Trabecular Meshwork in Pseudoexfoliation Syndrome With and Without Open-Angle Glaucoma
A Morphometric, Ultrastructural Study

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Purpose. To test the hypothesis that glaucoma in eyes with pseudoexfoliation (PEX) syndrome results from blockage of the outflow channels by PEX material, melanin granules from the iris pigment epithelium, or both, and to determine the origin of intratrabecular PEX material.

Methods. Trabecular meshwork tissue was obtained from five surgically enucleated eyes with PEX glaucoma, ten autopsy eyes with PEX syndrome without evidence of glaucoma, and six age-matched control eyes. Morphometric methods were used to measure the percentage area occupied by open spaces, cells, plaque material, PEX material, and melanin granules on electron micrograph montages of the entire filtration area and the juxtacanalicular tissue (JCT) area.

Results. Independent of the presence of glaucoma, most PEX deposits were located in the JCT adjacent to the inner and outer walls of Schlemm's canal, as well as in the uveal meshwork. Although ultrastructural evidence indicates the local production of PEX fibers in the JCT by endothelial and connective tissue cells, PEX material in the uveal meshwork is derived partly from the aqueous humor. A significant correlation could be established between the presence of glaucoma and the amount of PEX material in both the filtration area and the JCT, the average thickness of the JCT, and the mean cross-sectional area of Schlemm's canal. No significant correlation existed, however, between glaucoma status and the concentration of melanin granules or plaque material, and the cellularity.

Conclusion. In addition to a mechanical obstruction by PEX material of exotrabecular origin, the apparent production of PEX material by trabecular cells may be principally responsible for glaucoma development. Accumulation of locally produced PEX material in the JCT, followed by dysfunction of endothelial cells and disorganization of JCT and Schlemm's canal, appear to be causative factors in the development of a special type of secondary open-angle glaucoma in PEX syndrome. Invest Ophthalmol Vis Sci. 1995; 36:1750–1764.

One of the best known and most important clinical features of the pseudoexfoliation (PEX) syndrome, which is characterized by the widespread production and deposition of an abnormal fibrillar extracellular material, is the frequent association with open-angle glaucoma. This association has been well documented since the first description of the condition by Lindberg and Vogt. Although the reported incidence of glaucoma in patients with PEX syndrome varies considerably, ranging from 0% to 81%, it is widely accepted that glaucoma develops in approximately 50% of patients with PEX. Factors influencing this reported variation include patient selection, examination technique, and definition of glaucoma. The glaucoma that often accompanies PEX, also termed "glaucoma capsulare," manifests as a severe type of chronic open-angle glaucoma that differs from primary open-angle glaucoma (POAG) by higher intraocular pressure (IOP) values, more rapid loss of visual field, and poorer response to medical treatment.

In spite of many clinical and a few histologic investigations of the outflow system, the pathogenetic...
mechanism of the chronic elevation of IOP remains a controversial subject, and the debate as to whether PEX is a coincidental finding in POAG or is actually the cause of glaucoma continues. One widely accepted view is that capsular glaucoma is a type of secondary open-angle glaucoma resulting from obstruction of the aqueous outflow system by PEX material, melanin granules liberated from the iris pigment epithelium because of abrasive movements of the pupillary iris against the irregular lens capsule surface, or both. This idea was supported by electron microscopic observations of PEX material and melanin granules within trabecular meshwork tissue, although the origin of intratrabecular PEX deposits remained unclear.

To test the concept of trabecular blockage, we performed a morphometric, ultrastructural study to measure the concentration of extracellular materials and other parameters in trabecular meshwork tissue of PEX eyes in correlation with the presence or absence of glaucoma. Morphometric evaluation was performed on more than 1500 electron micrographs taken from the entire filtration region and juxtacanalicular tissue (JCT) region of trabecular meshwork specimens obtained from 15 PEX and 6 control eyes. In addition, we determined the origin and distribution of PEX material deposits in the trabecular meshwork by transmission electron microscopy.

MATERIALS AND METHODS

In this study, we used only trabecular meshwork specimens obtained from whole eyes for optimal preservation of tissue architecture, because artifactual distortion is almost unavoidable with trabeculectomy specimens. Moreover, this material allowed comparative measurements along the whole circumference of the globes.

Specimen Collection

Trabecular meshwork tissue was obtained from three groups of subjects:

1. Ten eyes of seven subjects with PEX syndrome were obtained at autopsy (mean age, 79.2 ± 2.2 years) and fixed between 4 and 15 hours of death (mean, 8.5 ± 4.2 hours). The PEX syndrome was diagnosed at autopsy on macroscopic examination of the globes by the characteristic PEX material deposition on ciliary processes, zonular fibers, and posterior iris surface, and it was confirmed histologically by light and electron microscopy. The eyes had no history or morphologic evidence of glaucoma as recognizable by macroscopic evaluation of the optic disks. Additionally, 1-μm semithin cross-sections of the optic nerves, approximately 1 mm posterior to the lamina cribrosa, were made and were stained with toluidine blue to exclude regional atrophy of axons. No histologic, glaucomatous, optic nerve damage existed in all eyes of this group. Meridional wedges from different quadrants were further processed for conventional electron microscopy.

2. Five eyes of five patients with clinically diagnosed PEX syndrome were enucleated because of painful advanced or absolute open-angle glaucoma and were fixed immediately after removal (mean age, 81.2 ± 5.9 years). On clinical examination, all eyes revealed PEX material deposits at pupillary margin and anterior lens capsule, pigment dispersion after mydriasis, open chamber angles with marked pigmentation of the trabecular meshwork, and Sampaolesi’s line. Complete clinical histories were available for four of the glaucomatous eyes (Table 1); one eye was sent to our institution for routine histopathologic examination from a private ophthalmology practice, and only incomplete clinical information was available. All eyes received long-term, anti-glaucoma medication, and some eyes underwent multiple surgical procedures before enucleation. There was no history or gonioscopic evidence of laser trabeculoplasty in any of the glaucoma specimens. Eyes with angle closure or rubeosis iridis, such as neovascular glaucoma and hemorrhagic secondary glaucoma, were excluded from the study. A central block, including the optic nerve, was taken for routine histopathologic examination. Meridional wedges were taken from the cleft of the globes and were processed for standard electron microscopy.

3. Six normal, age-matched control eyes with no history or morphologic evidence of PEX syndrome or glaucoma were obtained at autopsy (mean age, 79.5 ± 8.9 years) and were fixed between 1 and 2 1/2 hours after death. They had no known ocular disease, and death resulted from trauma, cerebral vascular accidents, or carcinoma.

The research followed the tenets of the Declaration of Helsinki and was approved by the local ethics committee. Informed consent was obtained from the patients undergoing surgical enucleation.

Tissue Preparation

All eyes were fixed by immersion in a solution of 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M/l phosphate buffer. Immersion fixation included corneal trephination (3 mm in diameter) in five PEX eyes without glaucoma and four control eyes to allow...
### TABLE 1. Clinical Records of Patients With PEX Syndrome and Glaucoma (Group 2)

<table>
<thead>
<tr>
<th>Number</th>
<th>Age (years)</th>
<th>Diagnosis</th>
<th>IOP (mm Hg)</th>
<th>Medication</th>
<th>Surgical Procedure</th>
<th>Contralateral Eye</th>
</tr>
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<tbody>
<tr>
<td>80</td>
<td>79</td>
<td>Advanced chronic open-angle glaucoma*</td>
<td>40</td>
<td>Epinephrine, β-blockers, pilocarpine, acetazolamide</td>
<td>Goniotrephination, trabeculectomy, pars plicata diathermy</td>
<td>PEX syndrome, no hypertension</td>
</tr>
<tr>
<td>1059</td>
<td>87</td>
<td>Absolute chronic open-angle glaucoma</td>
<td>20</td>
<td>Catapressan, β-blockers, epinephrine</td>
<td>Pars plicata diathermy</td>
<td>PEX syndrome, no hypertension</td>
</tr>
<tr>
<td>1175</td>
<td>75</td>
<td>Absolute chronic open-angle glaucoma</td>
<td>40</td>
<td>Pilocarpine</td>
<td>None</td>
<td>No PEX, no hypertension</td>
</tr>
<tr>
<td>1185</td>
<td>78</td>
<td>Absolute chronic open-angle glaucoma</td>
<td>38</td>
<td>Catapressan, pilocarpine, carbachol, epinephrine, β-blockers</td>
<td>None</td>
<td>PEX syndrome, open-angle glaucoma</td>
</tr>
<tr>
<td>1040</td>
<td>85</td>
<td>Absolute chronic open-angle glaucoma</td>
<td></td>
<td>Pilocarpine, epinephrine</td>
<td>Trabeculectomy</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Duration: 10 years</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Duration: 5 years</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Duration: 3 years†</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Duration: 6 years</td>
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</tr>
</tbody>
</table>

PEX = pseudoexfoliation; IOP = intraocular pressure.
* Residual light perception without projection.
† Perforating injury 50 years ago.

Fixative to enter the interior of the eyes. Meridional tissue segments measuring 1 to 2 mm and containing the trabecular meshwork were taken from all four quadrants, postfixed in 2% osmium tetroxide in phosphate buffer, dehydrated, and embedded in epoxy resin (Epon) according to standard procedures. To ensure that the entire trabecular meshwork and Schlemm's canal were completely represented, 1 μm-sections were prepared for orientation and were stained with toluidine blue. By histologic checking for scar tissue formation, precautions were taken to avoid areas in three glaucomatous eyes that had already undergone surgery. Ultrathin sections were collected on coated slot grids, stained with uranyl acetate-citrate, and examined with a Zeiss EM 9A electron microscope (Zeiss, Oberkochen, Germany).

**Morphometric Evaluation**

Two tissue blocks from different quadrants served for morphometric measurements per individual subject. The specimens were coded throughout the measurements. A first series of overlapping electron micrographs was taken at an original magnification of X1700. The micrographs were enlarged to a final magnification of X5300 and assembled into composite photomontages comprising 40 to 60 single prints and representing the entire filtration region between the inner wall of Schlemm's canal and the anterior chamber. Figure 1A shows the boundaries of this area sparing the scleral spur. On each montage, the boundaries of the whole filtration area, aqueous channels, and PEX material deposits were outlined and traced with a cursor. Using a planimeter (Morphomat 30; Zeiss), we measured the whole filtration area and the percentage areas occupied by aqueous channels or intertrabecular spaces, PEX material deposits, intracellular and extracellular melanin granules, as well as length and width of Schlemm's canal. Further, we assessed the cellularity, which is defined as the number of nuclei per total tissue area, by hand counting. The concentration of the various components was then obtained by dividing the area occupied by the respective constituents by the total area of the filtration region.

A second set of sequential electron micrographs (original magnification, X3400) was enlarged to a final magnification of X8300 and assembled into photomontages comprising 15 to 30 single prints and representing the entire JCT of each specimen. The JCT area was defined as the connective tissue layer limited by the inner wall endothelium of Schlemm's canal and by the outermost clearly recognizable corneoscleral lamellae. The first intertrabecular space between JCT and first corneoscleral beam was not included. On each montage, the boundaries of the single constituents were distinctively marked (Fig. 1B). By planimetry, we measured the whole JCT area and the percentage areas occupied by PEX material deposits, intracellular and extracellular melanin granules, cellular...
elements, and two types of extracellular "plaque" material—electron-dense plaques (elastic-like fibers and their collagenous sheaths, corresponding to types II and III plaques of Rohen) and amorphous ground substance (homogenous, basement membrane-like material usually found adjacent to Schlemm's canal endothelium, corresponding to Rohen's type I plaques). The concentration of the various constituents was calculated by dividing their area by the JCT tissue area. The area of open spaces in the JCT was obtained by subtraction of cellular elements and extracellular materials from the total JCT tissue area. The average thickness of the JCT was obtained by multiple measurements taken at 5-μm intervals.

Forty-two montages (two per subject) comprising more than 1500 single prints were evaluated morphometrically. The data from three patients in group 1, in whom both eyes were available, were used separately.
RESULTS

Ultrastructural Findings

Independent of the presence or absence of glaucoma, PEX material was found in the trabecular meshwork of each eye with PEX; the histopathologic changes in both groups were similar, differing mainly in the degree of changes observed. Changes observed repeatedly in the trabecular meshwork of persons with PEX without glaucoma were likely to correspond to earlier stages than changes observed in patients with a long history of glaucoma. Generally, most PEX material was found in considerable amounts in the subendothelial area of Schlemm’s canal and in the JCT, as well as in the uveal meshwork, whereas the corneoscleral portions adjacent to the JCT, as well as in the uveal meshwork, whereas the main part of the corneoscleral meshwork remained largely uninvolved.

At an initial stage, small foci of PEX material could be observed in the subendothelial area of Schlemm’s canal, predominantly along the inner wall endothelium. The PEX fibers, which had a characteristic diameter of 28 to 35 nm and were often intermingled with a finely fibrillar component of 6 to 10 nm, were localized within invaginations of the basal cell surface of the endothelial cells (Fig. 2A).

The endothelial cells frequently disclosed hypertrophied cell organelles, for example, prominent and dilated cisterns of rough endoplasmic reticulum and increased numbers of mitochondria, as well as a highly irregular outline (Fig. 2B). PEX fibers were found exclusively in the extracellular space, and they accumulated within endothelial surface infoldings created by formation of cell processes (Fig. 2C). Direct cell–fiber contacts were restricted to minute loci (Figs. 2A, 2C) that were occasionally characterized by a dense thickening of the plasma membrane and membrane pits (Fig. 2D). At places, a process of PEX fiber maturation from finer fibrils to typical electron-dense PEX fibers was evident along the subendothelial region. Extracellular components of the JCT further comprised plaques of amorphous ground substance subjacent to the endothelial lining (type I plaques) and electron-dense, elastic-like fibers (type II plaques) with their collagenous sheaths (type III plaques). The PEX fibers appeared in close association with these normal extracellular matrix components and intermingled predominantly with the granular material of the collagenous sheath type III plaques.

The PEX material accumulated within giant vacuole-like spaces protruding into the canal lumen along the inner and outer walls (Figs. 2E, 2F). Serial sectioning revealed, however, that these “pseudovacuoles” in fact represented invaginations of the basal endothelial cell surface only without open connections to the lumen of Schlemm’s canal. Accordingly, no PEX material was found in the canal lumen. The PEX aggregates were predominantly concentrated in the posterior regions of the JCT in all cases studied. Within the JCT, PEX clumps were additionally found in association with a number of apparently “activated” connective tissue cells, surrounding the PEX deposits with slender cell processes.

In the uveal portion of the trabecular meshwork, large PEX aggregates were focally present between the uveal cords or adhering to the inner surface of the trabecular meshwork. Although some PEX clumps were observed freely and uncovered on the surface or within the innermost intertrabecular spaces (Fig. 3A), other clumps were surrounded by cell processes of trabecular endothelial cells that became partly detached from their trabecular beams and contained PEX material within surface invaginations (Fig. 3B). The trabecular cells involved re-
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FIGURE 2. Electron micrographs showing PEX material (*) in the subendothelial area of Schlemm's canal (SC) of nonglaucomatous PEX specimens. (a) Small patch of PEX fibers in association with inner wall endothelium and type I plaque material (I); the PEX fibers appear to emerge from cell surface pits (arrow). (b) PEX fibers in association with type I plaques (I) and inner wall endothelium revealing prominent rough endoplasmic reticulum and mitochondria. (c) PEX fibers in direct association (arrow) with inner wall endothelium, creating extracellular compartments by cell processes (P). (d) PEX material is associated with an endothelial cell surface pit characterized by a dense thickening of the plasma membrane (arrow). (e) Accumulation of PEX material beneath the outer wall endothelium of SC. CO = scleral collagen fibers. (f) Accumulation of PEX material within "pseudovacuoles" of the inner wall endothelium.

revealed an irregular outline with cytoplasmic processes, frequently disclosed cell surface pits with emerging PEX fibers (Fig. 3C), hypertrophied rough endoplasmic reticulum (Fig. 3D), and phagocytosed melanin granules. At a given site, more than one cell was usually involved, in particular the trabecular cells directly adjoining the anterior chamber. Invaginations of the endothelial cell membrane containing PEX fibers were seen on both aspects facing the intertrabecular space (Fig. 3E) and the connective tissue core of the trabecular beams (Fig. 3F). In the latter case, other abnormal extracellular matrix material, such as multilamellar basement membrane, could be observed together with the PEX fibers (Fig. 3F). In
FIGURE 3. Electron micrographs showing PEX material (*) within the uveal meshwork of non-glaucomatous PEX specimens. (a) PEX clumps located freely between the uveal cords or adhering to the inner trabecular surface. AC = anterior chamber. (b) PEX aggregate surrounded by a superficial trabecular endothelial cell with phagocytosed melanin granules and prominent rough endoplasmic reticulum. (c) PEX fibers appear to emerge from cell surface pits (arrows) of trabecular cells. (d) PEX aggregate close to a trabecular cell with hypertrophied rough endoplasmic reticulum and phagocytosed melanin granules. (e) Invaginations of trabecular cell membrane with PEX fibers (arrows) facing the intertrabecular space (IS). (f) Deposition of PEX fibers together with multilamellar basement membrane material (BM) toward the connective tissue core of a trabecular beam.

the corneoscleral portion, small patches of PEX material were occasionally located within open intertrabecular spaces or within a few disorganized beams only in advanced stages of the PEX process. Most trabecular endothelial cells and trabecular lamellae in the uveal and corneoscleral meshwork were, however, ultrastructurally normal.

PEX Eyes With Glaucoma. In the advanced glaucomatous disease state, vast accumulations of PEX material were interspersed within the JCT and the adjacent corneoscleral lamellae that revealed pronounced structural changes: Masses of PEX material accumulated along the whole periphery of Schlemm’s canal causing its endothelial lining to bulge into the canal lumen (Fig. 4A). The endothelium appeared mostly attenuated and thinned, partly degenerative, and partly activated without remarkable giant vacuole formation. Focal occlusion of the canal lumen by contact
FIGURE 4. Electron micrographs showing PEX material accumulation (*) within the juxtacanalicular tissue (JCT) of glaucomatous PEX specimens. (a) Masses of PEX material cause the inner wall endothelium to bulge into the lumen of Schlemm’s canal (SC). (b) Focal collapse of SC by contact of inner and outer walls. (c) Fragmentation of SC with interstitial PEX masses. (d) Obliteration of SC with plaque material marking the original outline of the canal wall (arrowheads).

of inner and outer walls was observed (Fig. 4B). Splitting and fragmentation of Schlemm’s canal lumen by focal disruption of the endothelial lining resulted in its replacement by several smaller, endothelium-lined channels in four of the five eyes with glaucoma. The spaces between these mini-channels were filled by masses of PEX material (Fig. 4C). Partial obliteration of the canal lumen with PEX material, detached endothelial cells, and plaque material marking the original outline of the canal wall could be found in three subjects with glaucoma (Fig. 4D).

The JCT and the adjacent corneoscleral trabeculae were highly disorganized and contained active and degenerative appearing cells, and they were heavily interspersed with PEX material so that the normal architecture was disrupted and a typical JCT could no longer been identified.

Increased PEX material deposition was also evident in the uveal portion of the trabecular meshwork. Pretrabecular conglomerations of PEX material were either freely adhering to the surface or were surrounded by cell processes of trabecular endothelial cells. Small patches of PEX material were occasionally noted within intertrabecular spaces and a few damaged trabecular beams of the central portion of the corneoscleral meshwork. Focally, trabecular endothelial cells with large amounts of phagocytosed melanin tended to become detached from their beams to surround PEX clumps with slender cell processes. No melanin granules were found free in the intertrabecular spaces.

Further changes throughout the uveal and corneoscleral portions of the trabecular meshwork of most glaucomatous eyes comprised thickening of trabecular beams, deposition of multilayered basement membrane and long-spacing collagen, swelling and rounding off of some trabecular endothelial cells, and compression of intertrabecular spaces.

In all PEX eyes, except one, independent of the presence of glaucoma, the degenerative changes also involved the collector channels branching from Schlemm’s canal as well as the scleral aqueous veins, which revealed a focal overproduction of abnormal extracellular matrix material including PEX fibers in their periphery and partly collapse of their lumina (Figs. 5A, 5B).

In two eyes in the glaucoma group, proliferating and migrating corneal endothelial cells produced a pretrabecular sheet of abnormal extracellular matrix including collagen fibers, multilamellar basement membrane, and solid masses of PEX material coating the inner surface of the uveal meshwork (Fig. 5C). The cells revealed a fibroblast-like appearance with prominent rough endoplasmic reticulum, ribosomes, cytoplasmic filaments, and long cytoplasmic processes surrounding the PEX aggregates. The PEX fibers appeared to emerge from cell surface invaginations (Fig. 5D).
Quantitative Analysis

The circumferential variation of the JCT was tested in two eyes with and without glaucoma, respectively, and the coefficient of variation was compared for each parameter (Table 2). The largest circumferential variations were found in the concentrations of melanin granules (45% to 80%) and type I plaques (32% to 39%), the lowest variation in the open spaces of the JCT (7.2% to 7.8%) in both eyes examined. Regional variations in the parameters of cellular elements, types II and III plaques, and PEX material deposits were found to be within range of normal biologic variability (18% to 28.5%). These findings demonstrated a relatively equal distribution of PEX material deposits (coefficient of variation, 22.6% and 28.5%) around the circumference of nonglaucomatous and glaucomatous eyes, and they appeared to justify the morphometric evaluation of two sections only per subject.

The data obtained from our measurements in the total filtration area and the JCT area are presented in Tables 3 and 4. In general, a high variability in the measurements of individual specimens was obvious.

Total Filtration Area

Aqueous Channels. Regarding the whole filtration region of the trabecular meshwork specimens, the percentage area occupied by open spaces or aqueous channels revealed almost no difference between the control group (27.2%) and the PEX group without glaucoma (28.16%), but decreased significantly to 18.05% in the PEX group with glaucoma (P = 0.009) (Table 3).

Cellularity. No substantial difference in cellularity of the whole meshwork could be established among the three groups.

PEX Material. PEX deposits occupied 0.91% of the total filtration area in nonglaucomatous and 3.01% in glaucomatous PEX eyes; this difference proved to be significant (P = 0.01).

Melanin Granules. Although the concentration of
Trabecular Meshwork in Pseudoexfoliation Syndrome

TABLE 2. Coefficient of Variation*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Open Spaces</th>
<th>Cells</th>
<th>Type I Plaques</th>
<th>Types II/III Plaques</th>
<th>PEX Material</th>
<th>Melanin Granules</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEX without glaucoma</td>
<td>7.78</td>
<td>18.0</td>
<td>32.14</td>
<td>23.25</td>
<td>22.63</td>
<td>80.0</td>
</tr>
<tr>
<td>PEX with glaucoma</td>
<td>7.20</td>
<td>17.93</td>
<td>38.72</td>
<td>27.32</td>
<td>28.46</td>
<td>45.45</td>
</tr>
</tbody>
</table>

Values are percentages.
PEX = pseudoexfoliation; JCT = juxtacanalicular tissue.
* The measurements are based on 12 photomontages (three per quadrant) of the whole JCT of one PEX eye without glaucoma and one PEX eye with glaucoma.

(intracellular) melanin granules in the total filtration area increased from 0.21% in control eyes and 0.29% in nonglaucomatous PEX eyes to 0.78% in glaucomatous PEX eyes, these differences were not statistically significant.

Juxtacanalicular Tissue

PEX Material. Regarding the JCT separately, the percentage area occupied by PEX material increased significantly from 4.93% in the group without glaucoma to 12.23% in the group with glaucoma (\(P = 0.02\)) (Table 4).

Melanin Granules. The increase in the amount of (intracellular) melanin granules from 0.16% in controls and 0.20% in nonglaucomatous PEX eyes to 0.59% in glaucomatous PEX eyes was not statistically significant.

Plaque Material. No significant correlation could be demonstrated between the quantity of extracellular plaque material in the JCT and the presence of glaucoma: The PEX specimens revealed, however, a clear trend toward reduction of the total plaque material of all types, from 39.34% in the control group to 31.29% in the PEX group without glaucoma and further to 26.41% in the PEX group with glaucoma. Differentiating between the single types of plaque material in the JCT, it becomes evident that this decrease mainly results from a diminution of the amorphous ground substance (type I plaques) from 12.9% in control eyes to 5.43% and 4.51% in nonglaucomatous and glaucomatous PEX eyes, respectively. This decrease almost reached statistical significance. The decrease in types II and III plaque material from 26.45% in normal eyes to 25.85% and 21.89% in PEX eyes without and with glaucoma was less pronounced.

Aqueous Channels and Cells. On the other hand, the areas occupied by open spaces in the JCT (controls, 33.35%; PEX without glaucoma, 39.82%; PEX with glaucoma, 34.89%) and by cellular elements (controls, 27.31%; PEX without glaucoma, 23.95%; PEX with glaucoma, 26.47%) remained unchanged during the disease process.

JCT Thickness. The average thickness of the JCT increased from 7.62 µm in the control group and 8.08 µm in the nonglaucomatous PEX group significantly to 15.38 µm in the glaucomatous PEX group (\(P = 0.005\)).

Schlemm’s Canal Area

Degenerative changes of Schlemm’s canal (Table 4) were reflected by its morphometric measurements: the mean cross-sectional area of Schlemm’s canal was significantly reduced from 7052 µm² and 6562 µm² in control and nonglaucomatous PEX eyes to 2386 µm² in glaucomatous PEX eyes (\(P = 0.005\)). The incidence of giant vacuoles along the inner wall endothelium of

TABLE 3. Percentage of the Entire Filtration Area of the Trabecular Meshwork Occupied by Open Spaces, Cells, PEX Material, and Melanin Granules in Controls and in Nonglaucomatous and Glaucomatous PEX Eyes (One Measurement Per Individual Specimen)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 6)</th>
<th>PEX Without Glaucoma (n = 10)</th>
<th>PEX With Glaucoma (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open spaces (%)</td>
<td>27.20 ± 4.22</td>
<td>28.16 ± 5.95*</td>
<td>18.05 ± 4.37*</td>
</tr>
<tr>
<td>Cellularity (nuclei/1000 µm² total tissue area)</td>
<td>2.87 ± 0.62</td>
<td>2.65 ± 0.43</td>
<td>2.76 ± 0.51</td>
</tr>
<tr>
<td>PEX material (%)</td>
<td>—</td>
<td>0.91 ± 0.59†</td>
<td>3.01 ± 1.76†</td>
</tr>
<tr>
<td>Melanin granules (%)</td>
<td>0.21 ± 0.14</td>
<td>0.29 ± 0.16</td>
<td>0.78 ± 0.75</td>
</tr>
</tbody>
</table>

PEX = pseudoexfoliation.
* \(P = 0.009\); † \(P = 0.01\).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 6)</th>
<th>PEX Without Glaucoma (n = 10)</th>
<th>PEX With Glaucoma (n = 5)</th>
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<tr>
<td>PEX material (%)</td>
<td></td>
<td>4.93 ± 3.17*</td>
<td>12.23 ± 6.25*</td>
</tr>
<tr>
<td>Melanin granules (%)</td>
<td>0.16 ± 0.14</td>
<td>0.20 ± 0.21</td>
<td>0.59 ± 0.42</td>
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<td>Type I plaques (%)</td>
<td>12.90 ± 6.97</td>
<td>5.49 ± 2.89</td>
<td>4.51 ± 3.29</td>
</tr>
<tr>
<td>Type II/III plaques (%)</td>
<td>26.45 ± 4.77</td>
<td>25.85 ± 5.84</td>
<td>21.89 ± 4.64</td>
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<tr>
<td>Open spaces (%)</td>
<td>33.35 ± 8.11</td>
<td>39.82 ± 6.53</td>
<td>34.89 ± 8.07</td>
</tr>
<tr>
<td>Cells (%)</td>
<td>27.31 ± 4.0</td>
<td>23.95 ± 3.74</td>
<td>26.47 ± 5.31</td>
</tr>
<tr>
<td>JCT thickness (μm)</td>
<td>7.62 ± 1.59†</td>
<td>8.08 ± 1.30†</td>
<td>15.38 ± 5.74†</td>
</tr>
<tr>
<td>Schlemm's canal (μm²)</td>
<td>7052.08 ± 2768.20‡</td>
<td>6562.15 ± 2347.87‡</td>
<td>2386.07 ± 1645.64‡</td>
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JCT = juxtacanalicular tissue; PEX = pseudoexfoliation.

* P = 0.02; † P = 0.005; ‡ P = 0.005.

Schlemm’s canal was not morphometrically assessed because the formation of these structures is dependent on the IOP at the time of fixation, and part of the donor eyes investigated were subjected to corneal trephination before fixation.

**Regional Distribution**

In respect of the regional distribution, approximately half the total PEX material was concentrated in the JCT (PEX without glaucoma, 52.46% ± 20.92%; PEX with glaucoma, 44.66% ± 14.08%), whereas only 6.20% ± 5.25% and 13.64% ± 18.99% of the total melanin was localized in the JCT region.

**DISCUSSION**

The current study was designed to test the concept of trabecular blockage by a qualitative and quantitative analysis of extracellular material concentrations, open spaces, and other parameters in trabecular meshwork tissue of PEX eyes in correlation with the presence or absence of glaucoma. In the nonglaucomatous PEX eyes (incidentally detected at autopsy), we had a group that on the whole represented a relatively early stage of PEX, and we compared it with the established PEX glaucoma group whose history included medication and, in some cases, surgery. The PEX eyes were considered nonglaucomatous because macroscopic evaluation of the optic disks and histologic evaluation of optic nerve cross-sections revealed neither glaucomatous cupping nor areas of axonal atrophy. Elevated IOP, however, might well have existed in these eyes because mean IOP levels in PEX eyes were found to be generally higher than in age-matched control eyes or in the uninvolved contralateral eyes. Similarly, studies on aqueous humor dynamics revealed a higher resistance to aqueous outflow in PEX eyes with and without glaucoma compared to unaffected fellow eyes or age-matched control eyes, which in turn was primarily responsible for the elevated IOP. The obstruction theory presupposes a significant difference in the amount of PEX material, pigment granules, or both, in the trabecular meshwork of PEX eyes with and without glaucoma. Among the histopathologic studies on this subject, only two electron microscopic reports investigated trabecular meshwork tissue from one nonglaucomatous PEX eye in each case.

A clear relationship between the amount of PEX material in the trabecular meshwork and the glaucoma status has only been suggested by Ringvold and Vegge investigating one nonglaucomatous and two glaucomatous PEX eyes.

**Morphometric Analysis**

Generally, the measurements of the control specimens yielded results that agree with those obtained by other investigators examining the extracellular matrix concentration in the JCT from normal, older persons. According to the quantitative results of the current study, a statistically significant relationship between the amount of PEX material, both in the entire filtration area and the JCT, and the presence of glaucoma could be established, at first sight substantiating the hypothesis of obstruction of outflow channels. The significant decrease in aqueous channels in the glaucoma group might, however, also be caused by compression of intertrabecular spaces. A further correlation between clinical data (e.g. duration of glaucoma, type and duration of therapy) and the quantity of PEX material could not be made because of the limited sample size in the glaucoma group. An obstructional mechanism is supported by the regional distribution revealing approximately half the total PEX material concentration in the JCT area. A significant correla-
tion also could be established between the presence of glaucoma and the average thickness of the JCT, as well as the mean cross-sectional area of Schlemm’s canal.

Pigmentation of the chamber angle is a prominent aspect of PEX syndrome and may be a prognostic factor, but the extent of pigmentation and the severity of glaucoma do not always correlate. A positive, but statistically not significant, association between the melanin content and glaucoma was evident from our measurements in PEX trabecular specimens. However, the low quantity of melanin granules (less than 1% in the JCT of glaucoma specimens), their high circumferential variation, and their intracellular location make it unlikely that pigment contributes significantly to outflow obstruction. The high circumferential variation of the melanin concentration corresponds to the clinical observation of an uneven or splotchy distribution of pigment in the trabecular meshwork. Our findings are consistent with the observations of Richardson and Epstein showing minimal accumulation of intracellular pigment in the trabecular meshwork of PEX glaucoma eyes. Even in pigmentary glaucoma, quantitative evaluations and hydrodynamic models indicate that little flow resistance is generated by a buildup of melanin granules in the JCT, and factors other than pigment accumulation in the trabecular meshwork are believed to be responsible for the development of glaucoma.

PEX glaucoma is well differentiated from POAG not only clinically, but also histopathologically: Whereas a significant increase in juxtacanalicular plaque material was reported in POAG specimens, the PEX specimens revealed a clear trend toward reduction of extracellular plaque material in the JCT. No difference in plaque concentration to normal eyes was found in another semiquantitative study. Moreover, decreased cellularity of the trabecular meshwork appears to be a particular characteristic of POAG, but it does not seem to play a role in the pathogenesis of PEX glaucoma. The structural integrity of trabecular endothelial cells was mostly maintained in PEX glaucoma, and the cellularity remained unchanged. These histopathologic differences might help to explain the different response to medical therapy, the different responsiveness to steroids, and a more severe clinical course and management compared to POAG. Current evidence and this study, therefore, suggest that POAG and PEX glaucoma are separate, distinct clinical entities.

Origin of PEX Material in the Trabecular Meshwork

An important question is whether the PEX material within trabecular meshwork tissues was produced locally or entered the JCT through the aqueous flow. The possibility that PEX material may pass through the trabecular meshwork and may be trapped beneath the endothelium is unlikely because PEX material has not been observed in other parts of the meshwork in the early stages. Using thorotrast tracer substances, Inomata et al. noted 0.1-μm latex spheres within vacuole-like spaces of Schlemm’s canal endothelium of monkey eyes, but only rarely spheres of 0.5 μm or 1.0 μm in diameter, giving evidence of a sieve function of the JCT for particles larger than 1.0 μm in diameter, a size that is certainly exceeded by PEX clumps. Similarly, in pigmented glaucoma, surprisingly few melanin granules become trapped at the level of the JCT; usually they are found upstream in the uveal and corneoscleral portions.

Ultrastructural evidence for the in situ production of PEX material by trabecular cells are the formation of PEX fibers within cell surface invaginations and the clear emergence of PEX fibers from cell membrane pits characterized by a dense thickening of the plasma membrane; a marked elaboration of secretory organelles and mitochondria indicating stimulated cell function; an occasional maturation process from finer fibrils close to the cell surface to thicker fibers; intermingling of PEX fibers with newly formed abnormal basement membrane material; and the presence of PEX material in the outer wall of Schlemm’s canal and the involvement of collector channels and aqueous veins. Moreover, phagocytosis or intracellularly located PEX material has never been observed. Comparable features, such as fiber formation within invaginations of the cell surface occasionally characterized by a dense thickening of the plasma membrane, are known from the secretion of elastic fibers and collagen fibers.

Some material is brought by the aqueous humor in addition, but this is most probably already trapped between the uveal sheets and can be distinguished easily by its free location lacking surrounding cell processes. Figure 6 summarizes the localization of PEX deposits in the trabecular meshwork and their suggested endotrabecular and exotrabecular origin. Simultaneous mechanisms of local production and transport by the aqueous humor also have been suggested by Ringvold and Vegge.

Mechanisms of Glaucoma Development

In spite of the positive correlation between PEX material deposition and glaucoma status, it is much more complex than a simple mechanical obstruction of the outflow pathways. We tried to reconstruct the chronology of glaucoma development from the ultrastructural findings. Generally, the PEX eyes without glaucoma revealed less advanced histopathologic alterations than the glaucomatous eyes. The pathologic changes appeared to affect primarily the outer portions of the
trabecular meshwork and to proceed toward the inner regions, emphasizing the differential regional involvement.

We propose that the following sequence of events led to the development of the glaucomatous condition in PEX syndrome: The initial pathologic events appeared to start in the subendothelial area of Schlemm's canal along its inner and outer walls, where locally produced PEX material accumulated within endothelial cell surface invaginations. Later, cells of the JCT became involved, and PEX material accumulations extended into the JCT and the outermost corneoscleral lamellae. In contrast to melanin granules or other particulate materials responsible for various types of secondary open-angle glaucoma, the PEX material could not be removed by the cells lining the outflow tract. There was no evidence of phagocytosis of PEX material by trabecular cells nor of a transport of PEX material across Schlemm's canal endothelium into the canal lumen in this or previous studies. Instead, the endothelial cells of Schlemm's canal possess a special property for invagination of their basal cell surface in response to a pressure gradient or physical and chemical properties of the aqueous humor. The hydrostatic pressure of the aqueous humor might indent the endothelial cell walls, subsequently forming PEX material containing pseudovacuoles, which do not discharge their contents into Schlemm's canal. Thus, morphologic evidence makes it plausible that the functional integrity of giant vacuole formation by endothelial cells for the purpose of aqueous drainage is likely to be disturbed and drainage of aqueous humor into Schlemm's canal impaired.

The gradual accumulation of PEX material in the JCT and adjacent corneoscleral lamellae finally culminates in a significant swelling of this zone and a disorganization of the normal tissue architecture, which also is observed in glaucomatous eyes that have not undergone surgery. The endothelial cells lining Schlemm's canal seem to lose their ability to maintain a proper matrix because the considerably reduced or absent basement membrane-like ground substance is replaced by PEX aggregates. Lacking a proper cell-matrix interaction might contribute to focal endothelial disruption and focal collapse of Schlemm's canal. Splitting and fragmentation of Schlemm's canal into smaller channels are particularly advanced features notable only in glaucomatous eyes. Because the total cross-sectional area of Schlemm's canal appears to be considerably less than that of the control group, the remaining structures may not be able to accommodate normal aqueous drainage. Alternatively, the fragmentation could secondarily result from reduced flow of aqueous humor through an abnormal JCT region or from increased IOP compressing the trabecular meshwork and compromising the canal lumen. A smaller cross-sectional area of Schlemm's canal also has been reported in POAG eyes.

Because it is well established that the site of greatest resistance to aqueous outflow is localized in the JCT and the inner wall endothelium, the pathologic changes observed could well account for the increase in outflow resistance, and development of the glaucomatous condition in PEX may be considered primarily a quantitative problem depending on the degree of JCT involvement. Our findings fit well with the results of a study by Richardson and Epstein indicating that the condition involves disorganization and destruction of JCT and Schlemm's canal because of obstruction of the JCT by PEX material and degenerative changes of these structures.

Additionally, other factors have to be considered:
Trabecular Meshwork in Pseudoexfoliation Syndrome

Increase in flow resistance may be caused by indirect effects of PEX accumulation altering the physicochemical properties of the JCT. PEX material in the JCT may, for example, act like a layer of glue and lead to a nonspecific accumulation of serum proteins liberated from a disturbed blood–aqueous barrier adding to outflow resistance.

The structural integrity of trabecular cells and beams is, however, largely maintained, particularly in the corneoscleral portion of the meshwork. Additional changes in glaucomatous PEX eyes, such as thickening of trabecular lamellae, compression of intertrabecular spaces, disorganization of connective tissue cores with accumulation of long-spacing collagen, most probably represent nonspecific secondary changes from high pressure and antiglaucomatous medication.

The alterations in metabolic functions of trabecular meshwork cells are suggested to cause chronic, secondary, open-angle glaucoma in a first phase. This basic process might be superimposed by additional processes causing pressure peaks through acute clogging of the trabecular meshwork in a second phase. A transient rise in IOP may be provoked through diagnostic mydriasis, possibly because of dispersion and sedimentation of PEX material and melanin in the chamber angle, sometimes causing acute open-angle glaucoma.

Proliferating and migrating corneal endothelial cells may additionally produce a pretrabecular connective tissue membrane containing PEX and other extracellular materials over the chamber angle. Although this might be a sporadic, end-stage phenomenon not related to the primary mechanism of glaucoma development, it may contribute to pressure rise. But the primary disease appears to be the underlying metabolic disturbance leading to the abnormal production of PEX material within the trabecular meshwork that, in turn, leads to all the succeeding alterations and glaucoma development.

Key Words
electron microscopy, juxtacanalicular tissue, morphometry, open-angle glaucoma, pseudoexfoliation syndrome, trabecular meshwork

References