Observations on the drainage angle in man and rhesus monkey: A concept of the pathogenesis of chronic simple glaucoma

A light and electron microscopic study

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The structure of the anterior chamber angle of primate eyes (man and rhesus monkey) was examined by methods of light and electron microscopy. The anatomic observations indicated that the trabecular meshwork could be clearly and simply divided into two parts by an imaginary line drawn from the region of the scleral roll to the end of Descemet's membrane (Schwalbe's line). The outer layers may be termed the corneoscleral; the inner layers, the uveal. The corneoscleral layers are further subdivided into two parts: a small narrow band of tissue lying adjacent to the inner wall of Schlemm's canal, the juxtaocanalicular connective tissue, and a much larger portion, the remainder of the corneoscleral meshwork. Physiologic experiments were carried out in young rhesus monkey eyes by using coarse tracers (India ink) for light microscopy and fine tracers (ferritin molecules) for electron microscopy. The ferritin molecules were found to pass through the juxtaocanalicular connective tissue layer to reach the endothelial lining of Schlemm's canal. These endothelial cells took up a number of the ferritin molecules, many within small vesicles, and transported them across the cell cytoplasm, discharging the molecules into the lumen of the canal. Ferritin molecules were also observed to pass in similar fashion into the capillaries of the ciliary muscle and iris root. All of the observations made in this study support a simplified histologic terminology for this region more in conformity with function than terms in current use. Histologic examination of cases of chronic open-angle glaucoma led to the development of a concept of the pathogenesis of chronic simple ('primary' open-angle) glaucoma. The changes are considered to be aging processes within these tissues of the drainage angle, which hamper aqueous flow from reaching the lumens of these outflow vessels.

The drainage angle of the anterior chamber has long been of great interest to investigators of ophthalmic problems. This has been particularly true following the classic experiments of Leber, which pointed out the role of the anterior...
chamber angle in the outflow of normal aqueous. Based on these observations, Weber and Knies, independently and almost simultaneously, produced histologic evidence that adhesive obstruction of this chamber angle by the iris root was clearly associated with typical blinding (i.e., acute congestive) glaucoma. Later Priestley Smith, in an extensive series of experiments, was able to demonstrate clearly that mechanical obstruction of this angle would indeed result in a rise in the intraocular pressure.

It was clear, therefore, that "typical" glaucoma (i.e., acute congestive glaucoma) could be explained on the basis of closure of the drainage angle by apposition of the iris to the trabecular meshwork, which lies on the opposite side of the angle. These observations and demonstrations helped to crystallize a growing awareness of a second form of disease process, entirely lacking in dramatic clinical signs or symptoms, but still possessing the features of a progressive loss of the visual field and an increased intraocular pressure. With later histologic and gonioscopic examinations, it became evident that the concepts which answered so well many of the questions in acute congestive glaucoma no longer sufficed to explain this chronic disease, because the chamber angle did not appear closed or obstructed. The drainage-angle concept of aqueous outflow was then questioned. Was it no longer valid, was it inadequate, or was something perhaps being overlooked?

Today there is widespread acceptance of the validity of the anterior chamber angle as the main site of aqueous outflow. There is, I believe, also general acceptance of the concept of some form of chronic obstruction to the outflow channels that occurs within this drainage area, the so-called "retention theory" of glaucoma championed by the previously mentioned investigators. This obstruction is assumed by many to lie within the trabecular meshwork and is also assumed to be ultimately mechanical, although the basis for such mechanical obstruction may lie in biochemical or biophysical change.2

While the site of the normal resistance to aqueous outflow necessary to maintain intraocular pressure has not yet been conclusively pinpointed, the general impression one gains from the current literature is that in all probability it is within that part of the trabecular meshwork that forms the inner wall of Schlemm's canal—the area called the "pore tissue" by Flocks3 and the "endothelial meshwork" by Speakman. More recent observations of the anatomy of the chamber angle in normal human and rhesus monkey eyes and in a variety of open-angle glaucomas have left me unconvinced that this part of the trabecular meshwork is entirely or even predominantly responsible for the normal or the pathologic resistance to aqueous outflow.

It appeared that the entire trabecular meshwork, in the adult eye, may be divided into two major parts by the protruding scleral spur (or roll, as Salzmann termed it) (Figs. 1, 2, 8, and inset 10). If one draws an imaginary line connecting the scleral roll (spur) with Schwalbe's line, the trabecular meshwork is found to consist of a posterior uveal portion and an anterior corneoscleral portion. In both parts the intertrabecular spaces (lying between adjacent trabecular sheets) appear to course laterally, directing their contents (aqueous humor) away from Schlemm's canal. The anterior and posterior portions assume a different course at the scleral roll. There the posterior (uveal) portion sweeps backward along the recess of the anterior chamber angle to become continuous with the uveal tissue of the ciliary body and with the iris root. The anterior (corneoscleral) part of the meshwork passes through the substance of the scleral roll, coursing into the corneoscleral tissue that forms the lateral wall of Schlemm's canal. The lumens of these channels seem to disappear gradually within the scleral roll (Fig. 2) and the attached ciliary muscles. This striking appearance suggested
that the normal pathway of aqueous flow might not be limited to the sinuous route across the perforated trabecular sheets toward the canal of Schlemm but might be more direct laterally through the channels between the trabecular layers.

With all this in mind, a study was made in several parts that included:

1. Re-examination of the histology of the angle of the anterior chamber by conventional methods similar to those employed by the earlier histologists\(^1\)\(^2\)\(^3\) and by electron microscopy;

2. Repetition of some of the simple mechanical experiments carried out by some of the earlier investigators, coupled with a modified form of this type of experiment with more recent methods of electron microscopy; and

3. Application of the information so obtained to the histologic study of some cases of chronic open-angle glaucoma, with the aim of synthesizing a working hypothesis concerning the sites and manner of development of this obstruction.

**Materials and methods**

Ten human eyes ranging in age from 19 months to 61 years, removed surgically and prepared by methods outlined elsewhere,\(^1\)\(^3\)\(^4\) were used in this study. In addition, the eyes of several rhesus monkeys (approximately 18 months of age) were used. The latter were surgically removed under Nembutal anesthesia and prepared in a manner similar to the human eyes. While under anesthesia, the anterior chambers of two of the rhesus monkey eyes were gently perfused with 0.1 ml of ferritin.\(^4\)

With two separate syringes and needles, the aqueous was withdrawn from one side of the anterior chamber while the ferritin was perfused into the opposite side at an approximately equal rate. Removal of the obliquely inserted needles allowed the corneal perforations to close, maintaining the anterior chamber and the approximately normal intraocular pressure.

One eye was surgically removed 20 minutes following completion of the perfusion, the other after 40 minutes. One portion of each eye was then prepared and embedded in Epon, the other portion in 20/80 methyl butyl methacrylate. The representative electron micrographs that illustrate this work are from two human eyes from patients, aged 40 and 57 years, described previously in this journal,\(^1\)\(^4\)\(^5\) and from two experimentally treated rhesus monkey eyes described above.

The 40-year-old man had a malignant melanoma in the chorioid temporally. The remainder of the globe appeared clinically normal, and the intraocular tension just prior to enucleation was 10.2 mm Hg (Schiotz with 5.5 gram weight). The tension of the other eye was 15.9 mm Hg. The 57-year-old woman had a malignant melanoma limited to the ciliary body temporally. The remainder of the globe appeared normal by ophthalmoscopy and biomicroscopy. The pressure reading just prior to enucleation was 10 mm Hg (Schiotz 7.5 grams weight). The tension of the other eye was 17 mm Hg.

Thin (1 to 2 \(\mu\)) methacrylate-embedded sections of osmium-fixed tissue were stained for light microscopy\(^6\)\(^7\) for study and purposes of orientation for the micrographs. Other rhesus monkey eyes received perfusion of the anterior chamber with India ink while under Nembutal anesthesia. The eyes were enucleated one hour after the perfusion, fixed in formalin, embedded in paraffin, cut in conventional manner, and stained with alcin blue with a Kernechtrot counterstain. For re-examination of the normal anatomy by more conventional methods, two young rhesus monkey eyes were surgically removed under Nembutal anesthesia, promptly fixed in formalin, embedded in paraffin, cut in serial section, and stained with alcin blue.

Sections prepared for electron microscopy were either examined without further treatment (labeled "untreated") or were treated for 10 to 15 minutes in 1 per cent uranyl acetate in 50 per cent ethanol, washed with distilled water, drained, and allowed to dry before examination ("uranyl acetate treated"). The illustrative electron micrographs are all from such treated sections except where noted otherwise. The scale markers represent 1 \(\mu\) unless otherwise indicated.

Ferritin molecules as used in this study are composed of an electron-opaque central core of ferric hydroxide surrounded by a protein shell\(^8\)\(^9\). The entire particle measures approximately 100 \(\AA\) in its greatest diameter. The portion visible in the electron micrographs represents the ferric hydroxide core alone and measures approximately 50 to 60 \(\AA\). The narrow, more electron-lucent "halo" representing the protein shell can frequently be observed in the micrographs when the particle is suitably positioned and oriented (as in inset III, Fig. 15, B). The ferritin molecules were studied here in both methacrylate- and Epon-embedded tissue.

It is assumed that these ferritin molecules...
demonstrate, in part, the physiologic pathway taken by other protein molecules of similar size (100 Å). Significant aggregation of these particles does not occur, as it may in the case of India ink or some of the other electron-dense tracer materials (e.g., Thorotrast). As these ferritin molecules are essentially physiologic, they will occasionally be observed to be taken up by a cell, sequestered, and presumably metabolized (Figs. 10 and 11, A), as is considered to be the case in part elsewhere. The take-up of ferritin for intracytoplasmic sequestration and/or assimilation (“phagocytosis”) and the take-up of such particles (“micropinocytosis”) for subsequent transcytoplasmic passage are generally considered to be but variations of the same basic phenomena.

Observations

The observations are recorded as legends to each micrograph. Fig. 1, A indicates the general plan of orientation of all the micrographs.

Discussion

During the course of these investigations it became evident that some of the older terminology used for the trabecular meshwork was not clear and did not seem to correlate well with the evidence of direct histologic examination. Salzmann felt that the region could be best demonstrated by grossly separating the ciliary body from the scleral roll, followed by further separation of the iris and its adhering layers from this ciliary body preparation. The trabecular layers remaining adherent to the iris were called *lamina pectinata*, and the portion remaining adherent to the ciliary body the *trabeclular sclerocorneale*. H. Virchow gave the name of *uveal* to the former and *scleral* to the latter portions.

More recently Burian and others have proposed a division of this meshwork region into at least three distinct parts: the corneoscleral trabeculum near to the canal of Schlemm; the uveal trabeculum (meridional) extending directly from the meridional ciliary muscle and scleral spur to the cornea; and the uveal trabeculum (circular) passing from the radial and circular ciliary muscles to the ring of Schwalbe. The iris processes were found to be inconsistent in presence, thickness, and extent.

These observations are similar to those of Troncoso and Busacca, the latter giving the name of "trabeculocorneal lamella" to the layer extending over the face of the ciliary body to the iris root.

More recently Kupfer has added further support to these observations by noting even greater participation of the "tendons" of the ciliary muscle in the trabecular meshwork in fetal and infant human eyes.

From the physiologic experiments in the present study it became more evident that a slight alteration in nomenclature was necessary for a clearer appreciation of this region. The terminology proposed here, differing a little from that previously proposed, has, I believe, the advantage of being simpler and therefore more useful.

Despite the subtle histologic differences that may be found in the various trabecular layers of the adult human eye (Fig. 7), a clear division may be made between the very narrow layer of tissue lying in immediate relationship to the inner walls of the canal of Schlemm (Figs. 1, B, 2, and 3A) and the remainder of the trabecular meshwork as a whole. The tissue immediately adjacent to the canal (Fig. 1, B) contains a number of loose connective tissue cells and a quantity of intercellular material (Fig. 3A). This tissue, the *juxtacanalicular connective tissue*, differs from the remainder of the trabecular meshwork in which the continuity of the anterior chamber is maintained.

Throughout most of the trabecular meshwork, the endothelial cells are associated with a basement membrane and connective tissue ground substances on one surface, and are in free contact with the aqueous on the other surface. The spaces lined by these free surfaces represent the continuation of the anterior chamber. These spaces terminate in the juxtacanalicular connective tissue layer near the canal of Schlemm, where the loosely arranged cells are associated with connec-
Fig. 1, A. Hematoxylin and eosin stained meridional section (approximately 10 to 12 μ thick) of a celloidin-embedded normal human adult eye to illustrate the structures present in the drainage angle of the anterior chamber. The collagenous lamellae of the cornea and sclera are clearly seen at L. The endothelium-lined canal of Schlemm (CS) (sinus venosus sclerae) appears empty in this preparation. The trabecular meshwork is clearly seen between positions 1 and 2; its inner layers appear to run in continuity with the tissue of the ciliary muscle (LCM) and its outer layers appear to be continuous with the more unusual and circumferentially arranged collagenous bundles of the region of the scleral roll (SR). The densely cellular tissue of the anterior border layer of the iris possesses a number of interruptions or crypts (CR), and this layer ends abruptly at the first layer of the "neural trabecular meshwork." This latter relationship is more clearly seen in Fig. 8, B. The loose iris stroma, which contains large numbers of capillary-like blood vessels, is continuous with the loose stroma of the inner portion of the ciliary body. The iris pigment epithelium is continuous with the two-layered pigmented epithelium on the first ciliary process. AC, anterior chamber; LCM, longitudinal ciliary muscle. Position 1 indicates the region represented by Figs. 3 to 6 inclusive, and position 2, the region represented by Fig. 7. (×60. AFIP Neg. 64-771.)

B. Higher power of the region of Schlemm's canal (CS). The dense collagenous tissue of the sclera (SCL) lies adjacent to the outer endothelial lining of the canal. The inner endothelial lining, covered by a few red blood cells (R) in the lumen of the canal, is separated from the trabecular meshwork (TM) by a layer of loose connective tissue, the juxtacanalicular connective tissue (JCT), which varies in its thickness (arrows). The trabecular meshwork is easily identified by the collagenous cores covered by endothelium. They are separated from one another by spaces, the continuation of the anterior chamber. (Hematoxylin and phloxine, 1 to 2 μ section. ×530. AFIP Neg. 63-4154.)
Fig. 2. A thin section (1 to 1.5 μ) of methacrylate-embedded tissue stained for light microscopy to show the region of the canal of Schlemm (CS), the adjacent collagenous corneosclera (SC), a few red blood cells in the lumen (free arrows), the endothelial lining of the canal with the endothelial nuclei protruding into the lumen (EN), the subjacent loose connective tissue (JCT), the trabecular meshwork with its endothelial cells (TR), and a portion of the scleral roll (SR, broken outline) where the meshwork separates abruptly into two parts, the inner or uveal meshwork and the outer or corneoscleral meshwork. (See Salzmann for other definitions of these regions, as defined by gross dissection.) AC, anterior chamber; LCM, longitudinal ciliary muscle; CL, canaliculus of canal of Schlemm; U, uveal meshwork; C, corneoscleral meshwork. Forty-year-old man. (Hematoxylin and phloxine. ×420; reduced approximately 1/2. AFIP Neg. 63-4155.)

Fig. 3, A. Low-power electron micrograph to show the red blood cells in the lumen of the canal of Schlemm (CS), the endothelial cell lining of the inner wall of the canal (EN, I), and the juxtacanalicular connective tissue layer (JCT), in which are found loose connective tissue cells and extracellular materials, both fibrillar (F) and dense (D), the latter resembling the dense material found within the cores of the trabeculae. Spaces lacking a basement membrane lining and in continuity with the anterior chamber are seen at the lower edge of the micrograph. Forty-year-old man. (Uranyl acetate treated. ×6,160.)

Fig. 3, B. Higher power of inner endothelium of the canal to show two sites of abutment of cell to cell. They are separated by a space of approximately 150 Å that is common to most adjacent epithelial and endothelial cells. A "finger" of one endothelial cell is cut obliquely at its base at F. The fibrillar basement membrane material is seen at BM, and the intraluminal material lacking this filamentous appearance at PL. (Uranyl acetate treated. ×22,500.)
Fig. 3, A. For legend see opposite page.

Fig. 3, B. For legend see opposite page.
Fig. 5, A. For legend see page 618.

Fig. 5, B. For legend see page 618.
Fig. 4. Higher power of the inner wall of the canal of Schlemm in Fig. 3, A. This lining consists of a single layer of interlocking endothelial cells (END). The appearance of these interconnections may vary from a tongue-in-groove arrangement (1) to a more simple apposition of cell to cell (2). A portion of a red blood cell is present in the lumen, and patches of fixed plasma (PL) and possibly mucinous materials remain adherent to the endothelium and the red blood cell. A finely fibrillar, patchy basement membrane (BM) lines the inner side of the endothelium. Deep to this, the juxtacanalicular connective tissue is composed of cells and their processes and of a distinctly fibrillar intercellular material. This extracellular material presents at least three appearances: the coarse filaments (F), the denser patches of material (F • F) containing filaments, and the more lucent filamentous basement membrane material (F • B). The endothelial cells contain intracytoplasmic filaments and numbers of small vesicles derived from the surface that are better seen in Figs. 5 and 6. A few intracellular bodies of varying density are often present (DB). Forty-year-old man. (Uranyl acetate treated. x25,200.)

Fig. 5, A. Two simple junctions of adjacent endothelial cells of the inner endothelial lining of Schlemm's canal are present. Patches of intraluminal material are present near the cells on one side (PL). Beneath the cells are many delicate filaments cut longitudinally (L), and in cross section (X). A nearby connective tissue cell contains a dense pigment (melanin-type) granule. (Uranyl acetate treated. x28,000.)

Fig. 5, B. Junctions of adjacent endothelial cells of the inner wall are clearly seen. Within the cells, connected to the surface membranes on both sides, are small vesicular profiles (arrows) known in capillaries as "micropinocytotic" vesicles because of their presumed pinocytotic activity. CS, lumen of canal of Schlemm. (Uranyl acetate treated. x33,600.) Insets: Higher magnification of surface-connected vesicles to show the three-layered arrangement of the surface "unit membrane" (arrows) and the similar three-layered arrangement of the "unit membrane" of the vesicles (arrow). (x90,000.)

Fig. 6, A. The luminal surface of an endothelial cell from the inner wall of the canal has multiple invaginations, many of which are clearly in continuity with the surface membrane of the cell. (See insets.) A few segments of granular endoplasmic reticulum are present (ER), as well as a few clusters of dense RNP particles and a number of larger, less dense particles (G) which may represent glycogen. N, nucleus. Fifty-seven-year-old woman. (Uranyl acetate treated. x51,000.) Insets: Higher magnification of surface-connected vesicles to show the three-layered arrangement of the surface "unit membrane" (arrows) and the similar three-layered arrangement of the "unit membrane" of the vesicles (arrow). (<90,000.)

Fig. 6, B. Lower power of inner endothelial lining of the canal to show the presence of intracytoplasmic vesicles of irregular shape and size. Underlying the endothelial wall in the region of the juxtacanalicular connective tissue are patches of filamentous basement membrane (BM), larger filaments (F), and denser patches of granular to filamentous material (D). Many delicate cytoplasmic extensions of the connective tissue cells are seen (CY). Fifty-seven-year-old woman. (Uranyl acetate treated. x28,800.)
Fig. 6, A. For legend see opposite page.

Fig. 6, B. For legend see opposite page.
Fig. 7. An electron micrograph of portions of uveal trabeculae. The endothelial cells are clearly seen, as is one of their nuclei (N). This cell shows the Golgi apparatus (G), a few fragments of granular endoplasmic reticulum (ER), and a number of clusters ("rosettes") of free ribonucleoprotein particles (RNP) frequently found in most cells. The interdigitating junctions between adjacent endothelial cells are clearly seen. Beneath the endothelial cell lies its basement membrane layer (BM), deep to which are found the collagenous fibrils that make up the core of the trabeculae. Some of these collagenous regions possess foci of increased density (D). The spaces that are not lined by basement membrane and are devoid of collagenous fibrils represent the continuity of the anterior chamber through the meshwork. A second cell type, presumed to be a neurite (NE), is seen entirely enveloped by an endothelial cell. Intracellular filaments are present in this endothelial cell at FIL. Granules of varying density are frequently observed within these endothelial cells. Forty-year-old man. (Uranyl acetate treated. ×18,000.) The inset shows a portion of the collagenous core running through a trabecular layer. (×54,000.) Collagenous fibrils cut in varying directions of orientation are present (C). The slightly filamentous appearance of a patch of increased density is seen at D. The interfibrillar spaces are lucent (LU), denoting, presumably, a content of more lucent material or the space where a more soluble material was removed during preparation of the tissue.
Fig. 8, A. Thin section (1 to 1.5 μ) of a methacrylate-embedded eye of a 19-month-old child to illustrate anatomic arrangements of the drainage angle. The canal of Schlemm lies within the scleral furrow and here prominently displays its multiple lumens (CS) filled with red blood cells. Sections of blood-filled collector channels are seen within the sclera (CC). The sections of circumferentially oriented collagen bundles that represent the scleral roll (SR) are few in number and barely noticeable. The extracellular tissue between longitudinal muscle fibers (cells) passes directly into the trabecular meshwork region. The anterior border-layer of the iris ends abruptly at the iris root (arrow). Many blood vessels are present within the area of the iris root as well as throughout the ciliary muscle. (Hematoxylin and phloxine x90. AFIP Neg. 63-4363.)

Fig. 8, B. Thin section (1 to 1.5 μ) of methacrylate-embedded eye of a 57-year-old woman, to show the anatomic relationships within the drainage angle of the anterior chamber. The canal of Schlemm containing red blood cells is clearly evident (CS), as are a few sections of the collector channel, filled with red blood cells, within the adjacent sclera. The collagenous bundles of the scleral roll are not prominent. The prompt cessation of the anterior border layer of the iris (I) is particularly well demonstrated here. Apertures ("crypts") are clearly seen in this anterior border layer near the iris root (arrows). The corneoscleral trabeculae pass outward of the scleral roll, while the large mass of uveal trabeculae pass inward into the face of the longitudinal ciliary muscle. Many small blood vessels are clearly seen throughout the ciliary muscle. AC, anterior chamber; PC, posterior chamber. (Hematoxylin and phloxine x90. AFIP Neg. 63-4360.)
Fig. 9, A. Thin (1 to 1.5 μ) coronal section through the anterior part of the trabecular meshwork of a 56-year-old human eye stained with paraphenylenediamine. The round to oval cross-sectional lumens that lie between adjacent trabecular sheets are clearly seen (arrows). The space below represents the anterior chamber; the heavy collagenous layers above represent the corneosclera. (x530; reduced approximately 1/4. AFIP Neg. 64-563.)

B. Similar preparation to A but more posterior to show a portion of the canal of Schlemm (CS), the increased thickness of the trabecular meshwork, and the oval cross sections of the aqueous passageways (arrows). (x530; reduced approximately 1/4. AFIP Neg. 64-562.)

C. Similar preparation to A and B but more posterior and lower in magnification (x195). This coronal section shows the collector vessels (CV), filled with red blood cells, and the canal of Schlemm (CS) passing outward of the scleral roll (SR), which in this preparation clearly shows the circumferential arrangement of its collagenous bundles. The uveal meshwork is continuous with the ciliary muscle (CM) internal to the scleral roll. (x195; reduced approximately 1/4. AFIP Neg. 64-558.)

D. Higher power of C to show more clearly the circumferential bundles of the scleral roll (SR) and the continuity of both the trabecular layers and the intertrabecular spaces with the longitudinal ciliary muscle (CM). (x830; reduced approximately 1/4. AFIP Neg. 64-559.)
Fig. 10. For legend see page 626.
Fig. 11, A and B. For legend see page 626.
Fig. 10. Endothelial cells lining the inner wall of the canal of Schlemm (major canal). The myriad small uniform dots in the lumen of the canal (CS) represent the ferritin particles that were placed in the anterior chamber 40 minutes prior to sacrifice. Many of these particles are clearly seen on the anterior chamber side of the endothelium. Small clusters of particles within vacuoles are seen throughout the endothelial cell cytoplasm. Several ferritin particles appear either to lie freely within the cytoplasm (free arrows) or are contained within a very small vacuole. Some of the ferritin particles have been taken up by a dense intracytoplasmic body (DB). Small clusters of ferritin particles are seen near the lumen at FE, N, nucleus of endothelial cell. No ferritin particles are present within the intercellular space where the two endothelial cells abut. (The small irregular speck nearby in the micrograph is an artifact.) (Methacrylate-embedded, untreated. x68,500.) The inset is from an osmium tetroxide-fixed methacrylate-embedded young rhesus monkey eye cut meridionally at 1 to 1.5 μ and stained with hematoxylin and phloxine for light microscopy. The interconnecting lumens of the canal of Schlemm system are seen at CS. The densely packed cells of the anterior border layer of the iris (AB) are seen to end abruptly as the iris approaches the face of the ciliary body. The trabecular meshwork that occupies the remainder of the bow of the chamber angle may be conveniently divided into two parts, both triangular, with their bases placed posteriorly and their apexes anteriorly. The corneoscleral portion lies within the scleral furrow, while the remainder of the meshwork, for the most part in continuity with the ciliary muscle (CM), extends from the scleral roll ("spur") (SR), to the iris root (IR), where the dense surface layer of the iris appears. Note the continuity of the intertrabecular space (at the lettering TM) with the ciliary muscle. The numbers in this illustration indicate the approximate regions from which Figs. 10 to 15 were taken in the rhesus monkey experiments. 1, Figs. 10 and 11; 2, insets of Fig. 12; 3, Figs. 12, A and B; 4, Fig. 13; 5, Figs. 14, A and B; 6, Figs. 15, A and B. (>90. AFIP Neg. 63-4365.)

Fig. 11. A. Endothelial cells lining the inner wall of Schlemm's canal (major) fixed 20 minutes after placing ferritin in the anterior chamber. Ferritin particles are seen on the anterior chamber side of the endothelium and also adherent to the luminal side. A series of luminal invaginations (free arrows) suggests movement of vacuoles toward the luminal limiting membrane of the cell with particles taken up from the anterior chamber side. A small number of ferritin particles (FE) is found being taken up by a subjacent connective tissue cell. The inset shows more clearly the particles on the anterior chamber side (1), a cluster of three intracellular particles (2), and four particles forming a distinct line from within a surface-connected vesicle (3) through the open neck of the vesicle and within the lumen of Schlemm's canal. This suggests direction of passage of the particles through the cell cytoplasm via small pinocytotic vesicles and their discharge into the lumen. A few ferritin molecules appear to lie freely within the cell cytoplasm. Rhesus monkey. (Uranyl acetate treated. x66,500.) B, Endothelial junction along inner wall of Schlemm's canal. No ferritin particles are seen within the intercellular space, which here appears dense between two apposed cytoplasmic densities of the cells, closely resembling a terminal bar. Ferritin particles are clearly evident on both sides of the endothelial cell, and a few are present within surface (lumen)—connected cytoplasmic vesicles (arrows). Rhesus monkey, 20 minutes. (Uranyl acetate treated. ×44,900.) Inset: Endothelial lining at lower power to show relationship of lumen of canal of Schlemm (CS), the endothelium, and the adjacent juxtacanalicular connective tissue (JCT). Note the many tortuous villi and flaps of adjacent endothelial cells that may be present (arrow) in a region of focal widening of the intercellular space. (>8,700.)
Fig. 12, A and B. For legend see page 628.
Fig. 12. A. Endothelium from the outer wall of an early part of a collector channel. Ferritin particles are clearly visible within the lumen (L), within surface-connected vesicles (free arrows), and widely distributed through the adjacent corneoscleral tissue. Rhesus monkey, 20 minutes. (Uranyl acetate treated. ×58,000.) The inset in the upper left (I) shows a portion of endothelium lining the outer wall of the canal of Schlemm. A few ferritin molecules are seen on the surface of a cell projection into the lumen (I), within surface-connected vesicles, particularly on the far side of the cell (2), and within the dense extracellular connective tissue (3). (×50,700.) A second inset (II) in the upper right shows a number of lumen-connected cytoplasmic vesicles of the outer endothelium containing a number of ferritin molecules (free arrows). (×45,000.)

B. Outer endothelium of the early part of a collector channel showing a site of abutment of adjacent endothelial cells. A focal density resembling a terminal bar is present (TB). Ferritin particles are present within the lumen of the channel, within intracytoplasmic vesicles (FE), and throughout the subjacent corneal tissue. Long villouslike projections of the apposed endothelial cells are present. (×58,000.) The inset, from the outer wall of the nearby canal of Schlemm, a continuation of inset (1) A, shows some of the vesicles containing ferritin (free arrows) in continuity with the opposite (outer) limiting membrane of the endothelial cell. CS, lumen of canal of Schlemm. The free arrow indicates a single ferritin molecule on the cell surface membrane. (×50,700.)

Fig. 13. Capillary from the iris root just in front of the dilator muscle area. The lumen contains myriad ferritin particles embedded in the coagulated plasma protein. The ferritin particles are present outside the capillary, best observed embedded in the basement membrane (BM) of the vessel. Intracytoplasmic vesicles containing ferritin particles are seen throughout the vessel wall (arrows). Many long villous (or flap) processes (P) of the endothelial cells project into the lumen. A cluster of ferritin particles free of basement membrane is seen partially surrounded by a process of the endothelial cell at the free double arrow. A second region, which may represent a further stage in this process, is present at double arrow L. Rhesus monkey, 20 minutes. (Uranyl acetate treated. ×21,750.) Inset shows an enlargement of a junctional area between two endothelial cells. The intercellular density suggests the presence of an intercellular cement substance, while the adjacent cytoplasmic densities suggest that this portion is a terminal bar type of arrangement. No ferritin particles are seen within this intercellular space. Note ferritin particles within the enlarged intracytoplasmic vesicles. L, lumen of vessel. (×65,260.)

Fig. 14. A. A small portion of outer longitudinal muscle from well behind the scleral roll. Ferritin particles are clearly seen in the intercellular spaces (free arrow) which frequently contain a basement membrane-like material. The intracytoplasmic filaments of the smooth muscle are clearly seen (MF), as are the discrete, dense surface "attachment" plates (Z) characteristic of smooth muscle cells. Rhesus monkey, methacrylate-embedded, 20 minutes. (Untreated. ×36,250.)

B. Higher power of an area near A to show the ferritin molecules more clearly within the intercellular spaces of the ciliary muscle. (×58,000.)
Fig. 13. For legend see opposite page.

Fig. 14, A and B. For legend see opposite page.
Fig. 15, A and B. For legend see opposite page.
Fig. 15. A. Inset, upper right (×9,400), to show the wall of the blood vessel (capillary), the intraluminal red blood cells (R), basement membrane and adjacent collagen fibrils (C), and pigment (melanin) granules (P). This capillary is within the longitudinal ciliary muscle posterior to the scleral roll. The higher magnification of the capillary wall (×55,100) shows the accumulation of ferritin particles within the basement membrane (BM) of the capillary. A few intracytoplasmic ferritin particles are present (arrows), as well as a few within the lumen. Invaginations of both outer and inner limiting plasma membranes are seen at PL. Rhesus monkey, 20 minutes. (Uranyl acetate treated.) The inset in the upper left shows a portion of a red blood cell (RBC) in the lumen of another ciliary muscle blood vessel. The myriad ferritin molecules embedded in the coagulated plasma proteins in the vessel lumen are clearly seen. A number of ferritin molecules are also clearly seen on the surface of the red blood cell. (×53,000.)

B, Lower inset (I) is a low-power view (×10,100) of a longitudinal ciliary muscle capillary. The enlargement shows ferritin particles distributed throughout the basement membrane (BM) of this vessel. A peculiar laminated vesicular structure previously observed in rhesus retinal capillary walls is present here. Ferritin particles are present in the extracellular substances coating the luminal surface of the endothelial cells (arrows). (×38,400.) Upper inset (II) is an extension of the luminal surface to the left (×38,400) to show the continuation of the long villous or flaplike projection of the endothelial cell and the adherent coagulated luminal material. Ferritin particles are present here (arrow), better seen in the enlarged inset (III) to the right (×115,200). Note that the ferritin particles here clearly demonstrate "the halos" of their protein shells.
Fig. 16. Drainage angle of a young rhesus monkey eye enucleated approximately 60 minutes after placing India ink in the anterior chamber. The grossly clumped particles clearly occupy the intertrabecular spaces and are present well into the ciliary muscle (arrows). Gross particles therefore not only pass toward the canal of Schlemm system but also into the ciliary muscle. (Alcian blue stain, x115.)

Fig. 17. Photomicrograph diagram to indicate three main routes of physiologic aqueous outflow from the angle of the anterior chamber and its subsequent passage via aqueous and vascular channels to the episcleral and uveal vessels. Rhesus monkey.
tive tissue ground substances on all sides (Figs. 3A and 7). The trabecular meshwork, thus defined as the tissue containing the continuation of the anterior chamber, is clearly subdivided into two distinct parts by the scleral spur or roll. The corneoscleral fibers would then be represented by those layers extending from the cornea into the scleral spur or roll; the uveal layers, by those passing into the ciliary muscle and iris root. This distinction, while not very apparent in the youthful eye, becomes more so with increasing age (Fig. 8A).

In this study the older term "scleral roll" is used for the blunted scleral shelf that protrudes beneath (internal to) the canal of Schlemm (Fig. 2, broken line). The term "scleral spur" is reserved for the more prominent adult projection when present, or for the extreme exaggeration that may be present in pathologic material (Fig. 19, B).

It should be clearly understood that the term "anterior chamber angle" as used here refers not to the trabecular meshwork and canal of Schlemm alone but to the entire angle that stretches in an arc from the end of Descemet's membrane (Schwalbe's line or ring) over the "face" of the ciliary body to include the root of the iris (the entire iridocorneoscleral angle). The experiments with ferritin as a tracer of aqueous flow point toward at least three major sites of outflow, all of which lie within this anatomic angle of the anterior chamber (Fig. 17). The first is via the trabecular meshwork, in an anterolateral direction, through the juxta-canaliculare connective tissue and the endothelial cells lining Schlemm's canal into the lumen of the canal (Figs. 10 and 11). Additional lumens may be present posteriorly, protruding blindly into the corneoscleral meshwork or into the tissue of the scleral roll (Fig. 2). From here the passage is direct by collector channels to the episcleral region, where a number may be recognized as aqueous veins.

At this point in this study a more unexpected observation was made. The endothelium lining the outer wall of the canal and the collector channels also exhibited micropinocytotic activity, and the adjacent peripheral cornea and sclera were found heavily infiltrated with the ferritin molecules (Fig. 12, A and B). This observation suggests that this large canal and its drainage channels have a second function, that of assisting in the nourishment of the deep peripheral corneal stroma.

Studies with the coarser particles (clumps) of India ink (visible by light microscopy) showed deep penetration into the peripheral ciliary body (longitudinal ciliary muscle) (Fig. 16) and the iris root via crypts and between surface cells. This suggested either two more pathways or the possibility that the latter were secondary pathways, effective only when the primary route of Schlemm's canal was first severely obstructed by the coarse India ink.

The ferritin molecules, however, which do not aggregate in the manner of India ink, demonstrated that this second possibility was an unnecessary postulate; the molecules did indeed pass freely and primarily into the longitudinal ciliary muscle and iris root (Figs. 13 and 14).

Most of these observations are, of course, not entirely new, as they have in one way or another been made before. Re-examination of the drawings of earlier workers such as Priestley Smith and T. Leber showed that they often referred to the entire angle and not specifically to the "trabecular meshwork and canal of Schlemm." Leber was working mainly with rabbits, which possess no accommodative mechanism, hence no massive ciliary muscle. His pictures clearly show the passage of dyes into the entire angle tissue. The only distal passage of his finest tracer dyes that was clearly visible to him was the large canal, the coarser particles of a different color remaining apparently embedded in the angle tissue of the ciliary body.

*Resembling a more recent demonstration with nitroblue tetrazolium chloride.
Close attention to passage of these dyes into the large canal led to the concept of "pores" in the endothelium that conformed to the size of the particle, which apparently managed to pass its barrier. This concept of "pores" was championed by Schwalbe, although Leber, on the basis of his own observations, consistently refused to accept it.3, 30

Recent electron microscopic evidence28, 29, 31, 32 has been advanced to support Schwalbe's claim of apertures that pass through the endothelium, but these have not yet been observed in well-preserved material (Figs. 3 to 5).

There are a number of possible explanations for the apertures that are claimed to exist. The large villi and flaps that are often formed by the endothelium of a capillary, especially near its junction with an adjacent cell, may resemble large passageways (Figs. 11, B and 13). The canal of Schlemm, though lacking (normally) red blood cells, resembles in almost every way a large capillary, a large venule,33 or, even in some aspects, a large lymphatic. A second possibility is illustrated in Fig. 6B, where the tortuous interconnecting cytoplasmic system of tubules and cisterns known as the agranular reticulum has apparently begun to undergo dilatation, due to some slight delay between reduction of blood supply and fixation, as may be observed in experimental situations in which the blood supply has been retarded deliberately.34

Added to the observations of transcytoplasmic passage of ferritin molecules (and, presumably, related amounts of aqueous) in this study (Figs. 10 to 13) is another possible variation of this transport mechanism. This possibility is suggested by a number of observations here, of transcytoplasmic passage near the margins of endothelial cells in sites where impermeable terminal bars35, 36 are present. From a series of observations of static pictures (Fig. 13) it appears that this second mechanism may function by an envelopment of the ferritin molecules by peripheral flaps of adjacent endothelial cells, by a form of undulation of the cell margin, as in classic pinocytosis.37, 38 The cytoplasmic vacuoles so formed are then presumably transported across the cell cytoplasm to empty into the lumen of the vessel. This

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**Plate I.**

**Fig. 18,** A. Conventional (8 to 10 μ) meridional section of a formalin-fixed, paraffin-embedded eye of a young rhesus monkey, stained with the Masson trichrome method to bring out the dense collagenous bundles of the cornea, sclera, and scleral roll. The canal of Schlemm lies prominently within the scleral sulcus and here appears empty. A few layers of the trabecular meshwork continue the direction of the scleral roll to the corneal stroma, the corneoscleral trabeculae. The remainder of the meshwork passes into the ciliary muscle. The anterior border layer of the iris ends abruptly near its root. Small apertures are frequently present in the anterior border layer of the iris. The bundles of ciliary muscle cells are separated by a loose connective tissue composed mostly of collagenous fibrils, basement membrane, and other mucinous extracellular materials. (Masson trichrome stain. ×80. AFIP Neg. 60-4865.)

**B,** Thin section (1 to 1.5 μ) of methacrylate-embedded eye of a young rhesus monkey in which ferritin had been placed in the anterior chamber 40 minutes prior to enucleation and fixation. This section was stained for iron, and a nonspecific counterstain was used for contrast. The blue regions represent areas where ferritin has remained in sufficient concentration to show up for light microscopy (arrows). The ferritin here, therefore, is seen within the canal of Schlemm and its collector channels, between the muscle cells of the longitudinal ciliary muscle, and within the lumen of a vessel deep within the outer longitudinal ciliary muscle. A bluish tinge is also present within the peripheral corneal stroma at the anterior extremity of the trabecular meshwork and canal of Schlemm. Rhesus monkey, 40 minutes. (Ferritin, methacrylate-embedded, iron stain. ×80.)
Fig. 18, A and B. For legend see opposite page.
could be accomplished without there being at any time continuity between the anterior chamber and the canal of Schlemm. Such continuity has not yet been observed during these investigations.

A second site of outflow was found to lie on the opposite side of the angle. The ferritin was clearly observed to penetrate the anterior border layer of the iris, both by surface crypts and between closely associated surface cells to infiltrate the stroma. To be certain that there is meaningful activity here, it was, of course, necessary to demonstrate the ferritin not only in the basement membrane of a capillary, but also within the cytoplasm of the endothelial cell and within the lumen (Fig. 13).

A third site of outflow was the “face” of the ciliary body, which lies between the other two regions. The longitudinal ciliary muscle was diffusely infiltrated by the ferritin, which lay entirely outside the smooth muscle cells (Fig. 14, A and B). Capillaries lying within the outer parts of the longitudinal ciliary muscle and well back of the region of the scleral roll (“spur”) were found to contain the ferritin molecules within their basement membrane, their endothelial cytoplasm, and lumens (Fig. 15, A and B), clearly indicating specific activity on the part of these cells and not just “diffusion.” Drainage from here, although not important to this particular study, may be interpreted from previous work as occurring mainly in two directions—posteriorly and, especially, laterally via the ciliary plexus to join with the collector channels of the canal of Schlemm system within the sclera (Fig. 17).

The remainder of the iris, although functional, appears to play a small role in aqueous outflow in the normal eye. “Total” iridectomy (which presumably leaves the delicate angle tissue undamaged) apparently does not readily result in rapid development of an increased intraocular pressure.

The application of Poiseuille’s law to aqueous outflow on hypothetical grounds has long intrigued a number of workers. This principle cannot be readily applied to the current concept of flow of aqueous through an irregular tortuous pathway containing apertures of grossly varying sizes.

In this new light of posterior and posterolateral flow, however, the “tubes” are now relatively straight, short (Figs. 2, 8, and inset 10), and have either circular or elliptical lumens (Fig. 9) of considerable size. In this way, not only are some of the requirements for applying the law fulfilled, but one might proceed further to postulate relative rates of flow according to the straightness and shortness of the outflow “tubes” of a system, which might thus be called a system of “compound aqueous tubes.” The shortest and straightest tubes lie within this anatomic angle, but those passing out along the body of the iris become longer and more tortuous. From such a theoretical point of view alone (i.e., without considering the mucinous content of the iris or density of the anterior border layer), these latter passageways should be of lesser importance, a view that is supported by the well-known clinical observation described above. These latter passages may, of course, be of some importance secondarily, when the major outflow paths have become sufficiently embarrassed, or perhaps experimentally in perfusion studies.

The disease process known as chronic simple (chronic “primary” open-angle) glaucoma is generally a bilateral disease, which feature seems to correlate, as Priestley Smith and others have shown so well, with a single statistic, that of age. Because of this single correlation, Smith...
attempted to explain this disease by seeking some aging process that would tend to obstruct the angle, as he knew from his experiments that it must. He could conclude only that the growth of the lens, which was the only aging change of note, obstructed the angle by its pressure on the iris root. This concept was difficult to reconcile with latter gonioscopic evidence of an open chamber angle.

The correlation of the development of chronic simple (chronic “primary” open-angle) glaucoma with age continues to hold true. In the light of these investigations and observations, one might reasonably conclude that the obstruction is caused by a gradual aging process that takes place primarily within the connective tissue adjacent to Schlemm’s canal (juxtacanalicular connective tissue), within the longitudinal ciliary muscle, and within the stroma of the iris root (Fig. 20).

All of these aging changes have been well appreciated for a long time, and it is not at all unreasonable to consider these changes to be the cause of the slowly decreasing permeability of this drainage angle. It is likely that of the two major components of the connective tissue—cells and extracellular materials—the changes in the extracellular materials are, initially, the more important, as they would limit transport of nutrients and wastes to and from the cells.

These aging changes within the extracellular materials would therefore consist, at least in part, of the increasing inter- and intramolecular cross-linking of the collagen fibrils concomitant with a loss of the associated water-binding mucopolysaccharides. This would presumably decrease the permeability of the tissue on one count.

A decrease in permeability together with a slight increase in thickness of the base-
Fig. 19, A, B, and C. For legend see opposite page.
Fig. 20. Illustration to show approximate areas (indicated by the X's) where the tissues may undergo primary changes of aging along the paths of aqueous outflow. The region of the scleral roll (or spur) is prominent.

The region of the scleral roll has long been known to consist of bundles of collagen that appear to be cut more in cross section (on meridional section of the globe), indicating a more circumferential arrangement than most of the lamellae of the cornea and sclera. The well-developed scleral spur shows a remarkably similar arrangement (Fig. 19, B) which might be more readily understood by applying a principle of anatomy here. One can easily appreciate from this that these once-cellular extensions of the ciliary muscle functioning for years over the somewhat rigid fulcrum of the scleral roll become "fibrous." The superimposed collagenous cores of the trabeculae, losing their delicate (cellular) covering because of chronic pressure on the fulcrum of the scleral roll, appear in meridional sections of the eye as cross sections of circumferentially arranged bundles of collagen fibers, resembling closely the unusual bundles of the scleral roll and adjacent deep sclera.

Fig. 19, B. Exaggerated development of this spur would presumably add to the embarrassment of aqueous outflow posterolaterally as well as posteriorly into the longitudinal ciliary muscle (Fig. 19, B). This spur, a prominent development in primates con-
comitant with the prominent development of the ciliary muscles of accommodation, is most prominent in the human eye. It should be clearly noted that the concept outlined here applies specifically to that form of chronic open-angle obstruction almost always bilateral, which occurs in a small percentage of the aging population.

Other forms of acute or chronic obstruction to the surface of this drainage angle may appear secondarily. Extensive circumferential anterior cyclitis, sudden hemorrhage into the anterior chamber, or accumulation within these tissues of the drainage angle of debris or other materials such as macrophages filled with degenerating lens substances ("phacolytic glaucoma") may produce an acute glaucoma. Neovascularization proceeding from the ciliary muscle, overgrowth with endothelium that produces a fresh layer of Descemet-like material, the damage resulting from a contusion, or even an essential atrophy of the iris may produce a form of chronic open-angle glaucoma. Secondary forms of open-angle glaucoma have been considered elsewhere.

Consideration of these possible physiologic pathways now suggests a slight variation in our generally accepted concept of the mechanism of obstruction in primary acute congestive (i.e., narrow-angle obstructive) glaucoma. It has been known for some time that the most important part of the trabecular meshwork for drainage appears to be the part adjacent to the scleral spur and canal of Schlemm. Obstruction of this region alone is frequently considered to be sufficient to produce an elevated intraocular pressure. Many years ago Fuchs pointed out that the iris root could be lightly applied to the surface of the meshwork without causing a rise in pressure provided that the iris root be not compressed! It seems, therefore, that compression of the angle tissues (i.e., the posteriorly and posterolaterally directed aqueous tubes) on the scleral roll (or later spur) by the iris root might be an acceptable explanation for the seeming anomaly of an acutely increased intraocular pressure with the anterior part of the meshwork open.

A similar explanation might also be applicable to the phenomenon of steroid-induced glaucoma, an easily reversed, physiologic occurrence in a percentage of the population. The mucinous materials present in the iris root and anterior ciliary body might swell up under the influence of the topical corticosteroids and so close off these posteriorly directed channels by lateral compression, a closure that is soon released on cessation of the drug.

A final consideration arising from these observations suggests that the high percentage of favorable results of goniotomy in congenital glaucoma might result from exposure of these drainage tubes to the aqueous by rupture of a delicate covering layer of low permeability so that an adequate angle opening may form. This covering layer may consist of the first trabecular sheet itself, which, because of some developmental fault, has remained imperforate.

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*These explanations would also be in accord with the recent observations of Busacca, who discusses the effect of an inflammatory edema upon the region of the iris root and the "trabeculoconnective lamella" of the "sinus camerae anterioris."
meshwork as seen in tangential section, Arch. Ophth. 56: 708, 1956.


**Discussion**

Dr. Michael J. Hogan, San Francisco, Calif.

Dr. Fine has accomplished a considerable amount of work on the aqueous drainage system of human and monkey eyes. After sifting through the voluminous material presented, I find that he can make two conclusions:

1. There is no open passage between the anterior chamber and Schlemm's canal.

2. At all points where there is transfer of ferritin, it appears to be accomplished by an active process.

I would like to ask if the author presumes that aqueous moves through cells, or between cells, by the same mechanism as he observed with ferritin. If so, there is an impasse with respect to the reconciliation of aqueous outflow, which has repeatedly been shown to be pressure dependent (Poiseuille's law of laminar flow) with transcytoplasmic passage, which is largely independent of pressure, since it is an energy-requiring mechanism. The paper does not make this reconciliation. The "system of compound aqueous tubes" which is postulated by the author makes him feel that Poiseuille's laws are even more applicable than heretofore believed, indicating that he cannot dismiss all previous work on perfusion by Grant, Becker, Bárány, etc. This belief, then, is in contradiction to his findings. I would think that water does not flow in the same fashion as ferritin molecules, and that further work will be required to demonstrate how it actually departs from the eye. It is entirely possible that it passes between endothelial cells, through desmosomal connections, which then "snap" shut after transfer has been accomplished. Recent work by Farquhar and Palade shows that the junctional complex between cells may be of three forms. In this study
only desmosomes were shown, and these probably do not act as a barrier to movement of substances between cells.

With respect to the lack of openings through the pore tissue and endothelium into the canal of Schlemm, I think Dr. Fine probably is correct. Previous observations probably were based on artifact. It should be pointed out that rapidity of fixation, proper fixation, and careful preparation of tissues for sectioning are of utmost importance. Osmium is not the ideal fixative, particularly for the study of some tissues, and no attempt should be made to draw far-reaching conclusions except after many observations.

Little can be said about the author's "observations on the aging of tissues as a cause of glaucoma." As with all previous studies of this type, there is much smoke and little fire.

One comment about the perfusion of ferritin in these eyes. No attempt was made to control the perfusion pressure, except by visual observation of the depth of the anterior chamber. It is well known that numerous artifacts can be created during perfusion, and I think every effort should be made to perfuse at physiologic pressures in order to avoid such artifacts.

Another question concerns his statement that the "deep part of the trabecular mesh-work is entirely or even predominantly responsible for the normal or the pathologic resistance to aqueous outflow." If he believes this he should occasionally wonder why such a large channel as the canal of Schlemm, with its efferent system, should be placed in such proximity to the trabecular meshwork. Does he think the system exists only to provide nutrition to the regional limbal tissues, as the ferritin studies suggest in the collector system? Also, how does he explain the dissection studies of Grant in which resistance suddenly was lost when this zone was entered? And, finally, how about catheterization studies in which aqueous can be obtained in considerable amounts from the canal?

**REFERENCE**


**Dr. Fine (closing).** I would like to thank Dr. Hogan for his discussion of my paper. My primary concern in this study was not to answer all of the possible questions of transport across blood vessel walls, but rather to develop a concept of the pathogenesis of chronic simple glaucoma based on a combination of clinical, histologic, histopathologic, and experimental evidence that would be compatible with such evidence. This concept might then be tested by further observations and experiments. This concept has to my mind already produced a unifying theme for many hitherto puzzling entities (see page 642), and is in full conformity with the thesis that the obstruction lies somewhere in the tra-

Poiseuille's law may play in the mechanics of aqueous outflow. The purely mechanical portion of flow is not only dependent on changes in diameter of these channels (known to be produced by action of the ciliary muscle), but also upon pressure differences between entrance and exit. Increase in flow rate may therefore be accomplished as well by an increase in rate of aqueous removal at the terminus of these tubes. Increasing obstruction anywhere along these channels would produce a growing resistance to outflow. On the other hand, acceptance of the validity of pressure-dependent experiments demonstrating "normal" outflow rates and passageways should modify the criticisms, often advanced, of perfusion experiments conducted at other than rigidly maintained "physiologic" pressures. The limitations of my experiments are noted in the section on materials and methods.

In regard to the recent work of Farquhar and Palade; they demonstrate that a portion of the junctional complex found in various epithelia, known in light microscopy as terminal bars, and given by them the new name of "zonula occludens or adhaerens," has the property of keeping an intercellular space free from penetration by a material as fine as free hemoglobin. The junctional complexes described in my paper are considered to resemble terminal bars and not desmosomes, as stated in Fig. 11, B. On page 634 similar structures present in small capillaries or venules are often called terminal bars and they are described here as "impermeable." This is based on the analogous observation that the external limiting membrane of the retina has long been known to prevent movement of edema fluid either out of the retina into the subretinal space or the reverse. A rupture in this "membrane" is necessary for such transfer. A recent publication has shown the external limiting membrane to be, in reality, a series of terminal bars. This correlation of morphology with cytologic terminology, both old and current, and with well-established, long-standing observations on pathologic tissues is in full accord with these more recent experimental observations. As noted in my paper, terminal bars are not always present at these sites of endothelial cell abutment.

My primary concern in this study was not to answer all of the possible questions of transport across blood vessel walls, but rather to develop a concept of the pathogenesis of chronic simple glaucoma based on a combination of clinical, histologic, histopathologic, and experimental evidence that would be compatible with such evidence. This concept might then be tested by further observations and experiments. This concept has to my mind already produced a unifying theme for many hitherto puzzling entities (see page 642), and is in full conformity with the thesis that the obstruction lies somewhere in the tra-
becular meshwork, on the anterior chamber side of the effluent vessels.

I do not deny drainage of aqueous by the canal of Schlemm, but only point out a second function. I do not know its "primary" function or what percentage of aqueous exits here.

It is not inconceivable that an important function of this canal might be to nourish the adjacent deep corneal tissue which otherwise lacks a blood

wondered why this large canal is there, but these are teleologic questions which I have not discussed in my paper.

Dissection experiments as well as cannulation experiments are always subject to the problems of damage to the very delicate inner wall (endothelium) of the canal, damage which may artificially release a torrent of "aqueous" (or perfusion fluid) without light microscopic evidence of such a perforation. This is fully supported by Grant's statement that "India ink was found to flow readily out of the anterior chamber and into the episcleral vessels," upon opening the anterior chamber side of the canal of Schlemm.

A satisfactory cannulation may demonstrate that aqueous does flow out of the canal of Schlemm (which I do not doubt) but it remains to be seen if such experiments can quantitate this information in relation to total outflow in the normal eye in vivo.

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