Ultrahistochemical studies on tangential sections of the trabecular meshwork in normal and glaucomatous eyes

Elke Lütjen-Drecoll, Ryusuke Futa, and Johannes W. Rohen

The composition of the extracellular material of the cribriform meshwork was compared in five normal and 13 glaucomatous eyes of the same age group (58 to 70 years). Enzymatic digestion and histochemical methods applicable to electron microscopy were used. In both groups of eyes the ground substance was sensitive to chondroitinase ABC, and also the other methods showed no qualitative differences between the groups. In contrast, the fibrillar components of the extracellular material, which partly could be studied only after enzymatic treatment, showed qualitative differences between normal and glaucomatous eyes. In addition to the normal fibrous components, the glaucoma tissue contained large amounts of very fine fibrils. In seven eyes with end-stage glaucoma these fibrils filled the whole cribriform region. In these cases collagen fibers also appeared. Whether these were formed from the fine fibrils is not clear. (INVEST OPHTHALMOL VIS SCI 21:563-573, 1981.)

In normal eyes of elderly humans as well as in cases of chronic simple glaucoma the cribiform portion of the trabecular meshwork contains localized accumulations of extracellular material of three different types,1,2 the so-called "plaques." The main aim of this study is to analyze the composition of this material by means of various ultrahistochemical methods using sagittal and tangential sections through the inner wall region.

Material and methods

Five eyes enucleated because of posterior choroidal melanoma, seven eyes enucleated because of end-stage glaucoma, and six trabeculectomy specimens from patients with chronic simple glaucoma were examined. All patients were between 58 and 70 years of age.

From the enucleated eyes both sagittal and tangential2 sections were made; the trabeculectomy specimens were cut only in a tangential plane.

To identify the glycosaminoglycans, frozen sections (10 to 25 μm) of unfixed or briefly fixed specimens (30 min in 2.5% buffered glutaraldehyde) were incubated with Alcian blue and MgCl₂ according to the method of Scott and Dorling3 (Table I). Alcian blue 8Gx (No. 11-557-00; Fabrik Pasel, Frankfurt am Main) was used. The following incubation procedures were applied: (1) with Alcian blue (1% in 3% CH₃COOH solution) for 30 min at room temperature; (2) with 0.1% Alcian blue in Na-acetate buffer (pH 5.7) and 0.1M MgCl₂ for 24 hr at 4°C; (3) same as in (2), but with 0.3M, 0.8M, or 1.0M MgCl₂.

Other sections were incubated with the following enzymes (Table II):
1. Testicular hyaluronidase (No. 25118; Serva, Heidelberg) buffered with Sørensen's phosphate buffer to pH 5.6; incubation time 6 hr at 37°C.
2. Chondroitinase ABC (No. 32-021-1; Amano,
Fig. 1. For legend see facing page.
Table I. Differential staining of glycosaminoglycans by Alcian blue in MgCl₂ solution (Scott and Dorling⁶)

<table>
<thead>
<tr>
<th>Glycosaminoglycan</th>
<th>AB</th>
<th>AB 0.1</th>
<th>AB 0.3</th>
<th>AB 0.8</th>
<th>AB 1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyaluronic acid</td>
<td>+</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chondroitin sulfite</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Dermatan sulfite</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Heparan sulfite</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keratan sulfite</td>
<td></td>
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</tbody>
</table>

AB = Alcian blue 8GX; AB 0.1, 0.3, 0.8, 1.0 = Alcian blue 8GX with 0.1M, 0.3M, 0.8M, 1.0M MgCl₂; + = strong or moderate staining; (+) = weak staining; — = no staining.

Table II. Enzymatic determination of glycosaminoglycans

<table>
<thead>
<tr>
<th>Glycosaminoglycan</th>
<th>Hyaluronidase (testicular)</th>
<th>Chondroitin AC lyase</th>
<th>Chondroitin ABC lyase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyaluronic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chondroitin sulfite</td>
<td>(+)</td>
<td>+</td>
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<tr>
<td>Dermatan sulfite</td>
<td>-</td>
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<td>+</td>
</tr>
<tr>
<td>Heparan sulfite</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

Japan) buffered with Tris HCl buffer to pH 8.0; incubation time 2 hr at 37°C.
3. Chondroitinase ACII (No. 32-022-1; Amano, Japan); same buffer and incubation time as in (2).
4. Collagenase + chondroitinase ABC. Incubation with chondroitinase ABC as described in (2); afterwards *Clostridium histolyticum* collagenase (No. 17449; Serva), 0.1% in phosphate buffer, pH 7.3, was used; incubation time 18 hr at 37°C.
5. Pancreatic elastase (No. 20929; Serva) in carbonate buffer, pH 9.0, was used; incubation time 2 hr at 37°C.

In the enucleated eyes all methods described above with their buffer controls were applied. In trabeculectomy specimens only chondroitinase ABC or chondroitinase plus collagenase and the buffer control was employed. All sections incubated in the unfixed state were fixed after incubation with 2.5% buffered glutaraldehyde for 2 to 4 hr; the briefly fixed specimens were postfixed for 2 hr.

For optimal orientation the fixed sections were placed on a carbon-coated cover slip, dehydrated, and Epon-embedded in situ. The sections within the Epon sheet were then mounted for cutting and studied electronmicroscopically. No striking differences in the staining and digestion results were found between the unfixed and fixed specimens.

**Terminology.** The terminology used in this paper is explained in ref. 2.

**Results**

**Nonglaucomatous eyes**

*Trabecular lamellae.* Within the trabecular lamellae of the human trabecular meshwork exists a network consisting of elastic-like fibers. After being stained with Alcian blue for 30 min, the sheath of these fibers ap-
Fig. 2. Transmission electron micrographs of sections through the trabecular lamellae of an eye enucleated because of end-stage glaucoma at 79 years of age. A, So-called Gitterkollagen (curly or lattice collagen) consists of an electron-dense longitudinal and transverse portion with a periodicity of nearly 100 nm. B, After incubation with chondroitinase ABC and Clostridium collagenase, the longitudinal portion consists only of very fine fibrils that in places are lacking (arrowheads), but the transverse portion of the lattice remains unchanged (arrows).

peared nearly homogeneous (Fig. 1, A). After incubation with Alcian blue in the presence of 0.3M MgCl₂, a periodicity became visible within the previously homogeneous sheath. After incubation with Alcian blue in the presence of 0.3M MgCl₂ and digestion with testicular hyaluronidase or chondroitinase ABC, it became apparent that these periodic structures consisted of very fine longitudinal fibers and transverse, broad, electron-dense bands forming a lattice with a periodicity of about 50 nm adjacent to the central core and of
Fig. 3. Transmission electron micrographs of sections through the inner wall region of an eye enucleated because of choroidal melanoma (66 years). A, Sagittal section after incubation with Alcian blue for 30 min. The subendothelial region is filled with "plaque material" of types I, II, and III. S, Subendothelial region between inner wall endothelium (E) and the elastic-like cribriform network (EN). B, Oblique-section after incubation with chondroitinase AC. Immediately underneath the inner wall endothelium (E) some homogeneous material (asterisk) surrounding very fine fibrils remains. In the subendothelial region the homogeneous material of the types I and III plaques is dissolved. Only the connecting fibrils (F) are still visible.

about 100 nm more peripherally (Fig. 1, B).

To further analyze the periodic structures of the sheath, chondroitinases and collag-
genase were simultaneously applied. Digestion with these enzymes left the dark transverse bands completely unchanged, while part of the fine fibrils were removed. It is not clear whether the part removed was of the same nature as the part remaining.

After incubation with pancreatic elastase, the sheath and the dark components of the central core of the elastic-like fibers remained unchanged, whereas the light strands of the core had been digested (Fig. 1, C).

With the methods described above, the so-called Gitterkollagen (lattice-collagen, curly collagen, long-spacing collagen) of the uveal and corneoscleral trabeculae revealed the same fine structure and sensitivity to enzymes as the sheath of the quasi-elastic fibers. After digestion with chondroitinase ABC, the lattice also consisted of fine fibrillar components and dark transverse bandings. Digestion with chondroitinase ABC plus collagenase removed parts of the fine fibrillar components, while the dark periodic bands remained unchanged (Fig. 2, A and B).

The fiber material within the central core of the trabecular lamellae (elastic-like fibers, collagenous fibers, curly collagen) was embedded in a ground substance that was digestible with testicular hyaluronidase.

Cribriform meshwork. As seen in tangential sections, most of the previously described plaques in the cribriform meshwork underneath the first or second subendothelial cell layer consisted of the elastic-like network (cribriform plexus) with its sheaths ( plaque types II and III, Fig. 3, A).

The cribriform plexus had the same struc-
Fig. 4. Transmission electron micrographs of tangential sections through the corneoscleral trabecular meshwork of a trabeculectomy specimen of a glaucomatous eye. A, After incubation with Alcian blue for 30 min. The elastic-like fibers (EN) form broad, weblike plates (asterisk). B, After incubation with chondroitinase ABC. Many collagenous fibers are found adhering to the sheath of the elastic-like material. Most of these collagenous fibers are intermittently connected to each other by dark bands forming structures with a periodicity of 40 to 50 nm (arrows). EN, elastic-like fibers; asterisk, region of the weblike plates.

The type I homogeneous plaques located more superficially than II and III between inner wall endothelium and cribriform plexus could be dissolved by testicular hyaluronidase and by chondroitinase AC (Fig. 3, B). The remaining extracellular material of this region consisted principally of the “connecting fibrils” with their characteristic periodicity (Fig. 3, B). Only immediately underneath the inner wall endothelium was some homogeneous material left. This homoge-
Fig. 5. Transmission electron micrographs of tangential sections of trabeculectomy specimens after incubation with chondroitinase ABC and *Clostridium* collagenase. A, Section through the cribriform elastic-like network (chronic simple glaucoma, 78 years). Many fine fibrils (arrows) adhering to the elastic-like fiber network become visible. B, Section through the subendothelial region (chronic simple glaucoma, 57 years). Similar fine fibrils (arrows) as within the cribriform network are seen surrounding the connecting fibrils.

homogeneous material however was removed by chondroitinase ABC, which also digested dermatan sulfate. All that remained of this homogeneous material were a multitude of fine fibrils of unknown nature.

Glaucomatous eyes. After incubation with chondroitinase ABC, the elastic-like network in the trabecular lamellae looked the same as that in the nonglaucomatous eyes. However, in places where the elastic-like network formed broad weblike plates, large amounts of collagen fibers appeared after digestion with chondroitinase ABC. A large proportion of these fibers were bundled by dark bands.
Fig. 6. Transmission electron micrographs comparing a sagittal and a tangential section at the same level through the trabecular meshwork of an end-stage glaucomatous eye (57 years). A, Sagittal section through the inner wall of Schlemm’s canal (chondroitin ABC buffer control). The endothelial lining of Schlemm's canal (SC) as well as the other cellular elements are lacking because of the buffer incubation. The spaces within the corneoscleral trabecular meshwork appear empty. B, Tangential section after incubation with chondroitinase ABC. The areas within the cribriform elastic-like network (EN) are filled with collagenous fibers (arrows). No empty spaces are seen in this area.

showing a periodicity of 45 to 50 nm. This was especially so close to the sheath of the elastic-like fibers and in places where the bundles joined the sheaths (Fig. 4, A and B).

In the cribriform meshwork many fine fibrils adhering to the sheath of the cribriform plexus became visible after incubation with chondroitinase ABC + Clostridium collagenase (Fig. 5, A).

In the region between inner wall endothelium and the cribriform plexus, the homogeneous material (type I plaques) was chondroitinase ABC-digestible as in the nonglaucomatous eyes. In this region similar fibrils as described, adhering to the cribriform plexus, were found surrounding the connecting fibrils (Fig. 5, B). The nature of the fibrils described in the last two paragraphs is unknown. In our experience they are not found in nonglaucomatous eyes from the same age group.

In end-stage glaucoma, after incubation with chondroitinase ABC the meshes of the cribriform plexus were filled with collagenous fibers (Fig. 6, A and B). Collagenous fibers densely packed were also evident close to the connecting fibers, as well as immediately underneath the inner wall endothelium (Fig. 7, A).

In two out of seven cases of end-stage glaucoma we found some additional fibrous plaques within the subendothelial area that remained unchanged even after the standard incubation with chondroitinase ABC + collagenase (Fig. 7, B). The nature of these fibrous plaques could not be identified.

Discussion

Elastic-like fiber and its sheath. Normal elastic fibers are composed predominantly of elastin. The elastic-like fibers of the trabecular meshwork contain only a small amount of
elastase-digestible material, which may be responsible for the staining with elastin stains in light microscopy. The nature of the strongly osmiophilic material that forms the main portion of the fiber core could not be identified by the methods used in this study. The elastic-like fibers differ from normal elastic ones also by carrying a special sheath, the thickness of which can greatly exceed that of the core. The sheath consists of fibrils embedded in a ground substance. The fibrils are banded by osmiophilic material of an unknown nature forming structures with a periodicity of 50 to 100 nm. Some but not all of the fibrils are

Fig. 7. Transmission electron micrographs of tangential sections through two eyes enucleated because of end-stage glaucoma. A, Section through the inner wall of Schlemm’s canal of a 78-year-old eye. After incubation with chondroitinase ABC, clusters of fine fibrils (F) are visible immediately underneath the inner wall endothelium (E). The spaces between the connecting fibrils (CF) are filled with collagenous fibers (arrows). B, Section through the subendothelial region of a 79-year-old eye after incubation with chondroitinase and Clostridium collagenase. Clusters of a fibrous material of unknown nature are visible (arrows) between the connecting fibrils (CF).
digested by Clostridium collagenase. The osmiophilic bands are resistant to collagenase and chondroitinases. The ground substance is rich in glycosaminoglycans that can be totally digested by testicular hyaluronidase and chondroitinases. The composition of the collagenase-insensitive fibrils is unknown. As far as is known, Clostridium collagenase attacks all types of collagen.

The elastic-like fibers in the different layers of the trabecular meshwork are identical. 

Cribiform plexus and plaques. One to two cell layers below the inner wall endothelium, a network of elastic-like fibers has recently been described. In sagittal sections the fibers of this network, the cribiform plexus, can be cut in different ways: sometimes real cross-section containing sheath and core appears, sometimes only sheath is visible. In sagittal sections the more or less rounded profiles have been interpreted as plaques, according to previous nomenclature they would represent types II and III. Since in fact both these types simply are profiles of the cribiform plexus, the concept of plaque types II and III should be discarded. There remain the plaque type I, between the cribiform plexus and the inner wall endothelium. The digestion experiments indicate that this type consists of two different kinds. The one kind consists of chondroitinase AC or testicular hyaluronidase-digestible material surrounding the connecting fibers. The other kind of type I plaques immediately underneath the inner wall endothelium appears as bandlike deposits. The ground substance of these is completely digested by chondroitinase ABC but not by AC, indicating the presence of some dermatan sulfate. After removal of the ground substance a fine fibrillar material remains, whose nature is unknown. Thus the concept of type I plaques should also be dropped. It could be that the subendothelial material in fact is a basement membrane, but the presence of type IV collagen and basement membrane proteoglycans has not yet been established.

Chronic simple glaucoma. Electron microscopic studies of the cribiform meshwork in chronic simple glaucoma have utilized sagittal sections. The three types of “plaques” found were similar to those in normal eyes; differences observed were only quantitative. The digestion experiments in the present study show, however, that qualitative changes also exist. In the cribiform plexus the core and the sheath of the fibers are not different from those in the normal eye, but the sheath is covered by additional fibrils embedded in glycosaminoglycans. The fibrils of this material are distinct from those of the sheath proper. Also the connecting fibers between the cribiform plexus and the inner wall endothelium are covered by additional fibrils in a ground substance sensitive to chondroitinases and testicular hyaluronidase.

It is possible that the amount and also thickness of these fibrils increase with progression of the disease, because in all seven cases of end-stage glaucoma the entire cribiform region was filled with such fibrils and collagen fibers embedded in chondroitinase and hyaluronidase-sensitive glycosaminoglycans. It is possible that the collagen fibers are formed from the fibrils, but more work is needed to settle this question.

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REFERENCES


