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The experimental production of impaired outflow facility in the vervet monkey; an in vitro study

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THE EXPERIMENTAL PRODUCTION OF INCREASED OUTFLOW FACILITY IN THE VERVET MONKEY: AN IN VITRO STUDY

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THE EXPERIMENTAL PRODUCTION OF IMPAIRED OUTFLOW FACILITY IN THE VERVET MONKEY AN IN VITRO STUDY

by

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INTRODUCTION

Chronic simple glaucoma is an eye disease characterized by increased intraocular pressure sufficient to produce excavation and degeneration of the optic disc and degeneration of ganglion cells and nerve fibers in the retina. It is one of the leading causes of blindness in the United States, and it has an incidence of approximately 2% in unselected patients over the age of forty. (1) As such, it is one of the most important eye diseases encountered in the practice of medicine.

Intraocular pressure is determined by the rate of aqueous humor production and by the resistance to outflow of the aqueous from the eye, i.e.,

$$Pe \propto -\frac{F}{C}-$$

where Pe = intraocular pressure
F = the rate of aqueous humor production (μl/min) and
C = the facility of aqueous outflow (μl/min/mm Hg) or 1/resistance

The aqueous humor is secreted by the ciliary processes and flows from the posterior chamber through the pupillary aperture into the anterior chamber. It then exits from the chamber angle through the trabecular meshwork into Schlemm's

canal. Efferent collector channels, the aqueous veins, arise from the outer circumference of Schlemm's canal and drain to the anterior ciliary (episcleral) veins. In chronic simple glaucoma, the increased intraocular pressure is caused by an abnormal resistance to the outflow of aqueous humor, located in the trabecular meshwork or in the inner wall of Schlemm's canal. This results in the impaired outflow facility seen in this disease.

Although it is known that the decrease in the outflow facility results in an increase in intraocular pressure, it is not known how this increased pressure produces pathologic changes in the eye. For example, it is not known how long a given pressure must act before the characteristic histologic and ophthalmoscopic changes are produced in the posterior pole, nor at what point visual field loss is encountered or when it becomes irreversible. Even the regulatory mechanisms governing the control of intraocular pressure are incompletely understood. For example, what effect does the increased pressure have on the rate of aqueous humor production? Obviously, the elucidation of these and other interrelated problems remains a fertile area for intense investigation.

The National Institute of Health is currently sponsoring a collaborative study designed to yield information about the natural history of chronic simple glaucoma. It is hoped that the accumulation of a large series of clinical cases observed closely over time will provide answers to some of the many questions that exist. However, it will certainly take many years before even preliminary answers are forthcoming, and there is no assurance that this study will, or, indeed, even can, provide the necessary information.

It would seem desirable, therefore, to produce, in a suitable species, an experimental model with which to study the natural history of chronic simple glaucoma. There have been few previous attempts to establish such an animal model for glaucoma simplex.

Historically, the rabbit has been the most frequently used subject in such an attempt. A variety of techniques designed to increase intraocular pressure by obstructing the chamber angle have been used.

Kupfer (2) placed polyethylene tubing in the angle to block the outflow channels. In 60% of his rabbits, the intraocular pressure rose within twenty-four hours to

50-60 mm Hg and remained elevated for three months, with a gradual decrease in pressure to 30-35 mm Hg. Histologically, these eyes showed a selective loss of ganglion cells in the retina, loss of myelin from the optic nerve fibers, and deep excavation of the optic nerve head. There was fibrosis in the chamber angle.

Vessey (3) used three different methods in his attempt to obstruct the angle. Inserting either polyethylene tubing or surgical sutures into the angle, or suturing the root of the iris up against the cornea through a full 360 degrees, he was unable to demonstrate any increase in intraocular pressure. However, by his own admission, each of his methods had associated technical difficulties, and, consequently, the validity of his results is open to doubt.

Carvalho (4) blocked the angle by injecting cotton fragments into the anterior chamber. 57% of the eyes developed pressures of 38-48 mm Hg. Maintenance of this pressure for fifteen days resulted histologically in vacuolation and degeneration of ganglion cells; if maintained for more than thirty days, degeneration and atrophy of the retina and cupping of the optic disc resulted.

Flocks (5) produced increased intraocular pressure by applying a tight encircling rubber band around the equator of the eye. The pressure initially rose to 70-100 mm Hg, but then declined within forty-eight hours to 35-50 mm Hg, after which it declined more slowly. However, 33% of the eyes were lost because of panophthalmitis, and only 12% developed cupping of the optic disc. A constant finding was marked degeneration of all layers of the retina, in addition to the experimentally produced gross structural damage to the eye.

Chemical substances have also been used to produce angle block. Samis (6) and Kazdan (7) obstructed the angle with 0.5% methyl-cellulose. The maximum increase in intraocular pressure occurred within fifteen minutes to a level of 50 mm Hg. No information is presented concerning the duration of the hypertension or its subsequent level, and there are no histopathologic correlates. Hakim (8) injected argemone oil and sanguinarine subconjunctivally and intraocularly and produced a pressure increase to 30-50 mm Hg within fifteen minutes. This pressure was maintained for twenty minutes, but returned to normal thereafter.

Various other techniques have been employed to produce experimental glaucoma. Luntz (9,10) blocked the episcleral outflow channels by injecting a preparation of 5% phenol in almond oil subconjunctivally in all four quadrants. This produced an increase in pressure to 60 mm Hg over periods varying from five to fifteen weeks. A second group of animals reached levels of 30 mm Hg. Neither group was examined for signs of glaucoma nor were the eyes sectioned for histopathology.

Thomas (11) cut the extraocular muscles and produced "severe glaucoma" and a "transient miosis". He postulated that "the glaucoma" resulted from the release by the fifth cranial nerve of a miotic substance similar to histamine. However, histamine itself has been shown to cause a lowering of intraocular pressure. (12) "Non specific ocular insults" have been associated with an increase in pressure, and Thomas proposed that the trauma and proptosis associated with the surgery could have been implicated.

Levene (13) demonstrated a 40% decrease in outflow facility with an in vitro perfusion after in vivo retrociliary diathermy of the globe or posterior angiodiathermy of the long posterior ciliary vessels, unassociated with increased

intraocular pressure in vivo. The chamber angles were open and without histologic changes. Interference with the vascular supply to the structures of the anterior segment of the eye, with or without the liberation of substances toxic to the outflow tissues, is the explanation given.

Huggert (14) attempted to increase intraocular pressure using three of these different approaches. He tried diathermic cautery of episcleral aqueous veins near the limbus, ligation of trunks of vessels at the posterior pole, and injection of suspensions of particles into the anterior chamber. None of these methods, however, gave stable, enduring, and reproducible ocular hypertension.

In addition to the limitations of each of these approaches and the failings within each experiment itself, the use of the rabbit as the experimental model has obvious deficiences. The rabbit is so far removed from man phylogenetically that the validity of extrapolating results from this animal to man is certainly questionable. In this regard, the monkey eye would be a more ideal experimental model, as it more closely resembles the human eye. Yet, there have been very few investigations using this animal.

Rohen (15) injected a 0.5-1.0% p-cresol solution into the anterior chamber of monkeys and found that the pressure increased to 15-28 mm Hg within a few days in 60% of the eyes. No retinal degeneration or cupping of the disc was found after up to thirty-nine days. The trabecular meshwork in these eyes revealed marked thickening and hyalinization, with a cellular infiltration.

Kirsch (16) described a transient elevation of intraocular pressure in humans after cataract extraction associated with the use of alpha-chymotrypsin. Hamasaki (17), while studying the effect of alpha-chymotrypsin on the electroretinogram of monkeys, also noticed an increase in intraocular pressure in these animals, with cupping of the optic disc and atrophy of the optic nerve on routine histologic sections.

Following these leads, Kalvin (18) injected alpha-chymotrypsin into various anatomic parts of the owl monkey eye. He consistently raised intraocular pressure only with injections of concentrated enzyme into the posterior chamber with a miotic pupil. All (eleven) the animals treated in this way developed glaucoma, nine in the "severe range" (pressures greater than 61 mm Hg) and two in the "mild range" (pressures

of 24-40 mm Hg). Histopathologically, cupping of the optic disc and cavernous atrophy of the optic nerve were seen. However, except for a few representative figures, no correlation is made between the unmentioned duration of the ocular hypertension and the histopathology. Moreover, the enzyme produced severe damage to the photoreceptors and outer layers of the retina. The amount of elevation of intraocular pressure could not be controlled, and the relatively acute rise in pressures to above 60 mm Hg certainly does not simulate either the pressure level or the course commonly found in human chronic simple glaucoma.

Kalvin (19) also injected dental molding cement, talc, and liquid silicone into the anterior chambers of owl monkeys in an attempt to increase the intraocular pressure. The silicone produced only a transient increase in the intraocular pressure. The other two substances caused the pressure to be variably increased (26-70 mm Hg), with the subsequent production of cupping of the disc and atrophy of the nerve as with alpha-chymotrypsin, but without the retinal damage associated with the enzyme. Again, however, there is inadequate correlation between the duration of hypertension and pathology, and the

pressures were generally excessive.

Thus, these previous attempts to produce experimental glaucoma do not serve as a model for chronic simple glaucoma in man. What is needed is a model which would allow the production, without structural or inflammatory damage to the eye, of moderate ocular hypertension maintained over months or years.

Since $Pe \propto -\frac{F}{C}-$, an increase in the resistance to the outflow of aqueous humor from the eye, i.e., a decrease in C, outflow facility, would be expected to result in an increased intraocular pressure. It would be even better if it were possible to selectively increase the pressure to desired and reproducible levels so as to gain a fuller understanding of the pathogenesis of the ocular changes associated with chronic simple glaucoma.

This might be possible by obstructing varying percentages of the circumference of Schlemm's canal, a technique that has already been employed in anatomic studies of Schlemm's canal and the aqueous drainage paths. (20-24) In addition, Jocson (24) has determined the facility of outflow in eight human eyes before and after filling Schlemm's canal and the aqueous

outflow channels with silicone rubber. He reported that the facility dropped to very low levels, although he does not quantitatively correlate these values with the percentage of Schlemm's canal which was filled with silicone. Nevertheless, it certainly seems feasible to attempt to increase intraocular pressure by fractional filling of Schlemm's canal.

The choice of the experimental animal is obviously critical, both for success in the production of the model and for the applicability of its results to man. A species of monkey seems a particularly good choice, because of its phylogenetic proximity to man. In addition, the chamber angle of the higher monkeys is structurally similar to man's. (25) Rohen (26) even goes so far as to say that in Cercopithecus ethiops, the African green vervet monkey, "one finds almost the same chamber angle as in man." It is also known that glaucoma occurs naturally in the vervet, with histopathology very similar to what one finds in man. (25) Cercopithecus ethiops has certainly been widely used as an experimental model in ophthalmic investigation (27-38), and it therefore has some merit as the experimental animal in this research.

The present in vitro study was undertaken preliminary to

the establishment of the living model. It was designed to determine whether differential fractional filling of Schlemm's canal could indeed produce controlled and reproducible decreases in outflow facility. If successful, appropriate decreases could then be used in vivo to produce intraocular pressures in the range seen in chronic simple glaucoma.

Barany (39) has championed the validity of the results obtained by perfusing enucleated eyes. He argued that the structures which determine the resistance to outflow through the chamber angle - the trabecular meshwork, the endothelium of Schlemm's canal, and the collector channels - have never contained blood under physiologic conditions, and therefore make an ideal organ for perfusion, since their living conditions have not been changed by the cessation of blood flow. Accordingly, only post-mortem changes in the angle structures should influence outflow resistance. Indeed, Becker (40,41) has shown that in vitro and in vivo facility measurements are actually comparable.

MATERIALS AND METHODS

In order to determine outflow facility, a constant pressure perfusion system utilizing the fritted glass disc technique described by Grant (42) was set up. A reservoir of sterile 0.9% sodium chloride solution elevated 68 cm (pressure equivalent 50 mm Hg) above the eye served as the perfusion fluid. This flowed to the eye through an ultrafine fritted disc (pore size 0.9-1.4 μ) which was calibrated as a differential flow meter. The pressure in the eye was measured by an electric strain gauge transducer placed close to the eye in the infusion system and continuously recorded on a Sanborn electromonometer. The transducer-recorder system was calibrated and standardized before each perfusion. Standardization was accomplished by utilizing saline filled glass columns to give hydrostatic pressures equivalent to 0, 10, 25, and 50 mm Hg (elevations of 0, 13.6, 34.0, and 68.0 cm, respectively, above the eye).

In this system, the fritted disc acts as a pressure dependent differential flow meter, and as such gives the system its own facility value ($\Delta F/\Delta P$). Accordingly, the

facility, Cs, of the perfusion system is equal to the volume of effluent from the cannulation needle per unit time per unit pressure, or

(1) Cs (µl/min/mm Hg) = volume/time/mm Hg

Calibration was achieved by collecting a volume of effluent and weighing it. Since 1 mg = 1 µl, and since time and pressure were both known, Cs was readily calculable.

The rate of flow into a perfused eye at any given time is determined by the facility of the system and the effective perfusion pressure, the hydrostatic pressure from the reservoir minus the intraocular pressure, or

(2) Flow (µl/min) = Cs x (Pr - Pe)

where Cs = equation (1)
Pr = hydrostatic pressure from the reservoir
Pe = intraocular pressure as recorded

Outflow facility is, of course, equal to the outflow per minute divided by the outflow pressure gradient. When the intraocular pressure has reached and maintained a constant level, a steady state condition has been reached. (Experimentally this occurred within forty-five minutes, even in post-injection perfusions.) At this point, outflow is equal to inflow, and the outflow pressure gradient is Pe. Accordingly, the outflow

facility of the eye, Ce, becomes:

$$(3)\ Ce\ (\mu l/min/mm\ Hg) = \frac{Flow}{Pe} (equation\ 2)$$

Enucleated Cercopithecus ethiops eyes were kindly supplied by Wyeth Laboratories, Inc., Marietta, Pa. They were refrigerated in wet ice while in transit and in all cases perfused within twenty-four to forty-eight hours after enucleation.

The eyes were completely bathed in a 0.9% sodium chloride solution, and the anterior chamber was mechanically cannulated utilizing the spring gun and cannulation needles of Sears (43) Outflow facility (C1) was then determined as above, at room temperature.

Following the perfusion, the polyethylene tubing to the cannulation needle was clamped, maintaining the cannulation of the anterior chamber. The eye was examined under a Zeiss binocular operating microscope, and the aqueous veins were identified. One of these veins was cannulated using a specially prepared polyethylene cannula made by drawing PE-90 tubing heated over a hot plate into cannulas of very small diameter.

Room temperature vulcanizing orange colored liquid

silicone compound was then injected retrograde through this aqueous vein into Schlemm's canal. This injection material was a freshly prepared mixture of 1.0 ml MICROFIL MV-117 liquid silicone compound, 1.0 ml MICROFIL MV diluent, and 0.2 ml curing agent dibutyl tin dilaurate. (Canton Biological Products, Swarthmore, Pa.) The mixture was injected by firm finger pressure from a 2 cc glass syringe attached to the end of the polyethylene cannula by a 23 gauge needle.

After injection, the eye was placed in a cold, moist chamber to allow the silicone to polymerize. The following day the eye was re-attached to the transducer and re-perfused to determine the post-injection outflow facility (C2). Some eyes were then perfused with a fluorescein tinted saline solution and examined under cobalt blue light to determine the number of aqueous veins remaining patent and functional.

Following this, the eyes were treated in such a way as to fix and clear the tissues. (24) A 5 mm hole was trephined through the sclera posteriorly to allow the bathing fluids to enter the eye easily and also to prevent shrivelling or distortion of the globe. The eyes were fixed in formaldehyde and dehydrated by immersion in 50%, 70%, 90%, and 100% ethyl

alcohol, and finally in ether. Each eye remained in each solution for twenty-four hours. They were then cleared by immersion in methyl-salicylate in which they remained. This treatment rendered transparent all tissues except the pigment layers and the orange colored silicone compound and allowed direct observation of the extent of the filling of Schlemm's canal and the aqueous venous network.

Photographs were taken of the eyes after the globes were cleared. (Figures 1-4)

RESULTS

The experimental results are presented in Tables I and II.

Table I lists the complete series of eyes. C1 is the initial facility value. C2 is the post-injection facility value. % decrease in C is (C1 - C2)/C1 x 100. % circumference filled is the % of Schlemm's canal filled with silicone, determined by inspection after clearing of the globe.

Table II correlates the % of circumference filled with the % decrease in facility.

The five eyes asterisked in Table I are not included in Table II. Two eyes (GME 8-10-67A-W and GME 8-15-67B-W) served as control eyes to determine what effect the time delay between perfusions had on C2. Accordingly, they were not injected with silicone and do not appear in Table II. One eye, (GME5 8-4-67-PI) when received, was found to have a cloudy cornea, protein precipitates within the corneal stroma, and wrinkling of the corneal endothelium. These abnormalities probably represented generalized degenerative and/or inflammatory changes also affecting the angle structures, a hypothesis supported by the extremely low value of 0.20 for C1. The validity of the subsequent results is thus in serious

doubt, and this eye was therefore omitted from Table II. Two eyes (GME2 8-4-67-PI and GME 8-8-67B-W) were both associated with paradoxical increases in facility after 40% and 20% filling of Schlemm's canal, respectively. This became apparent during the post-injection perfusions when steady state pressures lower than those for the initial perfusions were seen. Examination of the perfusion system revealed, in each case, a leak of perfusate from around the connection between the anterior chamber cannulation needle and the polyethylene tubing coupling the eye to the transducer. These, then, are also absent from Table II.

TABLE I

EYE	C1	C2	% decrease in C	% circumference filled
Experimental				
*GME2 8-4-67-PI	0.53	0.58	- 9	40
GME3 8-4-67-PI	0.62	0.07	89	100
GME4 8-4-67-PI	0.44	0.06	86	60
*GME5 8-4-67-PI	0.20	0.09	55	25
GME 8-8-67A-W	0.56	0.10	82	85
*GME 8-8-67B-W	0.43	0.64	-49	20
GME 8-10-67B-W	0.39	0.05	87	95
GME 8-14-67B-SF	0.35	0.24	31	40
GME 8-15-67A-W	0.43	0.13	70	70
GME 8-22-67A-W	0.68	0.58	15	25
GME 11-30-67A-W	0.46	0.04	91	100
GME 12-5-67A-W	0.33	0.05	85	95
GME 12-7-67A-W	0.40	0.10	75	70
GME 12-7-67B-W	0.42	0.06	86	90
GME 1-3-68A-W	0.45	0.10	78	75
GME 1-3-68B-W	0.45	0.15	67	60
GME 1-13-68A-W	0.45	0.21	53	50

TABLE I
(continued)

EYE	C1	C2	% decrease in C	% circumference filled
GME 1-13-68B-W	0.49	0.12	75	80
GME 1-14-68A-W	0.42	0.06	86	100
GME 1-14-68B-W	0.48	0.12	75	75
GME 1-19-68A-W	0.45	0.32	29	20
GME 1-19-68B-W	0.52	0.20	62	65
GME 1-25-68B-W	0.53	0.29	45	40
GME 1-31-68A-W	0.50	0.23	54	50
GME 1-31-68B-W	0.46	0.28	39	40
Control				
*GME 8-10-67A-W	0.43	0.44	- 2	
*GME 8-15-67B-W	0.42	0.46	-10	
MEAN **	0.46			
S.D.	0.08			
S.E.	0.02			

** GME5 8-4-67-PI is not included in this or the subsequent calculations.

TABLE II

% circumference filled	% decrease in C	Eye
20	29	GME 1-19-68A-W
25	15	GME 8-22-67A-W
40	31	GME 8-14-67B-SF
40	39	GME 1-31-68B-W
40	45	GME 1-25-68B-W
50	53	GME 1-13-68A-W
50	54	GME 1-31-68A-W
60	67	GME 1-3-68B-W
60	86	GME4 8-4-67-PI
65	62	GME 1-19-68B-W
70	70	GME 8-15-67A-W
70	75	GME 12-7-67A-W
75	75	GME 1-14-68B-W
75	78	GME 1-3-68A-W
80	75	GME 1-13-68B-W
85	82	GME 8-8-67A-W
90	86	GME 12-7-67B-W
95	85	GME 12-5-67A-W

TABLE II
(continued)

% circumference filled	% decrease in C	Eye
95	87	GME 8-10-67B-W
100	86	GME 1-14-68A-W
100	89	GME3 8-4-67-PI
100	91	GME 11-30-67A-W

DISCUSSION

The data presented in Tables I and II clearly show that the facility of aqueous outflow is reduced by obstructing Schlemm's canal with silicone rubber. In addition, it appears that there is a rough but definite correlation between the percentage of circumference filled and the percentage decrease in facility. (Table II) That the correspondence is not exact is easily understandable, as the anatomic location and grouping of the aqueous veins and their relative sizes are variable from eye to eye. Thus, filling of the same percentage of circumference in different eyes might be expected to leave unblocked different numbers and sizes of outflow channels, resulting in a difference in contribution to total outflow resistance of the external collector channels (aqueous veins) remaining functional. It might be expected that the poorest correlation would occur with the lesser percentages of filling of Schlemm's canal, and the data support this conclusion.

Perfusions with the fluorescein tinted saline solution confirmed the anatomic variability of the aqueous veins and the rough correlation between decrease in facility and filling

of circumference. GME 12-7-67A-W, with 70% of Schlemm's canal filled and a 75% decrease in facility, had three functionally draining aqueous veins. GME 1-13-68B-W, with 80% of Schlemm's canal filled and a 75% decrease in facility, also had three patent channels. In these two eyes, a difference in filling of 10% was associated with the same number of patent aqueous veins and the same percentage decrease in facility.

The correlation demonstrated in Table II thus forsees the plausibility of the use of this experimental technique to produce controlled, pre-determined, and reproducible decreases in outflow facility and associated levels of ocular hypertension in the living animal. For example, in vivo,

$$Po = \frac{F}{C} + Pv$$

where Po = intraocular pressure
F = rate of secretion of aqueous humor
C = facility of aqueous outflow
Pv = episcleral venous pressure

Assume, for convenience, that the normal animal has values of Po = 12, Pv = 6, F = 3, and C = 0.5. From Table II it can be seen that obstruction of 50% of Schlemm's canal is associated with roughly a 50% decrease in facility. This amount of filling in vivo would be expected to result in a facility (C)

of 0.25 and an intraocular pressure of 18 mm Hg. Similarly, 75% filling would be expected to produce a pressure of 30 mm Hg. This, then, would allow the use of the vervet monkey as a model of chronic simple glaucoma, enabling one to investigate the pathogenesis of ocular changes associated with moderate but prolonged increases in intraocular pressure.

The experimental data also provide strong evidence in support of the hypothesis that the main route for the drainage of aqueous humor from the anterior chamber is through the trabecular meshwork into Schlemm's canal and out the aqueous veins. Davson (44) has summarized the evidence for this in an excellent review. The observed correlation (Table II) and the very low facility values found with complete filling of Schlemm's canal argue convincingly for this hypothesis.

The mean facility value of 0.46 ± 0.02 found here is in close agreement with that reported for Cercopithecus ethiops in vivo by other workers. (31,38) This speaks for the anatomic determinants of outflow resistance being of great importance as opposed to whatever neurohumoral factors exist. It also provides evidence that the eyes, which were not perfused until twenty-four to forty-eight hours after enucleation,

had not undergone degeneration and were similar to living eyes, at least as regards the outflow mechanisms. Finally, the agreement between the pre- and post-injection facility values in each control eye shows that these values were not artifactually lowered by perfusion with 0.9% sodium chloride, as Barany (27) has described during long perfusions in the vervet monkey.

The fact that the facility did not decrease to zero with 100% filling of Schlemm's canal requires an explanation. Leakage of perfusate from within the anterior chamber around the cannula track in the cornea or leakage from any of the connections in the perfusion system are possibilities. However, the failure to observe such leakage, when tested for by perfusing with fluorescein, argues against this. The perfusate could conceivably have escaped from the posterior aspect of the globe. But, Grant (24) has never observed this in human eyes, and it was not seen to occur here either. This lack of drainage via the vortex veins makes it unlikely, therefore, that the uveal vascular system drains aqueous humor from the anterior chamber. However, Fine (45) has recently challenged this by demonstrating the passage of ferritin

particles from the anterior chamber into capillaries of the ciliary muscle and the iris root in the rhesus monkey. It is also possible that Schlemm's canal was not completely functionally obstructed and that leakage occurred around the silicone cast. However, no patent outflow channels were seen when these eyes were perfused with fluorescein. Perhaps the sclera was undergoing post-mortem changes which resulted either in a very slow and constant stretching of its fibers, so called scleral creep, or in a constant absorption of perfusate into its substance, so called scleral sop. Either mechanism could have caused the observed failure to achieve zero facility.

Another explanation is that there was a constant escape of fluid through an alternate drainage pathway, the uveo-scleral outflow routes described by Bill (34,46-50). Uveo-scleral outflow is a bulk flow from the anterior chamber through the interfascicular spaces of the ciliary muscle into the supra-choroid and then sclera observed after prolonged perfusions. It has been described in the vervet and calculated to contribute as much as 20% of flow out of the anterior chamber in vivo (34).

Uveo-scleral outflow is thought to be a relatively pressure

insensitive system in vivo, contributing very little to facility. However, in enucleated cynomolgus monkey eyes, it appeared to be pressure sensitive over a wider pressure range than in the living eye (50). If this is also true in the vervet, part of the effect of obstructing Schlemm's canal on the percentage decrease in facility would be masked. The amount masked would, of course, increase with increasing intraocular pressure within the pressure sensitive range. The largest possible value attributable to this effect would be that observed in association with the highest pressure, that produced by 100% filling of Schlemm's canal. This would be, at its maximum, 0.07 µl/min/mm Hg or 15% of the mean outflow facility. Thus, at its worst, a small error would be introduced. At lower pressures, this error (amount masked) would be proportionately less. In any case, flow through this pathway could explain the observed failure to achieve zero post-injection facilities.

Although this study was initiated to derive primarily physiologic data, it has also provided some interesting anatomical details. Jocson (24) has shown in enucleated human eyes that Schlemm's canal is drained by aqueous channels which course to the episclera without intrascleral connections to

blood vessels, although they anastamose freely with vessels, including anterior ciliary arteries, in the episclera.

In studying the relationships between blood vessels and the aqueous outflow channels in the rhesus monkey, Jocson (51) found Schlemm's canal to be drained by twenty to thirty large caliber and numerous fine intrascleral vessels which emptied into the anterior ciliary veins. There were no intrascleral connections between Schlemm's canal or its outflow channels and uveal blood vessels, nor were there any arterial connections with Schlemm's canal. Of particular interest, he demonstrated the existence of many direct arterio-venous shumts between the anterior ciliary arteries and veins in the episclera.

Such a shunt was demonstrated in GME 8-22-67A-W. During the course of the injection, as the silicone flowed into the cannulated anterior ciliary vein, it suddenly appeared in the adjoining anterior ciliary artery and continued to fill both vascular networks. After clearing of the globe, filling of the iris vascular arcade was observed in the same quadrant as that of Schlemm's canal. The vortex veins and the long posterior ciliary arteries were also filled with silicone. (Figures 5 & 6)

It thus seems that similar arterio-venous shunts occur in the vervet, although apparently much less commonly than in the rhesus. It is also possible that this phenomenon explains the filling of the medial long posterior ciliary artery in GME3 8-4-67-PI. Filling of this vessel was not observed during the injection of the silicone, but was found only after the globe had been cleared. The physiologic significance and full anatomical extent of these shunts has yet to be determined.

SUMMARY

Chronic simple glaucoma is an important, and yet an incompletely understood, eye disease. There exists a great need for the production of an experimental animal model with which to investigate the natural history of this disease.

Previous experimental attempts to increase intraocular pressure are discussed. A technique to produce controlled, pre-determined, and reproducible ocular hypertension by decreasing outflow facility through mechanical obstruction of Schlemm's canal and the aqueous outflow channels is described. In vitro results indicate the feasibility of using this protocol to produce a model of chronic simple glaucoma in the living animal.

The data from this study also indicate that the principal route for the drainage of aqueous humor from the anterior chamber is through the angle structures - the trabecular meshwork, Schlemm's canal, and the aqueous veins. Reasons for the failure to achieve zero facility with 100% filling of Schlemm's canal are presented.

Sources of possible variance and error in the experimental data are discussed. The relevance of this work to ongoing anatomic studies is described.

FIGURES

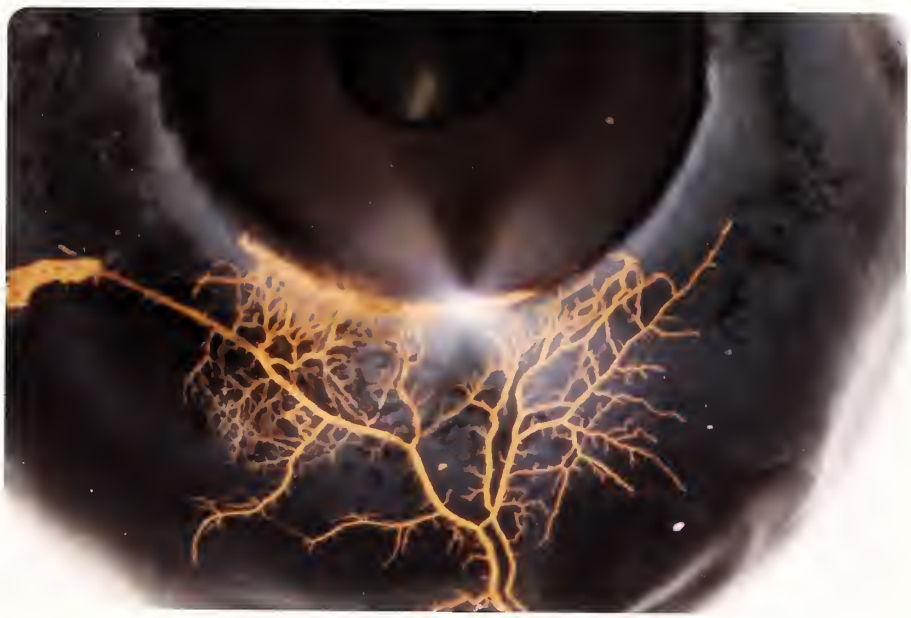

Figure 1. GME 1-19-68A-W (x 16)

Roughly 20% of Schlemm's canal is filled with the orange colored silicone compound. The episcleral network of aqueous veins is well demonstrated.

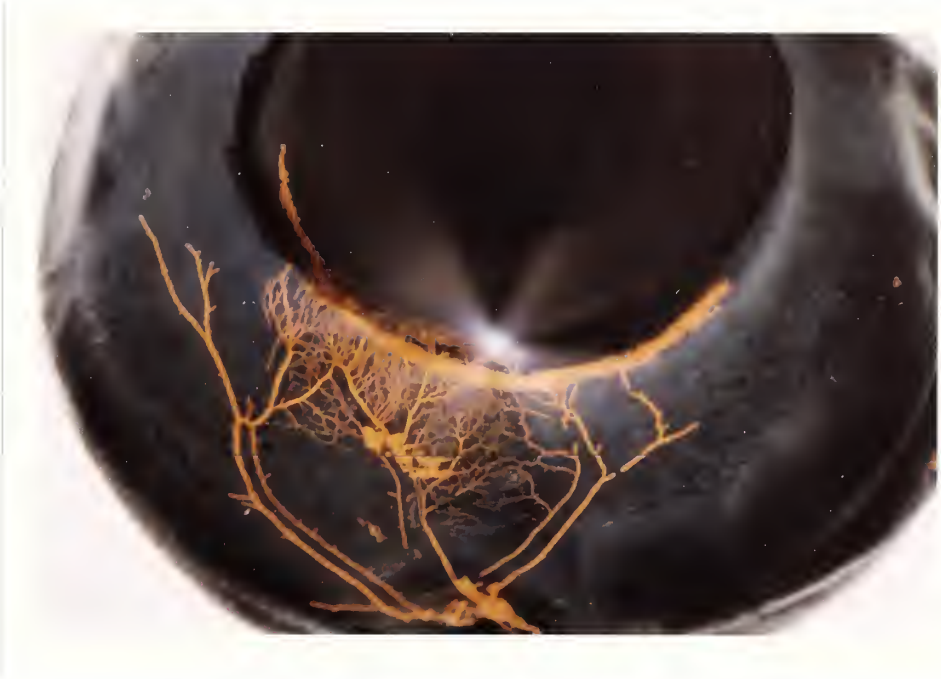

Figure 2. GME 1-31-68B-W (x 16)

Roughly 40% of Schlemm's canal is filled with silicone. The episcleral aqueous network is well demonstrated.

Figure 3. GME 1-19-68B-W (x 16)

Roughly 65% of Schlemm's canal is filled with silicone.

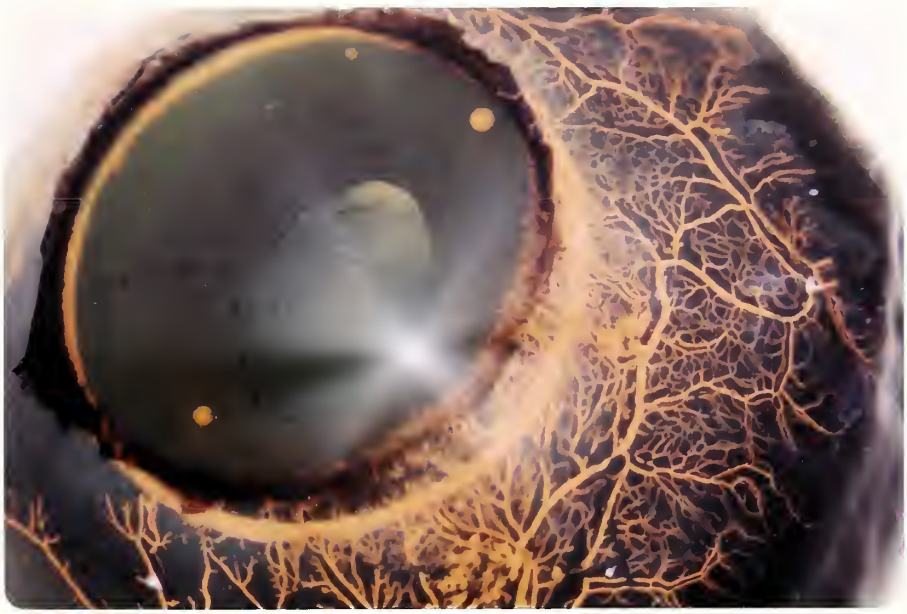

Figure 4. GME 1-14-68A-W (x 16)

The entire circumference of Schlemm's canal is filled with silicone. Perfusion with the fluorescein solution produced the yellow-green appearance of the iris. Spheres of silicone which squeezed through the trabecular meshwork during the injection are seen in the anterior chamber.

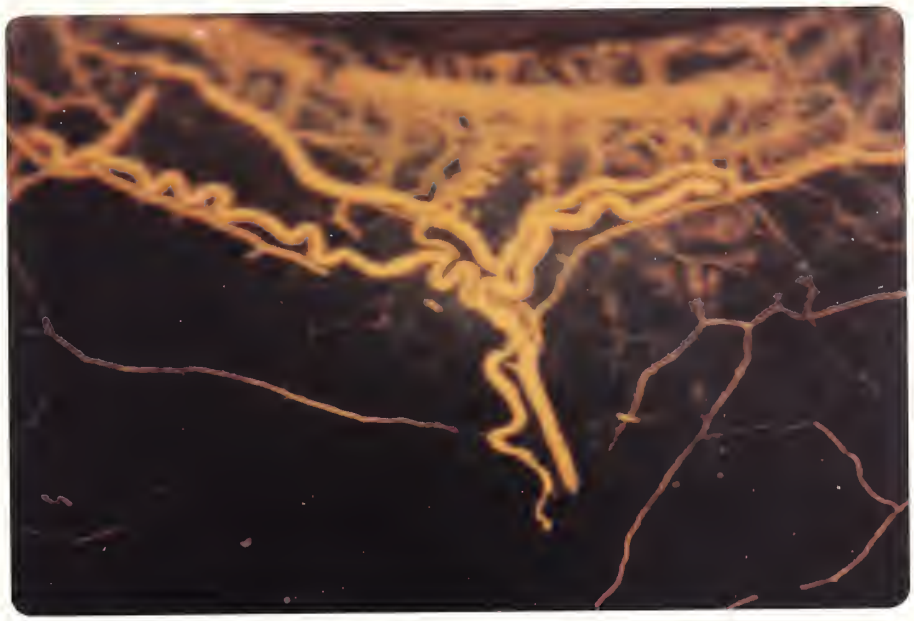

Figure 5. GME 8-22-67A-W (x 40)

The anterior ciliary vein and the adjacent tortuous anterior ciliary artery are each filled with silicone. Filling of the artery occurred during the course of the injection of the vein. Filling of Schlemm's canal can also be seen.

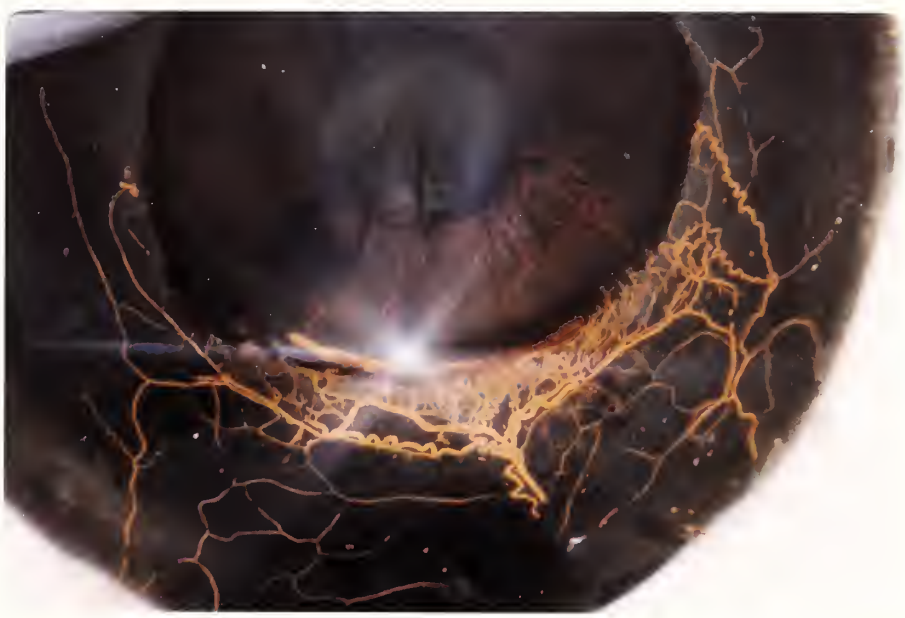

Figure 6. GME 8-22-67A-W (x 16)

The tortuous anterior ciliary artery and the adjacent anterior ciliary vein are each filled with silicone. The iris arterial system is filled in the same quadrant as is Schlemm's canal. The black staining on the cornea is an artifact produced during clearing of the globe.

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