×1 (5 fresh corneas)</td>
<td>5.04% (2.69 mm²)</td>
</tr>
<tr>
<td>×1 (4 M-K stored corneas)</td>
<td>6.15% (3.0 mm²)</td>
</tr>
<tr>
<td>×2-16 (7 fresh corneas)</td>
<td>2.75%/Stretch</td>
</tr>
<tr>
<td>Keratoplasty</td>
<td></td>
</tr>
<tr>
<td>14 fresh corneas</td>
<td>14.7% (8.2 mm²)</td>
</tr>
<tr>
<td>12 M-K stored corneas</td>
<td>12% (6 mm²)</td>
</tr>
</tbody>
</table>

* Corneas that were removed with a narrow scleral rim immediately after animal sacrifice were designated as "fresh" tissues.
† Number of times injury was performed.
‡ Corneas that were maintained at 7°C for 48 hr in McCarey-Kaufman (M-K) medium.

### Table 3. Comparison of observed and expected endothelial stain

<table>
<thead>
<tr>
<th>Procedure</th>
<th>% Stain Observed*</th>
<th>% Stain Expected After Keratoplasty†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trephine injury</td>
<td>1.13%</td>
<td>1.13%</td>
</tr>
<tr>
<td>Rub injury</td>
<td>0.05%-0.07%/rub</td>
<td>0.88%-1.12%</td>
</tr>
<tr>
<td>Stretch injury</td>
<td>2.75%-5.6%/stretch</td>
<td>44%-89%</td>
</tr>
<tr>
<td>Keratoplasty</td>
<td>13.3%</td>
<td>20%-30%</td>
</tr>
</tbody>
</table>

* Determined from Table 2.
† Calculated by multiplying the % stain observed per injury by the number of times the injury would be expected to occur during an uncomplicated keratoplasty procedure. For both rub and stretch injuries, the % stain observed is multiplied by 16, which is the number of times the corneal button would be manipulated using 16 sutures in an interrupted pattern. The % expected stain after keratoplasty is estimated from the endothelial cell loss observed after keratoplasty in cats.
Fig. 8. Results of image analysis of patterns of endothelial damage after rub, stretch, and keratoplasty injuries. Keratoplasty results in endothelial damage that is long and narrow like the damage that occurs after stretching Descemet's membrane. This is in contrast to small and round patterns of damage that occur after rub injuries. Horizontal and vertical error bars are ±SD.

keratoplasty when a graft is placed over an intraocular lens to prevent contact between the endothelium and the implant. The use of 1% sodium hyaluronate in the phakic or aphakic eye without an implant may not be routinely justified, because endothelial cell loss is not reduced, but secondary glaucoma from the use of intraoperative 1% sodium hyaluronate can be an important complication. If sodium hyaluronate proves from subsequent studies to have a protective effect against homograft rejection, then the risk-to-benefit ratio may warrant its routine use in penetrating keratoplasty.

Key words: cornea, endothelium, sodium hyaluronate, penetrating keratoplasty, rejection

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References