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Outflow resistance of enucleated human eyes at two different perfusion pressures and different extents of trabeculotomy

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ABSTRACT

Aqueous outflow resistance of enucleated human eyes was measured at 7 and 25 mm Hg before and after partial and complete (12 clock hours) internal trabeculotomy. Following complete trabeculotomy, 71% of the resistance was eliminated at 25 mm Hg while only 49% was eliminated at 7 mm Hg. In contrast to published findings in eyes with intact trabecular meshwork where the resistance increased with increasing IOP (1), following complete trabeculotomy, the resistance decreased 2% per mm Hg with increasing IOP. Experiments with trabeculotomy limited to part of the circumference showed that a one hour trabeculotomy produced 41% (25 mm Hg) to 60% (7 mm Hg) of the effect of a twelve hour trabeculotomy.

The results indicate that a surprisingly high fraction of aqueous outflow resistance resides in the distal aspects of the outflow system at normal IOP, and that this distal resistance drops as IOP is increased.

INTRODUCTION

It is widely believed that in human eyes approximately 75% of the aqueous outflow resistance is located proximal to the outer wall of Schlemm's canal. However, the experiments on which this information is based (2-5) were performed at perfusion pressures of 25 mm Hg. Since episcleral venous pressure, normally 8 to 11 mm Hg, would be essentially zero in enucleated eyes, the pressure drop across the aqueous outflow system may have been unphysiologically high in these experiments.

Previous investigators have noted non-linearity of resistance change with varying lengths of trabeculotomy in rhesus monkeys at physiologic pressure drops (6) and in enucleated human eyes at higher perfusion pressures (2,5).

We have been interested in the following questions: (1) What would be the effect on flow resistance of limited and total trabeculotomy in enucleated human eyes under steady state conditions at a physiological pressure drop (7 mmHg)? (2) How does the nature of the pressure-flow relationship change following limited and total trabeculotomy?, and (3) How do the resistance changes with extent of trabeculotomy vary with perfusion pressure?

MATERIALS AND METHODS

Nine pairs of normal human cadaver eyes were utilized in the experiments. No eyes were used if any history of glaucoma, ocular injury, surgery, or laser therapy was indicated. Ages of donors ranged from 59 to 76 years. Five pairs were from males, and four were from females. Causes of death included sepsis associated with chronic obstructive pulmonary disease or cirrhosis, myocardial infarction, cerebrovascular accidents, lymphomas and leukemia. Enucleations were performed within four hours of demise, and the bodies had been refrigerated within two hours of death. The eyes were placed in a moist chamber with normal saline and refrigerated at 4°C until brought to room temperature immediately preceding simultaneous paired perfusions. Storage at 4°C ranged from 9 to 38 hours, and all eyes were perfused within 48 hours of demise.

Constant pressure perfusion technique (2) was employed at room temperature with Dulbecco's phosphate-buffered saline augmented with 5.5 mM glucose as the perfusion fluid. The perfusion system has negligible flow resistance as compared to that generated by the aqueous outflow system. After conjunctiva and Tenon's capsule were excised, a 4.5 mm central corneal trephination was performed, followed by a radial iridotomy to the periphery to facilitate total removal of the iris by gentle traction. After the anterior chamber was irrigated with perfusion fluid to remove released pigment and debris, Grant corneal fittings were placed in the corneas, and one eye of each pair was perfused for one hour at 7 mm Hg, and the other at 25 mm Hg to obtain baseline outflow resistances. The assignment of the eyes to elevated or lower pressure was randomized.

A one clock hour (30°) trabeculotomy was then performed under direct microscopic control through an air-filled anterior chamber. A specially modified Von Graefe cystotome, with a shortened, rounded, smoothed end, yet retaining a sharp point and blade, was employed for all trabeculotomy incisions. The incisions created an opening between the anterior chamber and Schlemm's canal. Care was taken to avoid excessive incision depth to minimize trauma to the outer wall of Schlemm's canal or collector channel ostia. The eyes were then re-perfused for another hour, following which 3 additional one clock hour (30°) trabeculotomies were performed, each 90° from each other, resulting in a total of 4 clock hours (120°) incised. After another one hour perfusion to obtain outflow resistance, the eyes received complete 12 clock hour (360°) trabeculotomies, followed by another perfusion for one hour. Previously incised areas were not further manipulated during the subsequent trabeculotomies. Approximately 15 to 25 minutes elapsed between perfusions, due to experimental manipulations associated with the trabeculotomy.

After this sequence, the perfusion pressures were reversed between the eyes, so the eye previously perfused at 7 mm Hg was now perfused at 25 mm Hg and vice versa. In 3 pairs of eyes, a second pressure reversal was performed at the conclusion of the experiment. Finally the eyes were fixed at their final pressure with 3% glutaraldehyde with sodium cacodylate buffer for light microscopy. Representative sections were evaluated to assess patency of the incision and the possibility of inadvertent damage to adjacent structures.

RESULTS

Mean baseline resistance was slightly higher at 25 than at 7 mm Hg, as seen in previous studies (1), but the difference was not statistically significant. For both the elevated (25 mm Hg) and lower (7 mm Hg) perfusion pressures, each sequential step in the trabeculotomy protocol resulted in progressive elimination of outflow resistance (Table 1 and Fig. 1). Of note, however, is the difference in outflow resistance eliminated by a 360° trabeculotomy between the two perfusion pressures. This was calculataed as $100*(R_{baseline}-R_{subsequent})/R_{baseline}$. While 71% was eliminated at 25 mm Hg, only 49% was eliminated at 7 mm Hg ($p<0.02$). Statistical comparison were done using the paired Student t test. Other statistical results are

TABLE 1

OUTFLOW RESISTANCE VS
EXTENT OF TRABECULOTOMY

PERFUSION PRESSURE (mm Hg)	RESISTANCE mm Hg/(µl/min)				
	Baseline	1 Clock Hour	4 Clock Hours	12 Clock Hours	Reversal
7	3.76 (±0.26)	2.64 (±0.20)	2.11 (±0.10)	1.84 (±0.16)	1.01 (±0.08)
25	4.07 (±0.37)	2.86 (±0.37)	1.81 (±0.33)	1.15 (±0.16)	1.72 (±0.24)

N = 9 pairs
Mean Values ± S.E.M.

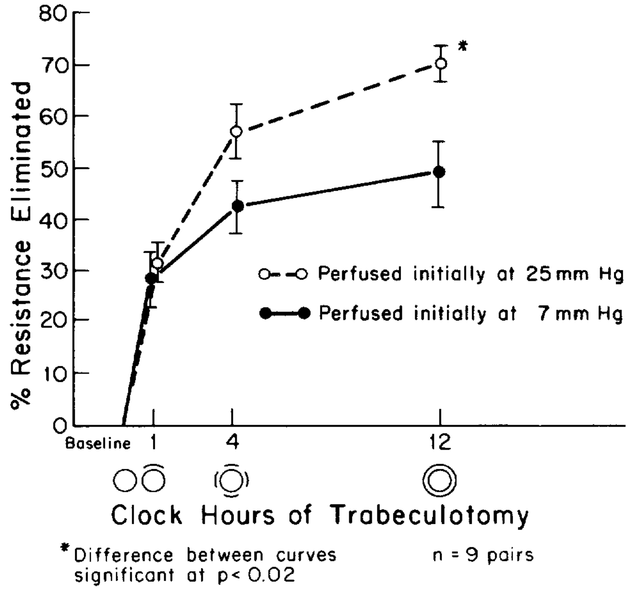

Figure #1: Percentage of baseline resistance eliminated by sequential 1, 4 and 12 hour internal trabeculotomy at 7 vs. 25 mm Hg perfusion pressure. Extent of trabeculotomy at each step indicated by circles at bottom of graph. Error bars are standard errors. Resistance units are (mm Hg/(μl/min)).

presented in Table 2.

This difference in resistance following complete trabeculotomy between the eyes perfused at 7 and 25 mm Hg was confirmed by the pressure reversal procedure that followed the 360° trabeculotomy. When the eyes previously perfused at 7 mm Hg had the pressure increased to 25 mm Hg, the mean outflow resistance decreased from 1.8 to 1.0 mm Hg/(μl/min) ($p<0.005$). In contrast, the eyes previously perfused at 25 mm Hg increased their outflow resistance from 1.2 to 1.8 mm Hg/(μl/min) when the pressure was lowered to 7 mm Hg ($p<0.01$). In the three pairs of eyes subjected to a complete trabeculotomy followed by a double pressure reversal, the same tendency was seen for a reversible pressure-induced change in outflow resistance (Fig. 2).

Another observation was the distinct nonlinearity in the elimination of resistance vs. the extent of trabeculotomy (Fig. 1). At both 7 and 25 mm Hg, a much greater proportional decrease in

TABLE 2
STATISTICAL SIGNIFICANCE OF
RESISTANCE CHANGES

EXTENT OF TRABECULOTOMY	ΔR	Stat Sig	% $\Delta R/R_0$ per clock hour	% $\Delta R/\Delta R_{max}$
7 mm Hg (baseline resistance = 3.76 mmHg/(μl/min))				
1 clock hour	-1.2	p<0.002	-32	60
4 clock hours	-1.7	p<0.05	-11	85
12 clock hours	-2.0	p>0.1	-4	100
reversal from 7 to 25 mm Hg	-0.8	p<0.005	-	-
25 mm Hg (baseline resistance = 4.07 mmHg/(μl/min))				
1 clock hour	-1.2	p<0.001	-29	41
4 clock hours	-2.3	p<0.0001	-14	79
12 clock hours	-2.9	p<0.01	-6	100
reversal from 25 to 7 mm Hg	+0.6	p<0.01	-	-

ΔR is computed from baseline except for pressure reversals. Statistical significance is computed using the change from the previous step.

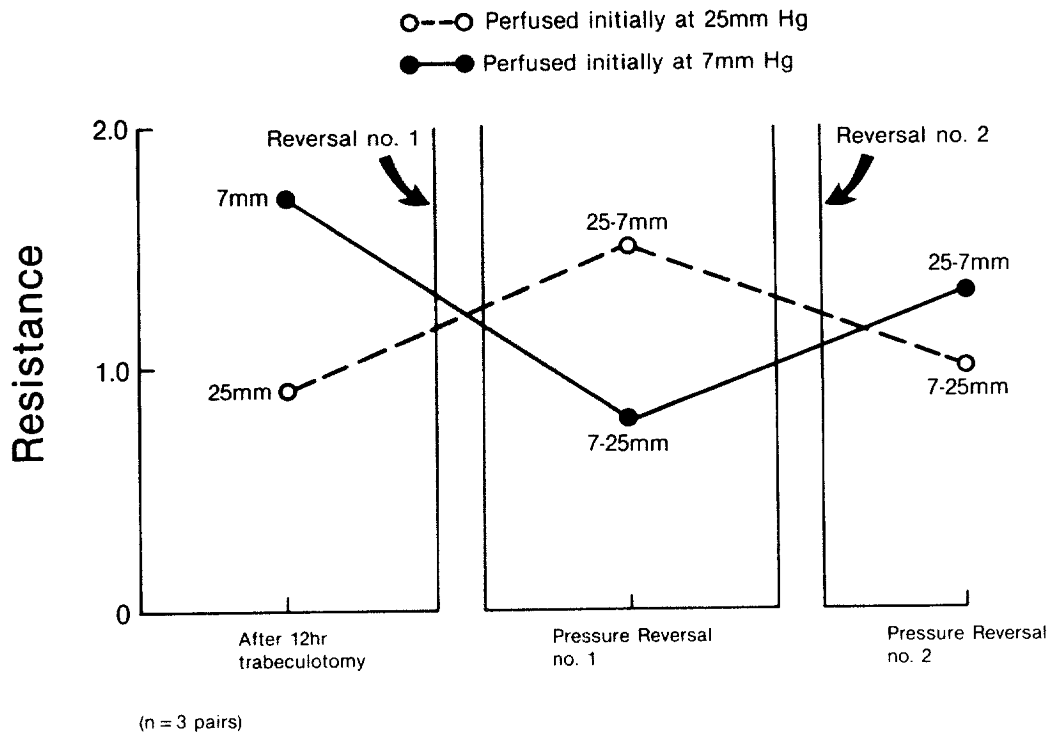

Figure #2: Double reversal of perfusion pressure following total internal trabeculotomy. Solid line represents eyes with 7 mm Hg - 25 mm Hg - 7 mm Hg perfusion pressure sequence. Dotted lines represent 25 mm Hg - 7 mm Hg - 25 mm Hg sequence.

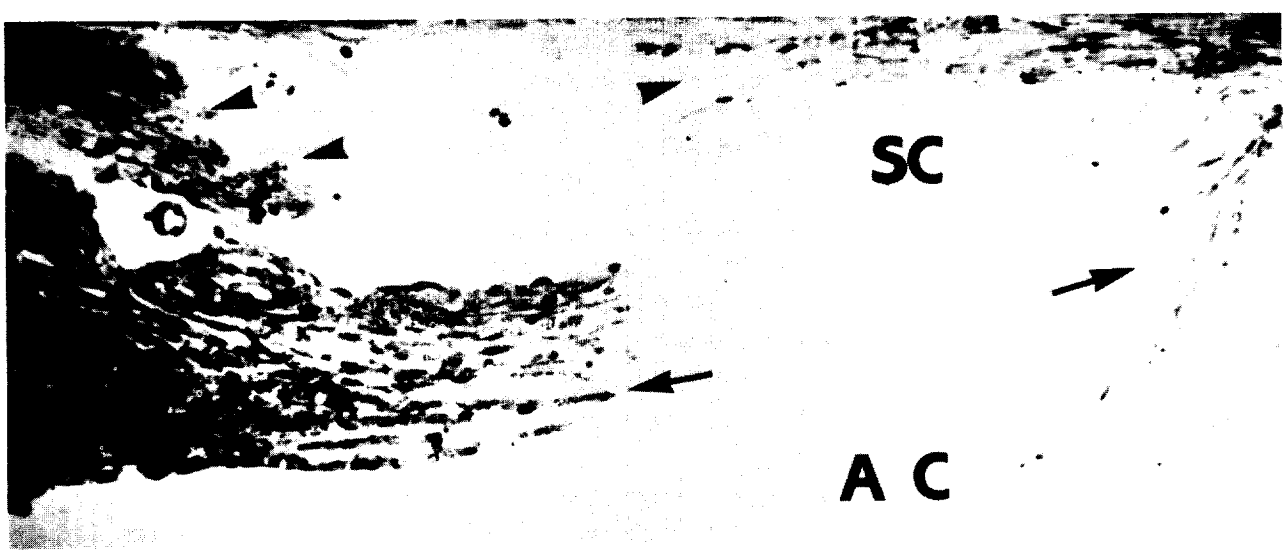

Figure #3: Internal Trabeculotomy -- In this section, a through and through trabeculotomy is evident (arrows), communicating Schlemm's canal (SC) with the anterior chamber (AC). Note cut into outer wall of Schlemm's canal (arrow-heads). "x 200".

resistance was noted with the initial 1 clock hour (30°) incision than was found with further incisions.

Histologic monitoring of the incisions revealed patent clefts from the anterior chamber to Schlemm's canal in 65 of 70 sections examined (Fig.

3). Only two of the eyes examined showed sections with non-penetration of the trabecular meshwork: one showed non-penetration in 2 of 8 sections, while the other showed non-penetration in 3 of 6 sections. Damage to the outer wall structures was occasionally noted.

DISCUSSION

Over twenty years ago, one of us (WMG) found that following complete internal cystotome trabeculotomy in enucleated human eyes, approximately 75% of aqueous outflow resistance could be eliminated (2,3). This has been confirmed by Ellingsen and Grant (4), Johnstone and Grant (7) and Van Buskirk and Grant (5). This observation has led many to believe that the principal site of aqueous outflow resistance lies proximal to the outer wall of Schlemm's canal; however, since the experiments were performed at comparatively high perfusion pressures (25 mm Hg), in retrospect it appears that the pressure drop across the outflow system was unphysiologically high in these experiments.

Later, Ellingsen and Grant (4,8) explored the relationship between perfusion pressure and outflow resistance and discovered that in human and primate eyes with an intact outflow system, elevated perfusion pressure caused an increase in outflow resistance. One aspect of their work that has received little attention was the observation that following 3, 6 or 12 clock hour internal cystotome trabeculotomy, increased perfusion pressure produced a decrease rather than an increase in outflow resistance. However, these experiments were performed with rapid step-wise pressure changes from 5 to 50 mm Hg, allowing only 5 minutes for each pressure increment, which likely prevented true steady state conditions.

Peterson, Jocson and Sears (6) investigated the effects of internal trabeculotomy on rhesus monkeys varying the extent of trabeculotomy from 5 to 100% at perfusion pressures of 5 to 12 mm Hg (mean of 8 mm Hg). Non-linearity of resistance changes with varying length of trabeculotomy was prominent, with a greater proportional resistance decrease occurring with minimal extent of trabeculotomy. Grant's data (2) and measurements by Van Buskirk and Grant (5) also indicated a greater proportional decrease in resistance with minimal extent of trabeculotomy.

The experiments performed in the current study involved hour long perfusions that ensured the measurement of steady-state conditions. While at a perfusion pressure of 25 mm Hg we found that 71% of the flow resistance could be eliminated by a trabeculotomy, in agreement with previous findings (2,3), we found that at a more physiological pressure drop across the aqueous outflow system (7 mm Hg), only about half of the total resistance could be eliminated by an incision between the anterior chamber and Schlemm's canal.

Furthermore, while the flow resistance of the intact eyes has been found to increase with increasing perfusion pressures (1-2%/mm Hg (1)), we found this trend to be reversed in eyes with complete internal cystotome trabeculotomy (a decrease of 2%/mm Hg). These values were calculated by determining the total percentage change in resistance divided by the change in perfusion pressure: $100*(1-R_{subsequent}/(R_{baseline})/(P_{baseline}-P_{subsequent})$. Since these differences in response persisted following reversal of perfusion pressure, we believe the effect to be mediated mechanically, and not the result of "washout" of substances or due to biochemical modification.

Experiments with partial trabeculotomy showed that a one hour incision produced 41% (25 mm Hg) to 60% (7 mm Hg) of the effect of a twelve hour trabeculotomy. These results suggested incomplete circumferential flow in Schlemm's canal. If there were very high resistance to circumferential flow in Schlemm's canal, and if the flow through the trabecular meshwork is uniform around the circumference, the increase in facility following partial trabeculotomy should be essentially linearly related to the amount of meshwork incised (2). On the contrary, if no resistance to flow in Schlemm's canal was present, virtually any opening between the anterior chamber and Schlemm's canal would result in functional bypass of the trabecular meshwork resistance and eliminate all "trabeculotomy-sensitive" resistance.

We hypothesized that four one hour incisions, placed 90° apart should be sufficient to bypass the flow resistance in Schlemm's canal. The results in Table 2 show that at 7 mm Hg, the changes in flow resistance from a 4 hour to a 12 hour trabeculotomy were not statistically significant ($p>0.1$), in agreement with the above prediction. At 25 mm Hg, there was a significant drop in flow resistance from a 4 hour to a 12 hour trabeculotomy ($p<0.01$). This may reflect higher flow resistances in the remaining segments of Schlemm's canal at the higher perfusion pressure (9). It should be mentioned here, however, that at both pressures, the additional drop in flow resistance resulting from a 4 hour trabeculotomy to a 12 hour trabeculotomy was far less than that resulting from the 1 hour and 4 hour cuts (see Figure 1).

Perhaps the most significant aspect of this study was the finding that only half of the outflow resistance, when measured at a physiological pressure drop of 7 mm Hg, could be removed by a complete internal cystotome trabeculotomy. Since patent incisions were noted between the anterior chamber and the lumen of Schlemm's canal (see Figure 3), we assume that we effectively eliminated the flow resistance of the trabecular meshwork and Schlemm's canal in the areas incised. Following complete trabeculotomy, with the elimination of the trabecular meshwork and Schlemm's canal for the full circumferential extent, the only remaining source of outflow resistance should reside in the collector channels and aqueous veins. Sears (10) also concluded that there was significant flow resistance distal to Schlemm's canal in experiments in which he simultaneously monitored pressure in the anterior chamber and in Schlemm's canal.

Based on the dimensions of the collector channels as noted by casting injection studies (11) or transmission electron microscopy (12-14), it is intriguing how any of these vessels can exhibit any functionally significant resistance. There is general agreement that there are approximately 30 of these vessels in each eye and that their path length through the sclera is approximately 0.1 cm. Using a flowrate of 2.0 μl/min and assuming a pressure drop of 1.25 mm Hg through the aqueous veins (25% of the total resistance in an average human eye; this was the generally accepted figure prior to the current study), Poiseuille's law can be used to calculate the expected diameter of these vessels. Assuming all aqueous veins to have the same diameter and assuming this diameter to be constant along their length, the aqueous vein diameter necessary to produce this hydraulic resistance is 20 μm; however, a single vessel 50 μm in diameter could carry the entire flow with the same pressure drop. Although vessels as small as 20 μm in diameter have been found in the eye (12), most vessels found are larger than this with diameters averaging approximately 50 μm (13). We thus conclude that, granting our assumptions, the aqueous veins could not generate the measured flow resistance unless they are collapsed, contain a localized constriction or are gel-filled. Nesterov (15) and Tripathi (16) reached similar conclusions.

Elevated perfusion pressure reduced the flow resistance following trabeculotomy, and these resistance changes were reversible. Thus we presume that the collector channels and/or aqueous veins may change their effective dimensions in response to changes in perfusion pressure. Since flow resistance is strongly dependent on vessel diameter (inversely proportional to the fourth power of diameter for Poiseuille's law), relatively small alterations in the effective diameter of the distal outflow system would produce sizeable changes in outflow resistance. One possibility is direct distension of the diameter of the vessels by increased intraluminal pressure, assuming some distensibility of the structures involved. Another contribution could result from pressure-induced stretching of the sclera, secondarily altering the effective diameter of the outflow channels.

A number of potential artifacts and shortcomings should be mentioned relative to the conclusions reached. Although we have no histological evidence of such, there is a possibility that during the experiments the trabeculotomized tissue fell back against the outer wall of the canal and occluded the collector channel ostia. It is also possible that extracellular matrix material, presumably present in the trabecular meshwork, could have been released by the incision procedure, although no signs of a 'wash-out' effect were noted. Finally, these experiments were on enucleated human eyes used 1 to 2 days post-mortem, and the perfusion pressures used were low due to the absence of an episcleral venous pressure. Furthermore, it is unknown what effect the lack of blood flow has on this preparation. Nonetheless, valuable information concerning the flow characteristics of the outflow system have been obtained in the past using similar methods (2,3,17-20).

No sham manipulations were performed in this study, but our group has previously demonstrated that sham manipulations of enucleated human eyes do not significantly affect the measured outflow facility (removal of uveal meshwork (3), sham trabeculotomy (7), lens depression (18), pressure cycling (19), long time perfusion (21)).

In earlier work, Grant (2) demonstrated in a small number of cadaver eyes with open angle glaucoma, that although the baseline resistances were elevated, following internal trabeculotomy, the final resistances were comparable to those of normal eyes. This suggested that the abnormal resistance in open angle glaucoma probably resides within the trabecular meshwork. If a technique can be devised to create a long-lasting patency between the anterior chamber and Schlemm's canal, a substantial lowering of resistance and intraocular pressure would result. Nevertheless, our results suggest that even following complete elimination of the trabecular meshwork and Schlemm's canal, outflow resistance from the aqueous veins would remain. In addition, of course, episcleral back pressure would remain operant.

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