Anatomic correlates of changing aqueous outflow facility in excised human eyes

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Five pairs of excised human eyes were examined for anatomic correlates of changes in aqueous outflow facility that result from changing the intraocular pressure (IOP) and from placing mechanical tension on the irido-corneal angle (lens depression). An increase in IOP from 0 to 40 mm Hg tended inconsistently to compress trabecular meshwork as a whole and to distend the juxtacanalicular tissue. The most constant and significant effect of increasing IOP, however, was to compress Schlemm's canal, thereby progressively diminishing its volume to its virtual collapse at 40 mm Hg IOP. Lens depression increased Schlemm's canal volume and partially prevented its collapse at high levels of IOP. The mean frequency of endothelial vacuoles was remarkably constant at IOPs from 2.5 to 40.0 mm Hg, with and without lens depression, but was significantly lower when the IOP was reduced to 0 mm Hg. When correlated with existing physiologic data, these anatomic findings suggest that Schlemm's canal collapse, but not vacuole frequency, is an important contributor to pressure-dependent changes in aqueous outflow facility. (INVEST OPHTHALMOL VIS SCI 22:625-632, 1982.)

Key words: outflow facility, intraocular pressure, lens depression, cyclotonia, Schlemm's canal, trabecular meshwork, morphometric analysis

Facility of aqueous outflow is an important determinant of intraocular pressure (IOP), and obstruction of outflow underlies many forms of glaucoma. Two factors that are known to affect facility of outflow are the IOP itself and mechanical tension on the scleral spur/trabecular meshwork complex, which in the living eye is applied by ciliary-muscle contraction. Cyclotonia has been simulated in vitro during quantitative aqueous perfusion of excised eyes by mechanically retrodisplacing the lens, thereby exerting tension on the iridocorneal angle through the lens zonules and ciliary body. This procedure causes reproducible and reversible increases in facility of aqueous outflow.1-3 In similar in vitro preparations, elevating IOP has reduced aqueous outflow facility, but this pressure-induced facility diminution can be largely blocked by the previous application of lens depression.2

In the present study, the morphologic effects of IOP and lens depression on the dimensions of the aqueous outflow pathways were measured to determine the location and mechanisms of interaction between these two physiologic factors.
Materials and methods

Five pairs of ostensibly normal human eyes from donors 49 to 70 years old were studied within 12 hr postmortem. A 5 mm button was trephined from the central cornea. With care taken not to injure the anterior capsule of the lens, a radial iridotomy was performed at the 12-o'clock meridian to prevent artificial deepening of the anterior chamber and to label the vertical meridian. A methacrylate perfusion fitting was placed in the corneal opening and connected to an open infusion reservoir via polyethylene tubing. Selected levels of IOP were established by adjusting the height of the reservoir above the corneo-scleral limbus. The eyes were placed in a small plastic cup and gently wrapped to the limbus in gauze saturated with Hanks' balanced salt solution (HBSS). One member of each pair of eyes was fitted with the previously described lens depression apparatus. The plunger was advanced 6 units or 3.3 mm, an amount that produces a maximal increase in outflow facility without disrupting the lens zonules. Both eyes of each pair were perfused for 1 hour with HBSS at the same level of IOP: 0, 2.5, 5.0, 20.0, or 40.0 mm Hg. After 1 hr, the HBSS was replaced with 3% glutaraldehyde in 0.1M cacodylate buffer for an additional 1 to 2 hr of perfusion. After approximately 30 min of intracameral fixative perfusion, glutaraldehyde was added to the cup holding the eye. The anterior chambers of the eyes fixed at 0 mm Hg IOP were cannulated with a 23-gauge needle that was connected, via polyethylene tubing, to a second reservoir level with the corneoscleral limbus to maintain the IOP at 0 mm Hg.

This procedure permitted both members of each pair to be fixed at the same level of IOP, one with maximal lens depression and the other with no lens depression. After 2 hr of fixation the eyes were disconnected from the corneal fittings; the
Fig. 3. Representative photomicrographs from four eyes fixed without lens depression at the IOPs indicated in the lower right hand corners. The trabecular meshwork as a whole is relatively compressed and juxtacanalicular tissue relatively distended as the IOP is increased to 20 mm Hg, but both components are markedly compressed with collapse of Schlemm’s canal at 40 mm Hg. The section at P20 shows a transcanalicular septum containing an arteriole.

anterior segments were excised from the globes at the pars plana and divided into 12 equal-sized, wedge-shaped pieces from which the limbal areas were excised. Each limbal segment was postfixed in osmium tetroxide, dehydrated in a graded series of alcohols and embedded in Epon (Electron Microscopy Sciences, Fort Warrington, Pa.) with Pelco molds (Ted Pella, Inc., Tustin, Calif.). Orientation and meridional identification of each segment were carefully maintained during tissue preparation (Fig. 1). Sections 1 pm thick were cut with a Porter-Bloom ultramicrotome, stained with methylene blue—azure II, and photographed with a photomicroscope at magnifications of 250× and 400×.

Examination of tissue. Each of the 120 microscope slides and photomicrograph transparencies was coded with a random five-digit number and arranged in the numerical sequence of the code number. Each photomicrograph was projected at constant magnification on a calibrated rear-projection grid (Fig. 2). The boundaries of trabecular meshwork, of its juxtacanalicular component (JCT), and of Schlemm’s canal were traced with a felt tip marker on the grid, and the respective cross-sectional areas were measured by counting the numbers of squares within the outlines. Lines were drawn through the axis of the collagen fibers of the corneoscleral wall and through the core of the scleral spur, and the angle was measured with a protractor.

Each photomicrograph was also assessed for the presence of complete transcanalicular septae and for anastomosis of collector channels with the lumen of Schlemm’s canal. Collapse of Schlemm’s canal was considered to be present when opposing walls of Schlemm’s canal in apposition for at least 75% of their length in the cross section. The vacuoles of the Schlemm’s canal inner-wall endothelium were counted.

After the measurements and the qualitative data were recorded for each coded section, the code was broken and the data were tabulated.
Fig. 4. Representative photomicrographs of four eyes fixed with lens depression at the IOPs noted in the lower right hand corners. Trabecular meshwork and Schlemm’s canal cross-sectional areas are greater than those in the fellow eyes shown in Fig. 3, except at 40 mm Hg. At this level the juxtacanalicular tissue is distended and the canal is nearly collapsed. An endothelial tubule of Johnstone is visible crossing Schlemm’s canal at 2.5 mm Hg IOP.

Results*

Trabecular meshwork cross-sectional area.
The trabecular meshwork typically appeared compressed at higher levels of IOP, with collapse of the intertrabecular spaces in the eye fixed at 40 mm Hg (Fig. 3), but the variability from section to section within the same eye was high and the differences between the mean trabecular meshwork cross-sectional areas at different levels of IOP were not statistically significant. The JCT of trabecular meshwork seemed to distend at 20 mm Hg but to collapse again at 40 mm Hg (Figs. 3 and 6). At 20 mm Hg IOP, juxtacanalicular cross-sectional area was significantly greater than at 0 mm Hg IOP (p < 0.01). Corneoscleral meshwork cross-sectional area (total trabecular meshwork minus juxtacanalicular tissue) tended to diminish linearly with increasing increments of IOP, but the differences were not significant.

Lens depression increased the mean cross-sectional area of both the juxtacanalicular and nonjuxtacanalicular trabecular tissue (Figs. 4 to 6), with the largest effect in the JCT at the middle pressure range (Fig. 6). In many of the sections, even at 40 mm Hg, the JCT appeared relatively distended in the lens-depression eye compared with the fellow eye, but the difference was statistically significant only at 5 mm Hg (p < 0.05) (Figs. 4 and 6).

Schlemm’s canal dimensions. Mean cross-sectional area of Schlemm’s canal was higher

*Absolute values and confidence limits of the various parameters are presented in Figs. 5 to 10. Student’s t test for paired data was used to derive all p values.
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in the eyes fixed at 2.5 and 5 mm Hg than that in the eye fixed at 0 mm Hg and was significantly smaller in the eyes fixed at 20 and 40 mm Hg (p < 0.05) (Fig. 7). The average median width of Schlemm's canal was also diminished at higher levels of IOP. The eyes fixed with lens depression regularly had a larger Schlemm's canal cross-sectional area than that of the fellow eye fixed without lens depression (Figs. 3, 4, and 7) (p < 0.05 at all pressures except 40 mm Hg).

There was no apposition of opposing walls of Schlemm’s canal at pressures of 2.5 or 5 mm Hg (Fig. 8), but there was collapse at the higher pressures. The collapse was reduced by lens depression. Schlemm’s canal appeared to be collapsed in all sections from the eye fixed at 40 mm Hg IOP without lens depression, suggesting that the opposing canalicular walls were in contact virtually throughout the circumference in this eye (Fig. 8). With the lens depression, the canal was narrowed at 40 mm Hg but not completely collapsed in five of the 12 segments, which, interestingly, turned out to be contiguous (from the 3-o’clock to the 8-o’clock meridians) (Fig. 8). At 20 mm Hg IOP without lens depression, Schlemm’s canal was collapsed in four of the 12 sections.

Transcanalicular septae and aqueous collector channels. Transcanalicular septae were observed in 65 of the 120 sections examined (54%). Some appeared to consist primarily of trabecular meshwork (corneoscleral or jux-
Fig. 8. Histogram showing the number of sections out of 12 in which the opposing walls of Schlemm's canal were in contact for at least 75% of their length in cross section. L.D., Lens depression.

tacanalicular), and in others the septae more closely resembled external wall and sclera. Of the 19 sections with collector channels visibly anastomosing with Schlemm's canal, 17 (89%) also had a transcanalicular septum (Figs. 3 and 4).

Schlemm's canal endothelial vacuoles. An average of 1.5 vacuoles per section was counted along Schlemm's canal inner wall in the eyes fixed without lens depression at 0 mm Hg. At all pressures above 0, the mean vacuole counts in the other four eyes fixed without lens depression did not differ significantly from each other, varying from 9.6 to 12.4 vacuoles per section and 2.5 to 4.2 vacuoles per 100 μm cross-sectional length (Fig. 9) (p > 0.10 in all cases). All were significantly higher than the vacuole counts observed at 0 mm Hg IOP (p < 0.001). Vacuole counts varied widely from section to section for a given eye, with the index of variability ranging from 45.2 to 65.5 among the four eyes fixed at IOPs above 0 mm Hg. When the vacuole counts for all eyes fixed without lens depression were pooled, there was no significant difference among the vacuole counts taken from the superotemporal, superonasal, inferotemporal, or inferonasal quadrants of the canalicular circumference. The mean vacuole count (7.8) in sections containing collector channels was not significantly different than that from other sections.

At each level of IOP studied there was no significant difference among vacuole counts in the eyes fixed with and without lens depression, except equivocally at 2.5 mm Hg (p < 0.05).

Scleral spur angle. In four of the five pairs of eyes studied, the angle of the intersection of the axis of scleral spur with the sclera was greater in the lens-depressed eye than that in the fellow eye. The difference was significant only at the low levels of IOP (0 and 2.5 mm Hg, p < 0.05) (Fig. 10).

Discussion

The most demonstrable anatomic findings from this study were that increases in IOP tend to compress Schlemm's canal with apposition of the canalicular walls at higher levels of IOP and that lens depression opens the canal. These findings may provide the anatomic basis for the physiologic observa-
tion that lens depression virtually eliminates the pressure-induced facility decrease until 40 mm Hg IOP is reached. Hence observed pressure-induced reductions in the outflow facility may not result so much from a reduction in Schlemm's canal volume, but rather in part from the collapse of opposing canalicular walls (thus reducing access of occluded meshwork to collector channels). This reduction in outflow facility becomes most evident at pressure levels where the canal begins to collapse (at about 20 to 25 mm Hg). By the same token, lens depression has its maximal effect at those levels where collapse can be entirely prevented.

Effects on the trabecular dimensions were less readily demonstrable, in part because of the small number of measurements and also because of uncertainty in identifying the borders of trabecular components. The use of a single observer and coded sections minimized but undoubtedly did not eliminate such artifacts. Although not proven statistically by the measurements, it seems that increasing pressure or flow tends to compress the trabecular beams together, but to distend juxtacanalicular connective tissue. This latter effect is diminished at high levels of IOP (40 mm Hg) by collapse of the canal and by compression of the whole meshwork into the canalicular lumen. Lens depression appears to permit greater distension of the juxtacanalicular tissue by displacing the meshwork internally and thereby permitting more outward distension into the opened lumen of the canal. A similar increase in the subendothelial space (JCT) occurs with the establishment of transtrabecular flow. These data, however, did not show the number of vacuoles to be determined by the rate or pressure head of that flow, although other studies have suggested this quantitative relationship. In either event, the vacuoles are unlikely to be an important site of resistance because, in studies showing a quantitative relationship, higher vacuole and pore frequency are present at high levels of IOP where resistance is increased, not decreased.

Progressive occlusion of Schlemm's canal with apposition of its opposing walls might contribute to outflow obstruction in some cases of glaucoma. Additionally, some forms of therapy may operate by providing tension on scleral spur and trabecular meshwork, thus separating the walls of the canal and permitting distension of the subendothelial space. This sequence of events may account for resultant lower outflow resistance in glaucomatous eyes treated with cyclotonic agents. However, to apply conclusions from this experimental model to the pathophysiologic or pharmacologic features of the human eye is precarious, since the model does not
account for the multiple biochemical, physio-
chemical, and neurophysiologic mechanisms
also likely to be operational in the living
eye. The present data from the experimental
model, however, do correlate well with the
physiologic observation of the model itself,
where increasing IOP increases outflow resis-
tance and where that effect is alleviated by
lens depression.

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