Viscocanalostomy in Rhesus Monkeys

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**Objective:** To examine structural changes and aqueous humor outflow after viscocanalostomy in live normal monkey eyes.

**Methods:** Viscocanalostomy surgery was performed in 1 eye of each of 4 rhesus monkeys. Outflow facility was determined before and after surgery. All eyes were fixed and examined by light and/or electron microscopy 36 or 63 days postoperatively.

**Results:** Schlemm canal was replaced by scar tissue at the surgical site. The juxtacanalicular zone contained homogeneous material, probably high-molecular-weight 1.4% sodium hyaluronate. The sclera external to Schlemm canal was overhydrated, and remains of a scleral lake were present in 1 animal. Multiple defects were present in the endothelial lining of Schlemm canal inner and outer wall. Fine fibrillar material and sheath-derived plaque material partly bridged the defects. Along the inner wall, aggregations of thrombocytes covered some defects in the endothelial lining of the canal. At 90° to 180° from the surgical site, small and fewer breaks in the inner wall were seen. Postsurgery outflow facility (n=2) was approximately 30% higher in the treated eye than in the contralateral control, corrected bilaterally for presurgery baseline.

**Conclusions:** The most likely explanations for the increase in outflow facility in monkeys after viscocanalostomy are focal disruptions of the inner wall endothelium of Schlemm canal and disorganization of the juxtacanalicular zone, resulting in direct communication of juxtacanalicular zone extracellular spaces with the lumen of Schlemm canal. The continuous presence of sodium hyaluronate might prevent repair of these defects by interfering with thrombocyte function.

**Clinical Relevance:** In nonhuman primates, viscocanalostomy appears to decrease outflow resistance through persisting focal disruption of the inner wall endothelium and opening of the juxtacanalicular or cribriform region of the trabecular meshwork, the tissue most affected by pathologic changes in primary open-angle glaucoma in humans.

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A NTERIOR CHAMBER DRAIN-age surgery is an essential tool in glaucoma management. Nonpenetrating techniques are being refined to reduce the postoperative complications associated with more traditional drainage procedures. Viscocanalostomy and a related procedure called deep sclerectomy have garnered much attention recently. Although more difficult and perhaps slightly less effective at lowering intraocular pressure (IOP) than standard trabeculectomy, they lessen the risk of some of the postoperative problems of trabeculectomy and thus seem attractive.

Stegmann et al recognized the need for a technique that would be successful in areas with a high risk of postoperative infection. They proposed the viscocanalostomy procedure, based on Krasnov’s and Zimmerman and coworkers work on nonpenetrating “trabeculectomy.” Ideally, there is no obvious or intentional invasion of the anterior chamber and no iridectomy. These patients typically have quiet eyes postoperatively, do not become hypotonous, and, in 1- to 5-year follow-up, have good IOP control, albeit not as low as with trabeculectomy. They often have no clinically visible filtering bleb, or they may have a small, relatively flat bleb, quite different from results with trabeculectomy. Antimetabolite is not used.

For editorial comment see page 1868

Critical components of the surgical procedure are creation of superficial and deep scleral flaps, unroofing of Schlemm canal and Descemet membrane followed by cannulation of the cut ends of Schlemm canal and intracanalicular injection of high-molecular-weight 1.4% sodium hyaluronate (Healon GV; Pfizer Inc, New York, NY), and excision of the deep scleral flap. The hypothesis was that aqueous humor leaves the anterior chamber by percolating through Schlemm canal endothel-
Tissue Fixation

Group 1

After perfusion inflammation had subsided (21 to 28 days after perfusion, 63 days after surgery), both anterior chambers of each monkey were exchanged with cationic 5-nm and non-cationized 10-nm gold solution as tracers to delineate flow pathways and to label extracellular matrix, at an IOP of approximately 15 mm Hg, and then perfused at 25 mm Hg with Ito solution from an elevated reservoir. Under deep general anesthesia with intravenous pentobarbital sodium, 15 mg/kg, these animals were then perfused through the heart with phosphate-buffered saline, 0.1 mol/L (pH 7.4), followed by Ito solution. The eyes were enucleated, windows were cut in the cornea and sclera, and the eyes were placed in the same fixative and sent to Germany for electron microscopy.

On arrival, the eyes were placed in cacodylate buffer (pH 7.4) for 24 hours to wash out fixative. Each eye was bisected and the anterior halves were cut into quadrants by meridional sectioning. Each quadrant was further dissected into wedge-shaped specimens 1.0 to 1.2 mm wide that contained TM, ciliary muscle, iris, and adjacent cornea and sclera. In the superior quadrant, the distance of each specimen to the 12-o’clock limbus, which had been marked by a suture during enucleation of the eye, was identified and documented. All wedges were dehydrated in ascending concentrations of alcohol and embedded in epoxy resin according to standard protocols. One-micrometer semithin sections were cut from each specimen of the superior quadrant (6–8 per monkey) and from at least 3 specimens of the other 3 quadrants. All semithin sections were stained with toluidine blue O and examined by light microscopy. Subsequently, ultrathin sections were cut from each specimen that had been investigated by light microscopy and stained with lead citrate and uranyl acetate. Both semithin and ultrathin sections were assigned a unique identifying number and were examined by a masked observer (E.R.T.).

Group 2

Tonographic outflow facility determination at day 35 after viscocanalostomy was followed by perfusion through the heart with phosphate-buffered neutral formalin on day 36. The eyes were enucleated and placed in 10% formalin for processing and analysis by one of us (D.M.A.). The eyes were histologically processed, embedded in paraffin, and serially sectioned at 5 μm. Every 10th section was stained with hematoxylin–eosin and covered. The eye sections were assigned a unique identifying number and a masked observer examined the hematoxylin–eosin–stained sections under a microscope. The eyes were examined and several histologic features were recorded: (1) the patency and integrity of Schlemm canal, (2) evidence of breaks in the TM, (3) the presence of scleral lakes, (4) the presence and degree of inflammation, (5) the presence and degree of fibrosis, and (6) any other unusual histologic ocular features. Emphasis was placed on the area of, and adjacent to, the surgical site, as well as the area 180° from the surgical site. The surgically treated eyes from group 2 monkeys were compared with the contralateral control eye from one of the animals.

Anesthesia and Antibiotics

Intramuscular ketamine hydrochloride anesthesia (10 mg/kg, supplemented every 20–30 minutes as needed with 5 mg/kg) was used for all procedures. In addition, animals received intravenous pentobarbital sodium (10–15 mg/kg) for perfusion outflow facility, or acepromazine maleate (0.2–0.5 mg/kg in-
SURGICAL TECHNIQUE

Phase 1: Preparation of the Surgical Field

After a bridle suture was passed under the superior rectus muscle, a fornix-based conjunctival flap was prepared. To avoid damage to Schlemm canal, collector channels, and the sclera itself, hemostasis was maintained by repeated irrigation with omnipressin solution, 5 IU/ml. (Por 8; Sandoz, Basel, Switzerland), so as to use as little thermal coagulation as possible.

Phase 2: Preparation of the Intrascral Chamber

With a 20-gauge diamond knife, a 5×5-mm limbus-based parabolic incision was made in the sclera, and a 200-µm superficial flap was dissected with a single-use bevel-up spatula (both from Grieshaber, Schaffhausen, Switzerland). By the same tech-

nique, an inner concentric 4×4-mm limbus-based scleral flap was sculpted beneath the previous one, keeping the surface of the cut so close to the choroid as to have a dark reflex. When the cut was advanced lim tally, Schlemm canal was deroofed and the 2 openings of the canal remained patent at the edges of the cut.

Phase 3: Injection of Viscoelastic in Schlemm Canal

The ostia of Schlemm canal were then cannulated with a 190-µm-diameter blunt cannula (Grieshaber) and filled with high-molecular-weight 1.4% sodium hyaluronate. To limit damage to the Schlemm canal walls, the injection of sodium hyaluronate was started while the ostia were approached, the cannula insertion did not exceed 1 mm, and a small amount of sodium hyaluronate was slowly injected on each side 3 to 4 times. Approximately 275 µL of sodium hyaluronate was injected. Most of it did not go into Schlemm canal because of high reflux.

Phase 4: Realization of Window Overlying Anterior Chamber

By gently pulling the inner scleral flap upward and delicately depressing the floor of the canal and Descemet membrane with the tip of a cotton swab, the membrane itself was cleaved anteriorly from the cornea for approximately 1 mm (Figure 1), and aqueous was seen to percolate through this window and to enter the lake. As soon as the window was completed, the inner scleral flap was excised by means of Vannas scissors.

Phase 5: Sealing of the Lake

The ostia of Schlemm canal were cannulated again and sodium hyaluronate was gently injected 2 to 3 times in each opening. Finally, the outer flap was tightly sutured with seven 10-0 nylon stitches, and sodium hyaluronate was injected underneath the flap to temporarily fill the intrascral lake and prevent it from collapsing and scarring in the early postoperative period. Finally, 2 wing 8-0 silk sutures were passed to hold the conjunctiva in place.

SODIUM HYALURONATE

In a separate experiment, approximately 25 to 30 µL of sodium hyaluronate was injected into the anterior chamber of 1 eye of an adult female cynomolgus monkey (Macaca fascicularis). Approximately 25 to 30 µL of aqueous humor was then removed and the IOP was checked to ensure that it was not elevated. Slit-lamp examination was performed 24 hours later to assess the amount of inflammation, which was minimal. Fixation for this animal was similar to that of the animals in group 1. Under deep general anesthesia with intravenous pentobarbital sodium, 15 mg/kg, the animal was perfused through the heart with phosphate-buffered saline, 0.1 mol/L (pH 7.4), followed by Ito solution. The eyes were enucleated, windows were cut in the cornea and sclera, and the eyes were placed in the same fixative and sent to Germany for electron microscopy. Wedge-shaped specimens from each quadrant of the anterior eye were processed for light and electron microscopy as described in “Tissue Fixation, Group 1.”

RESULTS

OUTFLOW FACILITY

One animal had preoperative and postoperative perfusion outflow facility determinations (monkey A110). Post-
operative facility (35 days after surgery) increased by 50% in the treated eye and 22% in the control eye of this animal compared with baseline. The ratio of facility in the viscocanalostomy-treated and control eyes (V/C) changed from 0.78 before to 1.22 after surgery. Tonographic outflow facility was determined before and 34 days after surgery on 2 animals (monkeys AI09 and AI10). Facility increased by 68.5%±0.5% in the viscocanalostomy-treated eyes and 42.5%±0.5% in the control eyes. (Results are given as mean±SEM unless otherwise indicated.) The V/C facility ratio changed from 0.66±0.10 before to 1.21±0.16 after surgery. Tonographic outflow facility data were collected 1 day before perfusion outflow facility on 3 occasions and 9 days before in 1 other. One animal had both preoperative and postoperative testing done (AI10) and so accounted for 2 of the 4 test points. The ratios of V/C facility for both types of facility measurement. The ratio of V/C facility for tonographic outflow was 0.86±0.18. The ratio of V/C facility for perfusion outflow was 0.84±0.12. The ratio of tonographic facility to perfusion facility was 1.00±0.07.

**INTRAOCULAR PRESSURE**

The presurgery IOP for the 4 eyes that underwent the viscocanalostomy procedure was 17.3±1.6 mm Hg. The IOP at time of death for these eyes was 16.3±0.6 mm Hg, a nonsignificant decrease of 6%. The presurgery IOP for the 3 fellow eyes was 17.3±1.2 mm Hg, while the IOP at time of death was 16.7±0.3 mm Hg, a nonsignificant decrease of 4%.

**ELECTRON MICROSCOPY**

The eyes of 2 monkeys that underwent viscocanalostomy in 1 eye were examined at 63 days after surgery. The entire circumference of operated-on and control eyes was investigated. In the operated-on eyes of both animals, Schlemm canal and juxtacanalicular TM had disappeared in the center of the superior quadrant, where the operation had been performed (Figure 2). In an area of about 2 mm in circumferential width, irregular whorls of elastic and collagenous fibers occupied the position where Schlemm canal originally had been located (Figure 2). At the inner aspect of the TM, uveal trabecular lamellae covered by TM cells remained intact and the chamber angle was open.

Adjacent to the operated-on area, Schlemm canal was present (Figure 3A) in both operated-on eyes. In none of the eyes was Schlemm canal abnormally dilated. There were, however, marked structural changes in the outer parts of the TM along a distance of about 2 to 6 mm next to the operated-on site. In this area, trabecular lamellae had become replaced by a network of fibroblastlike cells that were embedded in a loose collagenous matrix (Figure 3A). This tissue appeared not to impede flow of aqueous humor, as giant vacuoles were present along the inner wall of Schlemm canal (Figure 3A). In places, the juxtacanalicular zone was filled with homogeneous material that was not seen in control eyes. By electron microscopy, this material was electron dense and had a fine granular structure (Figure 3B). The inner wall endothelial lining of Schlemm canal frequently showed intercellular openings approximately 100 nm to 2 µm in diameter (Figure 3B and C). Through these openings, extensions of the fine granular material protruded into the lumen of Schlemm canal. Both 5-and 10-nm gold particles that were embedded in the fine granular material were seen at the outer side of the inner wall next to the openings, as well as in the part of the material that extended through the openings (Figure 3C). We hypothesized that the fine granular material was sodium hyaluronate that had been injected into Schlemm canal during surgery and was pressed through the openings in Schlemm canal endothelium into the juxtacanalicular region of the TM. To support this hypothesis, we performed an additional experiment and injected sodium hyaluronate directly into the anterior chamber of the eye of an addi-
Twenty-four hours after injection, sodium hyaluronate was observed by light microscopy in contact with the surfaces of ciliary processes and iris as homogeneous material that stained intensely with toluidine blue O (Figure 4A). By electron microscopy (Figure 4B), the same material was electron-dense and finely granular, and showed essentially the same ultrastructural characteristics as the material that was found in the juxtacanalicular TM of monkeys that underwent viscocanalostomy. Similar electron-dense and finely granular material was observed after anterior chamber injection at the inner side of the peripheral cornea (not shown) and at the inner surface and the intertrabecular spaces of the uveal meshwork (Figure 4C and D), but not in the juxtacanalicular region.
In one of the viscocanalostomy-treated monkeys (A110), at a distance of about 2 mm from the 12-o’clock limbus, a large triangular defect was present in the sclera adjacent to the anterior portion of the ciliary muscle (Figure 5A). The defect had a circumferential extent of about 1 mm. The walls of the defect had a length of approximately 0.5 to 0.6 mm and were not covered by cells. We concluded that this defect resulted from the operation and was a remaining part of the intrascleral reservoir, the so-called scleral lake that was created by removing the inner layer of the sclera. Between the scleral lake and the TM, the sclera appeared to be markedly less dense than in other parts of the eye (Figure 5A). In the TM of this region, there were fewer trabecular lamellae, and large intertrabecular spaces were present (Figure 5B). The lumen of Schlemm canal formed protrusions toward the TM (Figure 5C and D). Similar protrusions were not observed in control eyes. On serial sections, it became evident that these protrusions communicated directly with the intertrabecular spaces and were not Sondermann canals, which are blind diverticula in the inner wall of Schlemm canal that are completely covered by endothelium. Sondermann canals are extremely rare in monkeys (E.R.T., unpublished data, 2003), consistent with the findings in control eyes. A similar large scleral defect was not present in the same area of the other operated-on eye. However, in some distinct areas, larger defects, approximately 1 to 5 µm in length, in the endothelial
covering of Schlemm canal were observed that were not associated with the fine granular material nor with thrombocytes (Figure 6). Such defects were seen along the inner wall and, more rarely, in the outer wall of Schlemm canal in this area (Figure 6A and B). By electron microscopy, fine fibrillar material and sheath-derived plaque material was seen to partly bridge over the defects (Figure 6C). Numerous 5- and 10-nm gold particles were attached to this extracellular material (Figure 6D).

In a region 2 to 4 mm nasal from the 12-o'clock limbus and in an area where defects in the outer wall were frequently observed, the extracellular material between the outer wall of Schlemm canal and the outer side of the sclera and cornea was markedly less dense than in the control eye or in other regions of this eye (Figure 7). Thus, at the limbus, a sharp boundary was formed at the anterior end of the TM that separated corneal stroma of normal extracellular matrix density localized anteriorly to the TM from stroma of considerably less density that was localized opposite to Schlemm canal and TM. By electron microscopy of this area of diminished extracellular matrix density, large electron-empty spaces were seen between cells that showed typical ultrastructural characteristics of fibroblasts. The amount of collagenous fibers appeared to be greatly reduced.

At the nasal, temporal, and inferior sides of both eyes, approximately 90° to 180° from the center of the surgical site and beyond the extent of the Schlemm canal cannulation, obvious scarlike changes in the TM were not observed. Still, the normal contour of Schlemm canal had changed and often showed irregularly branched cul-de-sac–like protrusions that reached toward the inner parts of the TM (Figure 8). Such protrusions were not observed in control eyes. The inner wall in this area formed numerous giant vacuoles, and the juxtacanalicular area was again partly filled with homogeneous material. Along the inner wall, aggregations of thrombocytes were frequently observed that appeared to cover defects in the endothelial lining of the canal. By electron microscopy, individual thrombocytes were found at the outer side of the inner wall in close association with intercellular junctions of the endothelium, some of which appeared open.

*Figure 5.* Chamber angle of a monkey eye (monkey A10) treated by viscocanalostomy about 2 mm from the 12-o'clock limbus where the surgery was performed 2 months earlier (semithin sections, toluidine blue O stain). A, A large triangular defect (SL), probably representing a remaining part of the scleral lake created during surgery, is present in the sclera adjacent to the anterior portion of the ciliary muscle (CM). The walls of the defect are approximately 0.5 to 0.6 mm long and are not covered by cells. In a distinct area (solid arrows) between defect and trabecular meshwork (open arrow), the sclera appears to be markedly less dense than in other parts of the eye. AC indicates anterior chamber. B, In the trabecular meshwork of this region, the number of trabecular lamellae is reduced and large intertrabecular spaces are present (open arrows). SC indicates Schlemm canal. C and D, Serial sections of the trabecular meshwork, same region as in A and B. The lumen of Schlemm canal forms protrusions toward the trabecular meshwork (solid arrows) that communicate directly with the intertrabecular spaces. Bars indicate 90 µm (A), 22.5 µm (B), and 14.8 µm (C and D).
In addition, aggregates of thrombocytes were observed (Figure 9). In these areas, cytoplasmic protrusions of individual thrombocytes were observed (Figure 9). In these areas, cytoplasmic protrusions of individual thrombocytes were observed.
seen that filled the gaps between adjacent inner wall cells. Gold particles had become attached to extracellular fibers close to the openings, indicating that this region was used for flow of aqueous. In addition, gold particles were seen in intracellular vesicles in neighboring Schlemm canal endothelial cells next to the openings. In contrast, no gold particles were observed in association with the extracellular matrix in immediately adjacent areas that were separated from the lumen of Schlemm canal by an intact endothelial layer. More rarely observed were contracted thrombocytes that closed openings of the inner wall, or larger thrombotic plaques that consisted of numerous aggregated thrombocytes and fibrinlike material between them (Figure 9D and E).

Discontinuities in the periphery of Descemet membrane were observed at the base of the opercular area, an extension of Descemet covering the anterior TM, which is not present in humans (Figure 10). Between the ends of the discontinuous Descemet membrane, cells or smaller open spaces up to 5 µm in width were present. In other areas, Descemet membrane was interrupted by a small slit only. These discontinuities are normal for monkeys and were present in both control and experimental eyes and distributed equally around the circumference. No evidence of iatrogenic disruptions of Descemet membrane was observed in any eye.

Baseline physiological testing showed small differences in outflow facility between the eyes for each animal; the eye with lower facility was selected for viscocanalostomy surgery. As with clinical studies, there was a marked decrease in IOP after surgery. Still, in our monkeys this effect was transient and recovery to near baseline occurred within 30 days. Outflow facility, determined after surgery when the eyes were quiet, increased to a greater extent, relative to baseline values, in the eyes undergoing viscocanalostomy. By this time IOP had recovered to baseline levels. We assume that larger decreases in IOP as reported for human patients with glaucoma and larger increases in facility were not observed because preoperative IOP and baseline facility in our monkeys were within the reference range. In addition, the higher-than-physiologic pressure gradient...
ent or flow rate during external perfusion or tonography might contribute to the increase in outflow facility. During perfusion from an open reservoir, IOP can be up to 12 mm Hg higher than spontaneous IOP. The resultant higher pressure gradient and flow rate across the TM could further loosen already weakened (by the injection and retention of viscoelastic material) cell adhesions to each other and to their extracellular matrix in the inner wall and juxtaocular zone, relaxing the meshwork so as to facilitate flow through it, as well as driving fluid through the inner wall breaks in Schlemm canal. Under this scenario, the surgical procedure might be more clearly effective func-

Figure 9. Electron micrographs of monkey eyes treated by viscocanalostomy 2 months previously (monkey 81051). A, Thrombocytes (solid arrows) are found at the outer side of the inner wall of Schlemm canal (SC) in close association with intercellular junctions of the endothelium, some of which appear open (ie, the cells are separated; open arrows). B, Aggregated thrombocytes (arrows) adhere to the inner wall of Schlemm canal in the region of a larger gap between adjacent inner wall cells. C, Higher magnification of B. Thrombocyte processes fill the gap between adjacent endothelial cells (solid arrow). Gold particles measuring 5 and 10 nm attach to extracellular fibers close to the opening and are seen in intracellular vesicles in neighboring Schlemm canal endothelial cells (open arrows). D, A contracted thrombocyte (arrow) fills an opening in the inner wall. E, A thrombotic plaque (arrow) consisting of numerous aggregated thrombocytes and fibrinlike material between them closes an opening in the inner wall. Bars indicate 1.7 µm (A), 1.15 µm (B and D), 0.42 µm (C), and 2.15 µm (E).
tionally in patients with glaucoma with elevated IOP, especially if they are not receiving secretory suppressants.

Experimental glaucoma can be induced in monkeys by laser treatment of the chamber angle tissues. However, this procedure induces scarring of TM and Schlemm canal and would preclude cannulation and injection of sodium hyaluronate in Schlemm canal as required for effective viscocanalostomy.

In contrast to the modest changes in outflow facility, structural changes of the conventional outflow pathways that persisted for at least 1 to 2 months postoperatively were more pronounced. These changes included numerous breaks in the inner and outer walls of Schlemm canal endothelium that were observed over large parts of the circumference in all of the operated-on monkey eyes. Gold particles with a high affinity for extracellular matrix that were added to the perfusion fluid were observed in areas of disruption where underlying extracellular matrix bridged the defects or was partly protruding into the lumen of Schlemm canal, and were directly associated with this matrix. Because these particles must have been carried by aqueous flow, we assume that aqueous humor passed through the breaks in the endothelial lining of Schlemm canal. Comparable breaks were reported in a recent study on human and monkey cadaver eyes that had undergone viscocanalostomy. It is well established that acute disruption of the endothelial lining of Schlemm canal in monkey eyes, eg, by treatment with disodium EDTA, ethylene glycol-bis(2-aminoethylether)-N,N,N’,N’-tetraacetic acid (EGTA), or cytochalasin B, is associated with a marked increase in outflow facility. It has been calculated that the inner wall endothelium of Schlemm canal accounts for only less than 10% of total outflow resistance, since it forms numerous micrometer-size pores that allow relatively free passage of aqueous humor.

Therefore, the effects of inner wall disruption on outflow facility are thought to be due to a washout of extracellular matrix components from the juxta-canalicular or cribriform region of the TM into the lumen of Schlemm canal. Data from microcannulation experiments indicate that most of the outflow resistance is located in this region, at a distance of approximately 7 to 14 µm from the inner wall endothelium.

Figure 10. Discontinuities in Descemet membrane (arrows) at the opercular area of control eyes (A and B) and one eye treated by viscocanalostomy 2 months previously (C; monkey AI10) (semithin sections, toluidine blue O stain). A, The discontinuities are frequently observed at the base of the opercular area (Op), an extension of Descemet membrane covering the anterior nonfiltering trabecular meshwork (NFTM), not present in humans. The frequency and structure of discontinuities do not differ between control and viscocanalostomy-treated eyes. Between the ends of the discontinuous Descemet membrane, cells (A) or smaller open spaces up to 5 nm in width (B) may be present. In other areas, Descemet membrane appears to be interrupted only by a small slit (arrow, C). Bars indicate 20 µm.
this evidence, there is still uncertainty as to the exact site and nature of the resistance, with most advocating the jux-
tacanalicular region but some advocating the inner wall en-
dothelium. It seems reasonable to assume that focal dis-
ruption of the inner wall endothelium and resulting washout of extracellular material from the outer parts of the TM
caused the effects on outflow facility in our monkeys. Our
findings are informative regardless of the resistance distri-
bution between the sites in question; indeed, from the vis-
cocanalostomy mechanistic standpoint, both the extracel-
lular matrix washout and inner wall endothelial defects are
important.

It is surprising that these defects remained open for
at least 1 to 2 months after surgery. In the living eye, de-
fects in Schlemm canal endothelium that are larger than
the physiologic pore size of 0.25 µm to approximately 2
µm are occluded by platelet aggregation, a process that
occurs within minutes. This process appeared to be
ineffective, or at least significantly delayed, in our mate-
rial, as only some pores were observed that were com-
pletely or partially occluded by thrombocytes. Moreover,
it is unclear whether the thrombocytes were effective in
sealing the endothelial defects, as numerous gold par-
ticles were often seen associated with extracellular ma-
trix components close to occluded pores, but not in im-
mediately adjacent areas that were separated from the
lumen of Schlemm canal by an intact endothelial layer,
indicating that passage of fluid through the pores did still
occur. A likely explanation for the persistence of endo-
thelial defects in Schlemm canal could be a direct or in-
direct action of sodium hyaluronate on thrombocyte ag-
gregation. Indeed, we observed in some of the defects
homogeneous, granular, electron-dense material that was
not observed in control eyes. Although we do not have
direct molecular proof, we assume that this material re-
flects the presence of remaining sodium hyaluronate, since
sodium hyaluronate that was directly injected into the
anterior chamber of a monkey in a parallel experiment
showed similar ultrastructural characteristics. After an-
terior chamber injection, sodium hyaluronate was found
in the posterior chamber, on the inner surface of the pe-
ripheral cornea, and on the inner uveal parts of the TM,
but not in the juxtacanalicular region. This distribution
indicates that in a structurally intact TM, sodium hya-
uronate is too viscous to enter the fluid pathways of the
juxtacanalicular region, which are considerably smaller
than those of the corneoscleral and uveal TM. Hyalu-
ronan has been shown to inhibit platelet adhesion and
aggregation, and sodium hyaluronate might have similar
effects on the adhesion of platelets to Schlemm canal
endothelium. Identification of the molecular processes
by which sodium hyaluronate might prevent healing of
Schlemm canal defects could provide important infor-
mation on how to increase the effectiveness of agents
that may be used to disrupt the endothelial lining of Schlemm
canal to therapeutically decrease outflow resistance.

Reduction of IOP in viscocanalostomy putatively re-
quires aqueous humor to percolate into an intrascleral re-
ervoir (the so-called scleral lake) that is created by remov-
ing the inner layers of the sclera. From there it is thought
to enter the widened cut ends of Schlemm canal and/or
cut ends of collector channels. As a scleral lake was cre-
ated during viscocanalostomy of our monkeys, such a fluid
pathway may have existed for a time but was absent by
the time of our investigation 1 to 2 months after surgery.
At the site of surgery, Schlemm canal was obliterated and
open ends were not observed. In addition, a larger open
intrascleral reservoir next to Descemet membrane or
Schlemm canal endothelium did not remain in any of the
monkeys. There were, however, regions of hydrated sclera
next to Schlemm canal and close to the site of surgery in
some of the monkeys. These regions were filled with cells
expressing the typical structural characteristics of scleral
fibroblasts and loosely arranged collagen fibers, and might
represent healing stages of a former scleral lake, based on
surgical anatomy and postoperative ultrasound biomi-
croscopy studies in humans. In one of the monkeys, there
was an intrascleral open space at a site considerably dis-
tant from Schlemm canal, which was likely a remaining
part of the scleral lake created by removing scleral tissue.
Thus, scleral spaces that were generated by removing the
inner scleral layers were largely occluded 1 to 2 months
after surgery. However, the hydrated sclera indicates that
fluid was still moving through this region, which may con-
stitute a low-resistance pathway for egress of aqueous
humor from the anterior chamber, and could also be func-
tionally equivalent to a scleral lake. Ultrasound biomi-
croscopy data on human patients indicate that clear scleral
lakes may remain open until at least 7 to 9 months after
surgery. This might indicate that scarring and/or endog-
enous removal of sodium hyaluronate occurs at a much
faster rate in monkeys than in humans. Reasons for this
might be species-specific differences or the presence of more
active tissue repair mechanisms in monkeys vs human pa-
tients with glaucoma. On the basis of our data, we cannot
say whether collector channels were cut open during sur-
gery and whether they remained so despite the ongoing
healing process. Theoretically, an opening of collector chan-
nels could have had effects on outflow facility, as about
25% of outflow resistance appears to be localized distal to
Schlemm canal, probably in the aqueous veins. We did
not see clyodialysis in these animals despite step serial
sectioning in 2 different laboratories, in all probability ex-
cluding this as a possible factor for changes in outflow re-
sistance in our experiments.

We did see discontinuities in the periphery of Desce-
met membrane in the opercular area (an extension of Descemet membrane covering the anterior TM, not pres-
et in humans), but these are normal for monkeys, were
present in control eyes as well, and were distributed equally
around the circumference. These are not related to the sur-
gical procedure, and, in fact, we saw no evidence of iat-
rogenic disruptions of Descemet membrane in any eye.
Because in both humans and normal monkeys aqueous humor
can be seen percolating through Descemet membrane dur-
ing viscocanalostomy surgery, this implies either that mi-
croperforations occur but were missed in our examina-
tion because of their infrequency, or that Descemet
membrane and the endothelium at the corneal periphery
are in fact leaky in the absence of covering stroma. The
latter seems especially likely in the monkey, given the
normal discontinuities in Descemet membrane. This could be
present but more subtle in the human. Indeed, small fis-
sures that are frequently associated with Hassall-Henle warts
have been described in Descemet membrane in human peripheral cornea. These fissures are predominantly localized on the endothelial side of Descemet membrane and may contain processes of endothelial cells or collagen fibrils. Some of these fissures have been observed to penetrate the entire thickness of Descemet membrane to the corneal stroma.

In summary, the most likely explanation for the decrease in outflow resistance in monkeys after viscoanalostomy is the focal disruption of the inner wall endothelium and the opening of the juxtaacinaricular or cribiform region of the TM. We hypothesize that a similar effect is the major reason for the decrease in IOP in human patients after this type of glaucoma surgery. Opening of the outer TM had only modest effects on facility in normal monkeys but might be very effective in human patients, where the cribiform region is the tissue that is most affected by the pathologic changes that occur during primary open-angle glaucoma.

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