Advances in morphologic glaucoma research

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This article presents and discusses recent information about new advances in morphologic glaucoma research. Recent publications indicate that patients with pseudoexfoliation syndrome might have a similar fibrillopathy in extraocular tissues. Cell cultures of iris tissue from human eyes with pseudoexfoliation syndrome were shown to synthesize pseudoexfoliative material. No new relevant studies dealing with morphologic changes in the trabecular meshwork of eyes with primary open-angle glaucoma have been published. However, the composition of the extracellular matrix in the trabecular meshwork of normal aging human eyes was further clarified with ultraimmunohistochemical methods. The distribution of collagen type VIII in the human eye was examined for the first time. Furthermore, various aspects of the cell biology of the trabecular meshwork were investigated. Experiments on the phagocytic ability of human trabecular cells were performed using perfusion organ cultures. A number of studies showed contractile properties for bovine trabecular meshwork cells, but the significance of these findings for human eyes has yet to be confirmed. Using cationized ferritin as a tracer, researchers demonstrated paracellular routes through the endothelium of the inner wall of Schlemm's canal. Studies of the extracellular matrix of the optic nerve head in glaucomatous human and monkey eyes found collagen changes that might be related to increased intraocular pressure.

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Ever since Rohen and Witmer [1] demonstrated that eyes with open-angle glaucoma show increased amounts of extracellular material in the cribriform region of the trabecular meshwork adjacent to Schlemm's canal, numerous studies on the morphology of the extracellular matrix in the human trabecular meshwork have been undertaken by various investigators.

Pseudoexfoliation

In glaucoma associated with pseudoexfoliation syndrome, deposits of the so-called pseudoexfoliative material are characteristically found in the trabecular meshwork [2]. Pseudoexfoliative material consists of fibrils 800 to 900 nm in length and 20 to 50 nm in diameter and has a banding periodicity of about 50 nm [3]. It has been suggested that this extracellular material originates from the lens capsule [4,5], but nonpigmented ciliary epithelium and iris tissue have also been discussed as possible sources [6,7]. In a recent study Ringvold and Bore [8•] reported on a patient in whom, 8 months after extracapsular cataract extraction and posterior chamber lens implantation, pseudoexfoliation syndrome developed as a distinct peripheral band on the anterior surface of the intraocular lens (Fig. 1). This case report, along with others [9,10], indicates that lens epithelium is not necessary for the production of pseudoexfoliative material.

In addition to its occurrence within the eye, pseudoexfoliative fibrillopathy has also been reported in the conjunctiva [11] and around a posterior ciliary artery [7]. These findings are further supported by recent studies that describe pseudoexfoliative fibrillopathy in skin [12••] and parabulbar structures (conjunctiva, extraocular muscles, vortex veins, and optic nerve sheaths) [13••,14] of patients with pseudoexfoliation syndrome. Streeten *et al* [12••] found a similar skin fibrillopathy in only one (a 78-year-old low-tension glaucoma patient) of 13 non–pseudoexfoliation syndrome control subjects. The authors mention, however, that in pseudoexfoliation syndrome patients over 70 years of age, the age-related dermal elastosis made evaluation difficult because the pseudoexfoliative nodules in the skin occur primarily along elastic fibers, and their morphologic character-

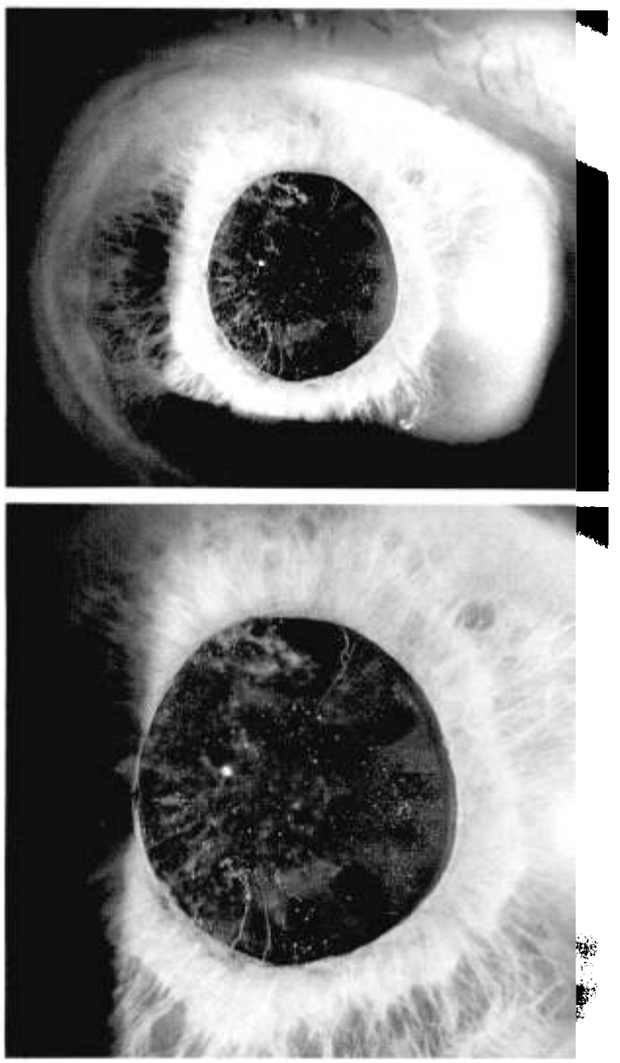

Fig. 1. Top, Peripheral pseudoexfoliation band on a posterior chamber intraocular lens. The central pupillary field is hazy due to secondary cataract. **Bottom,** Detail from *top panel* shows granular peripheral band with distinct oval defects. (*From* Ringvold and Bore [8•]; with permission.)

istics appear to be influenced by the elastotic process.

As only small quantities of pseudoexfoliative material are usually available, a detailed purification and characterization has been difficult. An interesting approach to pseudoexfoliative material production *in vitro* was introduced by Ringvold and Nicolaissen [15••], who cultured iris tissue from human eyes with and without pseudoexfoliation syndrome. Specimens from patients with pseudoexfoliation syndrome revealed typical pseudoexfoliative aggregates both within the native tissue and between the outgrowing cells. This model might be helpful for further studies on the nature of pseudoexfoliative material.

Primary open-angle glaucoma and age-related changes

The ultrastructure of the extracellular material in the cribriform region of eyes with primary open-angle glaucoma resembles that of the so-called sheath-derived plaque material that develops in aging human eyes [16,17]. The amount of this sheath-derived plaque material is, however, significantly higher in glaucomatous eyes than in normal age-matched control eyes [18].

Using antibodies against collagen types I through VI and laminin, Marshall *et al.* [19•,20•] studied the ultrastructural composition of this plaque material as well as that of other matrix compounds of the trabecular meshwork found in normal aging human eyes. In accordance with previous studies [21,22], this study found that type IV collagen is stained in the basement membrane of the trabecular beams and that types I and III collagen are stained in the striated collagen fibrils of the trabecular core and the cribriform tissue. Type II collagen was not identified. The authors showed for the first time positive staining of the so-called type I plaques (fine patches of fine filamentous material in the cribriform layer [1]) with antibodies against type IV collagen and laminin. In contrast to other studies [21,22], this study found no staining for laminin in the basement membrane of the trabecular beams. Laminin is a glycoprotein that is generally regarded as essential for the formation of all basement membranes in the human body [23]. The authors also could not confirm previous ultraimmunohistochemical findings showing that type VI collagen is stained in long-spacing collagen and the sheath-derived plaques [21]. Without question, immunohistochemical staining procedures depend on the binding sites, the affinity of the antibodies used,

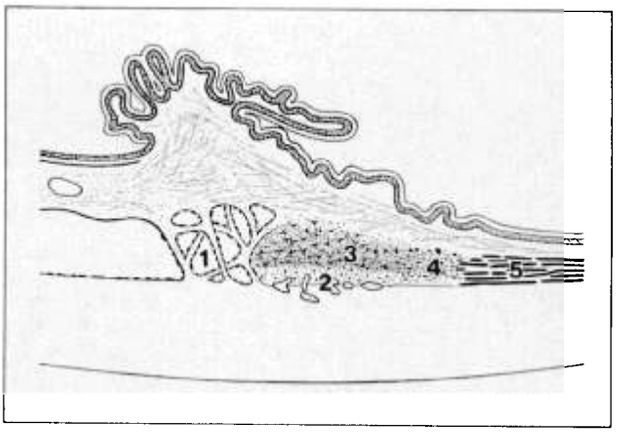

Fig. 2. Sagittal section through the bovine chamber angle of the superior or inferior quadrant. 1, pectinate ligament; 2, region adjacent to the aqueous plexus; 3, reticular meshwork; 4, transitional region between reticular meshwork and ciliary muscle; and 5, ciliary muscle. (*From* Flügel *et al.* [36••]; with permission.)

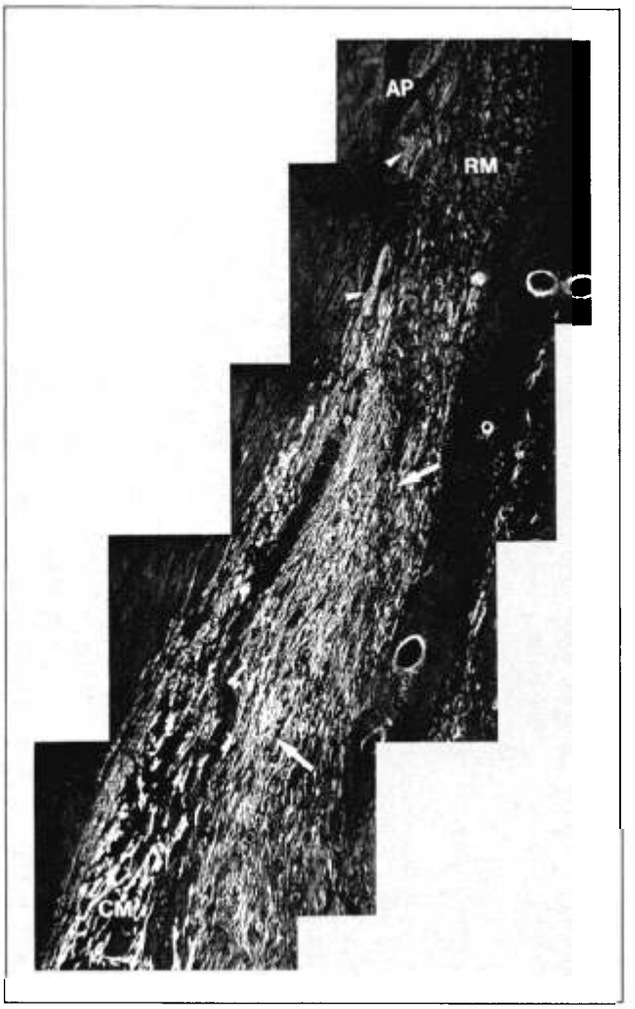

Fig. 3. Sagittal paraffin section through the bovine chamber angle, stained with antibodies against α-smooth muscle actin (the orientation differs from that of Figure 2, as cornea and reticular meshwork face the top and the ciliary muscle faces the bottom of the figure). The ciliary muscle (CM), region 4 (*arrows*), and cells of the posterior parts of region 2 (*arrowheads*) are positively stained. The presence of α-smooth muscle actin-containing cells can also be seen in the media of arterial vessels, whereas scleral fibroblasts and the inner reticular meshwork (RM) cells in region 3 remain unstained (original magnification, ×25). AP—aqueous plexus. (*From* Flügel *et al.* [36••]; with permission.)

and the masking of the antigen. Thus, lack of staining is not necessarily evidence for lack of antigens; further studies are needed to clarify each of these results.

Tamura *et al.* [24•] were the first to describe the distribution of type VIII collagen throughout adult and fetal human eyes. This collagen has been demonstrated ultrastructurally in the hexagonal lattice of Descemet's membrane [25]. Tamura *et al.* [24•] observed staining in Descemet's membrane, the trabecular meshwork, the walls of Schlemm's canal, Bruch's membrane, the choroidal stroma, the sclera, the cribriform plates of the optic nerve, and the intima of the central retinal artery. No distinct positive staining was seen in other blood vessels. When fetal eyes were investigated, significant differences in positive staining were found between adult and fetal sclerae. In fetal eyes, the posterior sclera stained strongly; however, the positive staining gradually decreased, and in the equatorial area, it disappeared completely. Because this study was performed in histologic sections only, the ultrastructural appearance of type VIII collagen, *eg*, in the trabecular meshwork, is still unknown.

Tripathi *et al.* [26•,27•] studied the distribution of noncollagenous matrix components in the trabecular meshwork. Using histochemical methods, they studied the localization of sialic acid moieties in the trabecular meshwork of normal human eyes. Furthermore, a biochemical analysis was performed and the total content of sialic acid in the meshwork was determined by a colorimetric assay. The authors concluded that significant quantities of sialic acid are present in the normal human trabecular meshwork as neuraminidase-sensitive, α-ketosidically linked terminal residues of polypeptides. Thrombospondin is a high molecular weight glycoprotein with terminal sialic acid residues. Tripathi *et al.* [27•] demonstrated *in situ* and *in vitro* that a thrombospondin-like cytoadhe-

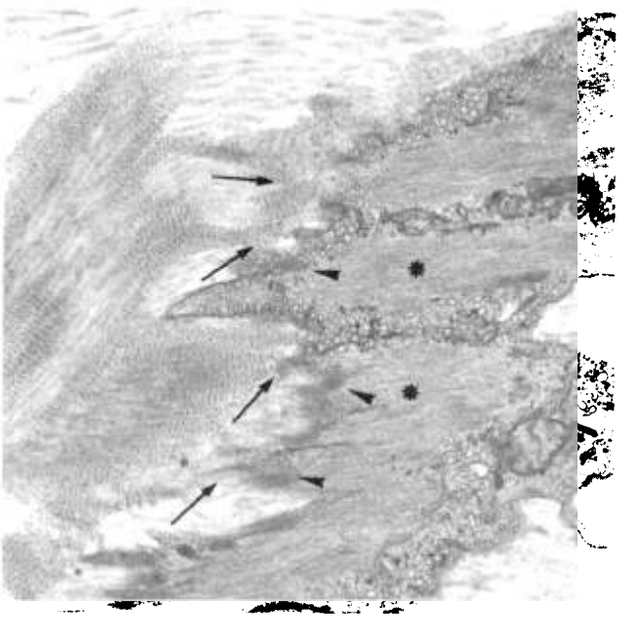

Fig. 4. Electron micrograph of a myofibroblast in the scleral spur of a human eye (tangential section). The cytoplasmic processes of these "scleral spur cells" form tendonlike structures with the elastic fibers in the scleral spur (*arrows*). In the region of contact, the cell membrane forms dense bands (*arrowheads*) where the intracellular 6- to 7-nm thin actin filaments (*asterisks*) attach (original magnification, ×33,600).

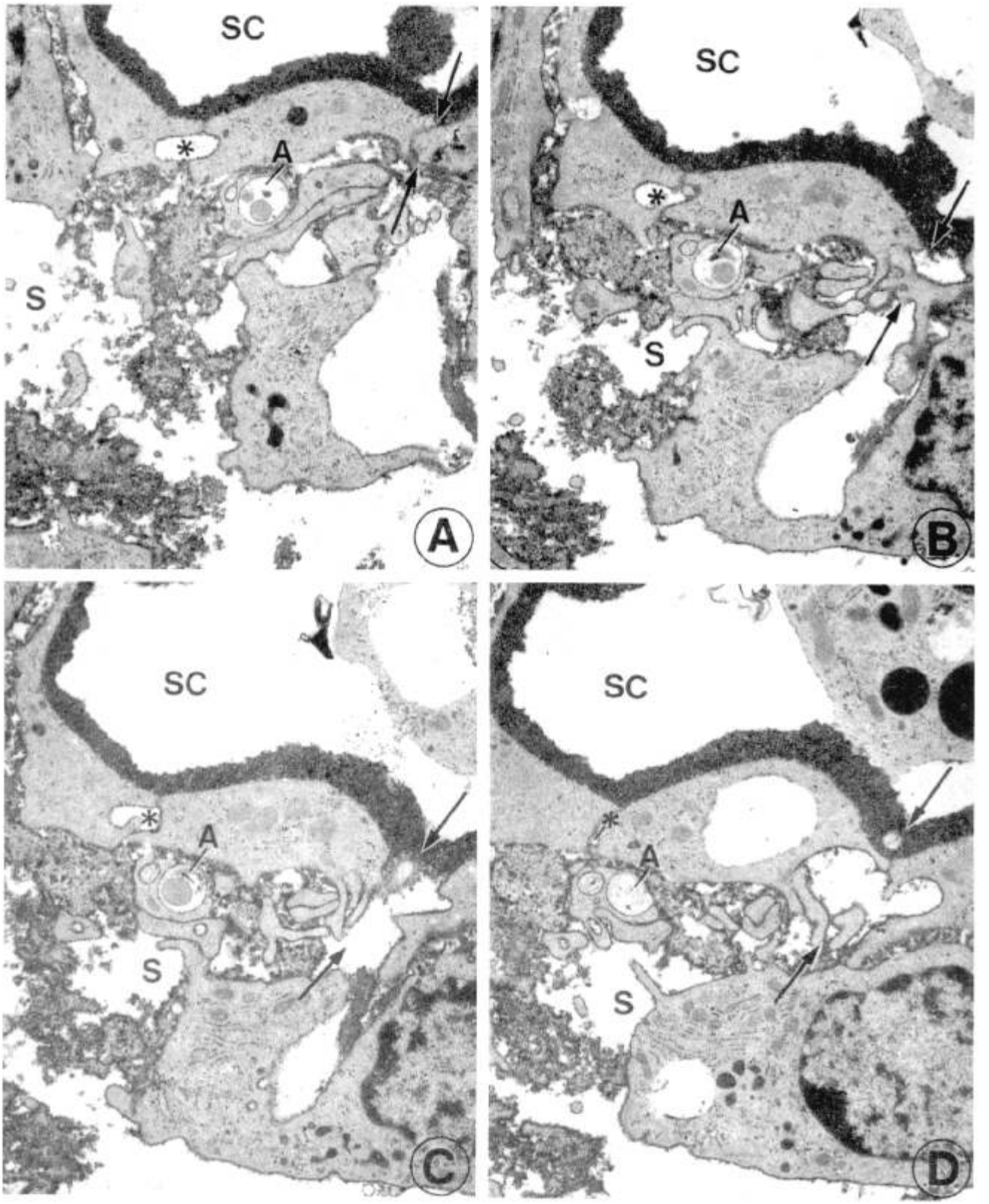

Fig. 5. Electron micrographs of sagittal serial sections through the inner wall of Schlemm's canal in a cynomolgus monkey after perfusion with cationized ferritin at an intraocular pressure of 30 mm Hg. Serial section 4 (*panel A*), section 6 (*panel B*), section 8 (*panel C*), and section 12 (*panel D*) are shown here. In *panel C* the intercellular space is completely open, whereas in *panel D* it is closed again (*arrows*). The electron-dense granular material is cationized ferritin, which shows intense labeling of the cell membranes (original magnification, ×13,600). *Asterisks* represent intercellular space that is distended in its middle part. A—subendothelial axon; SC—Schlemm's canal; S—subendothelial cell layer. (*From* Epstein and Rohen [40••]; with permission.)

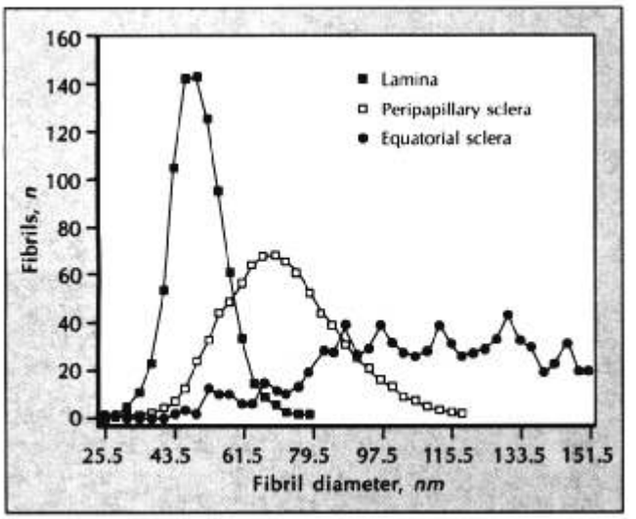

Fig. 6. The size distribution of collagen fibrils within the human lamina cribrosa compared with that of the peripapillary and equatorial sclera in normal human eyes. The graph shows the mean data from five eyes. Note the small uniform size of fibrils in the lamina, the broader distribution in the peripapillary sclera, and the wide spread in the sclera. (*From* Quigley *et al.* [41•]; with permission.)

sion molecule is synthesized by trabecular meshwork cells.

Biology of trabecular meshwork cells

The phagocytic activity of the trabecular meshwork has been amply documented by several researchers using *in vivo* and *in vitro* models [28,29]. Controlled investigations of the phagocytic ability of human meshwork cells *in situ* are still lacking. Such experiments have recently been conducted using a reliable organ culture system, which was developed by Johnson and Tschumper [30]. Buller *et al.* [31••] were able to show that human trabecular meshwork cells in these organ cultures ingest blood cells, latex microspheres, or zymosan particles. Interestingly, the phagocyting cells remained seated on the beams, and migration of the cells was only rarely observed. In another set of experiments the authors compared the phagocytic activity of feline meshwork cells *in situ* with that of eyes in organ culture. A great increase in phagocyting cells was observed when the eyes were left *in situ*. The authors discuss that the difference might be due to a lack of inflammatory response in the perfused eye. Therefore, this and other [32] perfusion organ culture systems might serve as an important tool for further studies on the complicated immunologic situation in the anterior segment of the eye.

Tripathi *et al.* [33•] further investigated the question of whether trabecular cells can phagocytose and, in addition, can present antigens similar to macrophages. The authors stained histologic sections of the anterior chamber of human eyes with antibodies against the major histocompatibility complex, class I and II antigens (HLA-A, -B, -C, -DR). At variance with other reports on human trabecular meshwork cells *in vivo* and *in vitro* [34,35], this report found staining for HLA I and II on most cells of the anterior segment of the eye. Further studies must be done to clarify this interesting question.

Another function of the trabecular meshwork cells is their contractility, which has been subject to a number of studies. Flügel *et al.* [36••] immunocytochemically studied the distribution of α-smooth muscle actin–positive cells in the different regions (Fig. 2) of the outflow tissue of bovine eyes. This actin isomer is normally found *in vivo* in smooth muscle cells, myofibroblasts, and pericytes only. In bovine eyes, α-smooth muscle actin was found in the region connecting ciliary muscle and the trabecular meshwork (region 4), as well as in a small area adjacent to the posterior capillary loops of the aqueous plexus (region 2) (Fig. 3). Ultrastructurally, these cells resembled myofibroblasts. In contrast to smooth muscle cells, the cells did not stain for the muscle specific intermediate filament desmin. The cells of the trabecular meshwork, which is more reticular than lamellar in structure, stained for vimentin, not for α-smooth muscle actin, and were found to have abundant rough endoplasmic reticulum and glycogen in their cytoplasm (region 3). These results show for the first time that different cell types exist in the bovine chamber angle.

Coroneo *et al.* [37••] cultured bovine trabecular meshwork cells. Phase-contrast microscopy revealed the

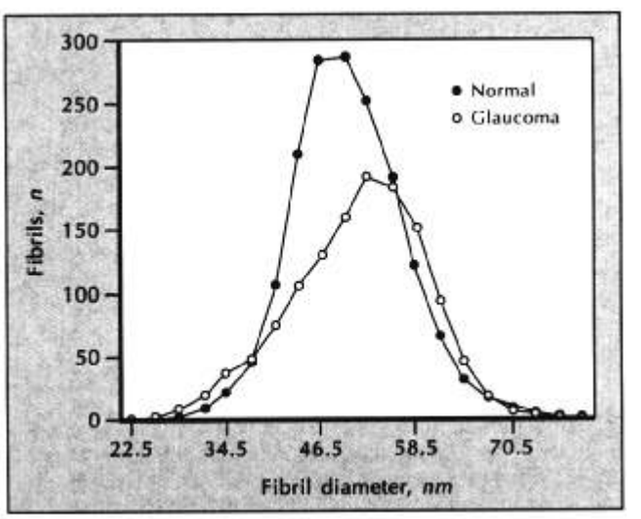

Fig. 7. The collagen fibril diameter of human glaucoma (mean of seven eyes) and normal lamina cribrosa (mean of five eyes) did not differ significantly; however, the distribution curves show a slight tendency toward fewer small fibrils in the glaucoma group. The curves are plotted in proportion to the density of fibers present in each group. (*From* Quigley *et al.* [41•]; with permission.)

presence of epithelial-like and spindle-shaped cells in their cultures. One millimolar Ba^{2+} induced an immediate depolarization of membrane voltage in these cells, with the onset of "overshooting" action potentials that were dependent on extracellular Ca^{2+} and Na^{+} but were not blocked by tetrodotoxin. Morphologically, the spindle-shaped cells stained for α-smooth muscle actin and showed ultrastructurally abundant intermediate and microfilaments. These data provide further evidence of multiple bovine trabecular cell types. Lepple-Wienhues *et al.* [38••] found contractile properties of bovine trabecular meshwork strips using an electromagnetic force-length transducer for isometric force measurements. Clearly, the significance of these data for human eyes has to be investigated.

Tamm *et al.* [39••] found a circular population of myofibroblasts in the human scleral spur, which also stained for α-smooth muscle actin but not for desmin and showed the ultrastructure of myofibroblasts (Fig. 4). They form individual tendinous connections with the elastic fibers of the scleral spur, which are continuous with the elastic-fiber network in the cribriform meshwork ("cribriform plexus"). Contraction of the scleral spur cells might modulate aqueous outflow.

A new concept regarding the outflow of aqueous humor through the endothelium of the inner wall has been presented by Epstein and Rohen [40••]. The authors investigated monkey eyes that were perfused through the anterior chamber with cationized ferritin at normal and increased intraocular pressures. The morphologic appearance of the endothelial lining of Schlemm's canal was analyzed using serial sections. The authors demonstrated that paracellular routes exist through which high molecular weight substances, such as ferritin and macrophages, can leave the anterior chamber (Fig. 5). The paracellular pathways appeared enlarged and were more easily identified at elevated perfusion pressure. Apparent giant vacuoles were often observed to be, not real intracellular vacuoles, but dilatations of the paracellular spaces. Probably both transcytoplasmic and paracellular mechanisms of aqueous outflow exist that vary under different conditions of pressure or flow.

Optic nerve head

Neither age-related nor glaucomatous changes of the extracellular matrix are confined to the outflow system: they are also found in the lamina cribrosa. Quigley *et al.* [41•] studied the density and fibril-size distribution of collagen and elastin fibrils in the optic nerve head and sclera in glaucomatous human eyes and in monkey eyes with experimentally induced glaucoma. The collagen fibrils of the normal lamina cribrosa are smaller and more uniform in size than those of the sclera (Fig. 6). In glaucomatous nerve heads, there is a major disruption of the lamina cribrosa beam structure, including a decrease in collagen density (Fig. 7). The peripapillary sclera undergoes collagen density changes similar to those seen in the optic nerve head in human glaucomatous eyes. In contrast to the collagen changes, elastin fiber density is unchanged in the glaucomatous nerve heads.

In another study [42••], the same group compared the composition of the extracellular matrix of the lamina cribrosa in glaucomatous monkey eyes with that in normal monkey eyes and eyes after optic nerve transsection. Immunohistochemically, they observed increased labeling for collagen type IV along the margins of beams in the lamina cribrosa. They also noted material in the pores of the laminar beams that labeled with antibodies to collagen types I, III, and IV, which was not observed after nerve dissection. Hernandez *et al.* [43] investigated the human optic nerve head in primary open-angle glaucoma and found a considerable increase in collagen type VI throughout the lamina cribrosa in glaucomatous eyes. Thus, an elevated intraocular pressure might influence increased formation of extracellular material in this region. In fact, Hernandez *et al.* [44] found greater gene expression for collagen type I in cell cultures from human lamina cribrosa cells when cells were exposed to high pressure. The authors have now applied *in situ* hybridization methods [45] to further study which kind of cells is responsible for the formation of the different types of collagen in the lamina cribrosa.

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Using an electromagnetic force-length transducer for isometric force measurements of bovine trabecular meshwork strips, the authors found evidence for contractility of bovine trabecular meshwork.

39. •• TAMM E, FLÜGEL C, STEFANI FH, ROHEN JW: **Contractile cells in the human scleral spur.** *Exp Eye Res* 1992, 54:531–543.

Within the scleral spur of human and monkey eyes, a population of equatorially arranged cells is found that stain for α-smooth muscle actin and express the ultrastructure of myofibroblasts. The cells form individual tendinous connections with the elastic fibers in the scleral spur, which are continuous with the elastic fiber network of the cribriform meshwork (cribriform plexus).

40. •• EPSTEIN DL, ROHEN JW: **Morphology of the trabecular meshwork and inner wall endothelium after cationized ferritin perfusion in the monkey eye.** *Invest Ophthalmol Vis Sci* 1991, 32:160–171.

Using cationized ferritin as a tracer, the authors demonstrate in serial sections that there are paracellular routes through the inner wall of the endothelium of Schlemm's canal by which high molecular weight substances can leave the anterior chamber. Apparent giant vacuoles were often observed to be, not real intracellular vacuoles, but rather dilatations of the paracellular spaces.

41. • QUIGLEY HA, DORMAN-PEASE ME, BROWN AE: **Quantitative study of collagen and elastin of the optic nerve head and sclera in human and experimental monkey glaucoma.** *Curr Eye Res* 1991, 10:877–888.

Quantitative studies of collagen density and fibril size distribution were carried out in the optic nerve head and sclera of glaucomatous human eyes and monkey eyes with experimentally induced glaucoma. In glaucomatous nerve heads, a major disruption of the structure of the lamina cribrosa beam structure is present, including a decrease in collagen density.

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The optic nerve head extracellular matrix was studied in monkeys with experimentally induced glaucoma or after optic nerve transsection. Glaucomatous nerve heads showed increased labeling for collagen types I, III, and IV, which was not observed following optic nerve transsection.

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