Analysis of Newly Synthesized Bruch's Membrane Proteoglycans

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Bruch's membrane may provide a selective filtration barrier for nutrients coming from the choriocapillaris to the outer retina. Because proteoglycans have been shown to have structural and filtration properties in other tissues, we have been investigating the deposition of newly synthesized proteoglycans into Bruch's membrane and how these may be affected by aging and pathology. Proteoglycans deposited in human Bruch's membrane were metabolically labelled with $^{35}$SO$_4$ and $^3$H-glucosamine using a whole-eye organ culture system. Labeled proteoglycans were extracted from dissected Bruch's membranes with 4 M guanidine and isolated by ion-exchange column chromatography on DEAE cellulose using a linear salt gradient. These molecules were subsequently chromatographed on Sepharose CL-4B and glycosaminoglycan content characterized by enzymatic and chemical degradation. The elution profiles for proteoglycans remains relatively unchanged with age, although in eyes from donors over age 70 there is a small change in size distribution toward higher molecular weights. However, the proportions of newly synthesized glycosaminoglycans remain unchanged with age, being approximately 75% chondroitin sulfate/dermatan sulfate and 25% heparan sulfate. Bruch's membrane proteoglycans from donors with different retinal pathologies, however, exhibited an increased proportion of heparan sulfate. Considering the structural and filtration properties of proteoglycans, such alterations could result in abnormal functioning of Bruch's membrane that could ultimately affect the maintenance of the outer retina. Invest Ophthalmol Vis Sci 30:478-486, 1989

Bruch's membrane is a complex extracellular matrix located between the choriocapillaris and the retinal pigmented epithelium (RPE). In addition to acting as a support element and an attachment site for the pigmented epithelial cells, it also appears to provide a selective filtration barrier for nutrients carried to the eye by the choriocapillaris. It is composed of five layers (see ref. 1 for a detailed description). The central, elastic layer is a continuous meshwork of intersecting elastin fibers. The outermost layers on both sides have the ultrastructural appearance of typical basement membranes. Between the elastic layer and each basement membrane are collagen-containing layers. The inner and outer collagenous layers contain loosely arranged, interwoven collagen fibrils that apparently are continuous through the elastic layer. These fibrils react with cationic markers at regular intervals, suggesting an interaction with such polyanionic molecules as proteoglycans.2,3 This structure has been known to undergo a variety of morphologically and histochemically identifiable changes with age in man. There is at least a three-fold increase in the thickness of Bruch's membrane with age, primarily due to changes in the inner collagenous layer.5,6 Overall, the periphery appears to thicken more than the macula.5 Aging is also correlated with increases in alcian blue reactivity, PAS positivity and basophilia of Bruch's membrane.5,7 In addition, the elastic area is also subject to fragmentation, crystal formation and calcification—particularly in the region of the macula.5,6 Drusen formation is another characteristic of aging in man. These are small nodules on the surface of the RPE basement membrane which in many cases contain basement membrane molecules elaborated by those cells.10 Normally, drusen are small, are found primarily in the periphery, and do not appear to impair visual function. In some cases, however, such as in the maculopathy of aging, large drusen will form in the macula. These are sometimes associated with the...
breaching of the normally avascular Bruch's membrane by vessels and can seriously compromise central visual function.\textsuperscript{11}

Considering its strategic location between the outer retina and its primary source of nutrition, the choriocapillaris, alterations in Bruch's membrane structure and composition could influence the filtration properties of the structure and, ultimately, the normal maintenance of the RPE and outer retina. Proteoglycans, composed of glycosaminoglycans covalently linked to a core protein, are found to be associated with the filtration properties of some basement membranes.\textsuperscript{12-14} Removal of the charged groups from glomerular basement membranes, for example, affects the filtration properties of this structure.\textsuperscript{12-14} Alterations of Bruch's membrane proteoglycans might, likewise, be expected to influence its permeability. This paper describes the newly synthesized proteoglycans isolated from the human Bruch's membranes of normal donor eyes of different ages and compares these findings with alterations found in association with pathology.

Materials and Methods

Materials

Coon's modification of Ham's F-12 nutrient medium and linacolin, a sulfate-free antibiotic, were obtained from Gibco (Grand Island, NY); bovine albumin and lincomycin, a sulfate-free antibiotic, were obtained from GIBCO (Grand Island, NY); bovine serum was from Hyclone (Logan, UT); ascorbic acid and glutamine were from Sigma (St. Louis, MO); Tris (hydroxymethyl)-aminomethane (Tris), guanidine-HCl, cesium chloride, and urea (all ultra-pure grades) were from Bethesda Research Laboratories (Gaithersburg, MD). Stock solutions of 10 M urea were deionized before dilution with the mixed bed resin, AG 501-X8 (BioRad, Richmond, CA). All other chemicals were of reagent grade from various chemical supply houses. \textsuperscript{[35S]}-Na\textsubscript{2}SO\textsubscript{4} (carrier-free) was from ICN Biochemicals (Irvine, CA); D-[6-\textsuperscript{3}H]-glucosamine hydrochloride (40 Ci/mmol) was from Amersham (Arlington Heights, IL); Whatman DE-52 anion exchange resin was from Sigma. Papain was from Sigma. Tris (hydroxymethyl)-aminomethane (Tris), guanidine-HCl, cesium chloride, and urea (all ultra-pure grades) were from Bethesda Research Laboratories (Gaithersburg, MD).

Culture System

Bruch's membrane proteoglycans were labeled in a whole-eye organ culture system.\textsuperscript{16} Each eye, from which the sclera had been removed, was placed into a 50 ml sterile conical centrifuge tube (Corning, NY) with 4 ml of Coon's modified Ham's F-12 medium, containing 0.2 mM MgSO\textsubscript{4}, 0.5 mM MgCl\textsubscript{2}, and 50 mg/ml glucose, supplemented with 20% dialyzed fetal bovine serum, linacolin (0.5 mg/ml), and ascorbic acid (50 \textmu g/ml). Following a preincubation for 30 min to reduce intracellular levels of sulfate and glucose, the medium was replaced with 4 ml of fresh medium containing \textsuperscript{35}SO\textsubscript{4} (0.25 mCi/ml) and \textsuperscript{3}H-glucosamine (0.05 mCi/ml) and incubated for 5 hr at 37°C. The chorioretinal complex, dissected free of the sensory retina, was incubated overnight in ice-cold distilled water containing protease inhibitors (0.1 M 6-aminohexanoic acid and 5 mM benzamidine hydrochloride). By shaking the complex in the water after incubation, most of the RPE and some of the choroidal cells are removed and remaining choroid becomes fluffy. The choroidal connective tissue and vessels can be removed by gentle teasing and peeling with fine forceps under a dissecting scope resulting in a semitransparent membrane with a small amount of choriocapillaris (Fig. 1).

Ocular Material

The eyes used in this study were obtained from the International Medical Eye Bank, Inc. (Baltimore, MD), and the National Disease Research Interchange (Philadelphia, PA), in cooperation with the National Retinitis Pigmentosa Foundation Fighting Blindness. All eyes used in these studies were obtained less than 10 hr postmortem, with most eyes having postmortem times of 4–6 hr. Only eyes that had been enucleated and chilled within 3 hr postmortem were used in this study. Rapid enucleation and cooling in a moist chamber appears to be more important than actual postmortem time since identical results were obtained from fellow eyes enucleated 1 hr postmortem if one was cultured starting 2 hr postmortem and the other 24 hr postmortem (not shown). Normally, only one eye from each pair was cultured. The condition of eyes was assessed by examination of the fellow eye, which was used in other studies, as well as examination of the organ-cultured eye at the conclusion of incubation. Most eyes, particularly from donors over age 30, contained small, peripheral drusen. However, only those eyes which were free of gross pathology, such as large macular drusen or extreme pigmentary changes, were used in the aging studies. In most cases, labeled Bruch's membranes from two to five age-matched normal human eyes were pooled and used for analyses.

Extraction and Isolation of Proteoglycans

Proteoglycans were extracted from Bruch's membranes with 4 M guanidine hydrochloride in 0.5 M sodium acetate, pH 5.8, containing the protease inhibitors 10 mM EDTA, 0.1 M 6-aminohexanoic acid, and 5 mM benzamidine hydrochloride.\textsuperscript{16-18} Extracts
were dialyzed against DEAE starting buffer (8 M urea, 50 mM Tris-HCl, pH 6.8) and chromatographed on a column of DEAE cellulose (1.6 x 10 cm) equilibrated in the same buffer. A linear salt gradient (0-1 M NaCl; 150 ml total volume) was used to elute bound material. Fractions (2.5 ml) were collected, and 0.2 ml aliquots of alternate fractions were assayed for radioactivity by liquid scintillation counting (Beckman (Fullerton, CA) MP scintillation fluid; Beckman LS 9800 counter programmed for spillover and dpm determinations). The elution position of proteoglycans was indicated by coincidence of the $^{35}$S and tritium peaks. $^{35}$S-containing fractions were pooled, dialyzed against distilled water and lyophilized.

Characterization of Proteoglycans

Lyophilized material was chromatographed on a column of Sepharose CL-4B (1.6 x 165 cm) in the presence of 4 M guanidine-HCl, 10 mM Tris-HCl, pH 7.0, plus protease inhibitors. In some cases, sulfated material was chromatographed on a column of Sepharose CL-6B (1.6 x 80 cm) both before and after treatment with papain, in order to establish the proteoglycan nature of these molecules.

Glycosaminoglycans were obtained by digestion of core protein with papain (1 mg/ml in 1.0 M sodium acetate buffer, pH 5.0, with 0.05 M EDTA and 1 mM cysteine, overnight at 52°C). Sulfated glycosaminoglycans were isolated from the digest by ion exchange on a small DEAE column (1 x 5 cm) and characterized by chromatography in 4 M guanidine-HCl on a column of Sephadex G-50 (1 x 50 cm) both before and after treatment with either chondroitinase ABC or nitrous acid. Glycosaminoglycans from unlabelled tissue were identified by cellulose acetate electrophoresis in 0.1 M barium acetate (pH 8.1; 150 V, 60 min) with and without the chondroitinase and nitrous acid treatments. The glycosaminoglycans were visualized by staining with 0.5% Alcian blue 8-GX followed by destaining in 2% acetic acid.

Cesium Chloride Density Gradient Centrifugation

Guanidine extracts of pooled, labeled Bruch’s membranes were prepared for ultracentrifugation using associative followed by dissociative conditions. Standard procedures for associative conditions were modified because a precipitate formed when high densities of CsCl were used with low guanidine concentrations. In this system, the guanidine concentration of extracts was reduced to 0.75 M by dialysis and the density of the solution was adjusted to 1.45 gm/ml by the addition of solid CsCl. Gradients were generated by centrifugation in an SW 50.1 rotor (Beckman) at 132,000 g for 68 hr at 10°C. Tubes were sliced into four fractions (A1-A4) and assayed for radioactivity. For dissociative conditions, the A1 fraction (which had the highest density and the most $^{35}$SO$_4^-$) was adjusted to 4 M guanidine; CsCl was added to obtain a density of 1.45 gm/ml. Centrifugation was as above and four fractions, designated A1-D1, A1-D2, A1-D3, and A1-D4, were collected and assayed for radioactivity.

Results

Separation of Newly Synthesized Components in Bruch’s Membrane

Eyes were labeled with $^{35}$SO$_4^-$ and $^3$H-glucosamine in whole-eye organ culture for 5 hr. Dissected Bruch’s membranes were extracted with guanidine and the extracts fractionated by ion-exchange chromatography to separate $^3$H-labeled glycoproteins from $^{35}$S-$^3$H-labeled proteoglycans (Fig. 2). Sulfated material eluted with approximately 0.45 M NaCl and there was no apparent change in elution positions of the peaks with age. There was some variation in the...
size of peaks (ie, degree of incorporation) between eyes; decreased incorporation appeared to be related as much to postmortem time as to the age of the donor. However, differences in the degree of total incorporation did not affect the outcome of the analytical results.

Characterization of Bruch’s Membrane Proteoglycans

$^{35}$SO$_4$-containing fractions from the ion-exchange column were pooled and chromatographed on a column of Sepharose CL-4B. Figure 3 shows the CL-4B profiles of proteoglycans extracted from Bruch’s membranes of different age groups. Normal eyes from infants to donors in their sixties had similar elution profiles. There was usually a small peak at the void, but the major portion of the labeled material eluted as a broad peak. With advanced age, there was some alteration in the profile, with the tritium peak shifting toward a higher molecular weight. The shift of the tritium profile can vary from a broadening of the tritium peak, to either a shoulder on its leading edge, a small but distinct peak near fraction 60, or the large peak shown in the top panel of Figure 3. This peak appears in the region indicated by the arrow in the second panel of Figure 3, a small peak often found in preparations from donors over age 40.

The sulfated material from the major peak was subsequently chromatographed on a column of CL-6B both before and after papain treatment. Samples from all age groups showed a shift toward a lower molecular weight when treated with papain (Fig. 4), indicating that the labeled glycosaminoglycans are linked to a protein core. The elution position of the digested sample (ie, the glycosaminoglycans) was the same regardless of donor age.

Glycosaminoglycans from labeled Bruch’s membranes were analyzed by chromatography on columns of Sephadex G-50 both before and after treatment with either nitrous acid or chondroitinase ABC. Chondroitinase ABC digested 75-80% of the labeled material (Table 1). This material was predominantly chondroitin sulfate, since 68-72% of the total labeled sample was sensitive to chondroitinase AC. The remainder of the labeled material (20-25%) was heparan sulfate. There appeared to be no alteration of this ratio with aging. The results with labeled samples is in contrast to the findings when total Bruch’s membrane glycosaminoglycans were analyzed from ten pooled, unlabeled Bruch’s membranes (Table 1). In the latter case, heparan sulfate was the predominant glycosaminoglycan. This variation in ratios between newly synthesized and accumulated glycosaminoglycans probably reflects differences in turn-over rates between the heparan sulfate and chondroitin sulfate/dermatan sulfate species.

Extracts of pooled, radiolabeled Bruch’s membranes were also analyzed by cesium chloride density gradient centrifugation using both associative and dissociative conditions. These procedures would be expected to give some information regarding the buoyant density of the proteoglycans as well as whether there are any interactions between these molecules and other components of Bruch’s membrane. When subjected to ultracentrifugation using associative conditions (0.75 M guanidine-HCl), approximately 90% of the $^{35}$S and 25% of the tritium from the total extract were contained in the A1
Fig. 3. Sepharose CL-4B column chromatography. The pooled fractions from the DEAE column were concentrated and chromatographed on a column of Sepharose CL-4B in the presence of 4 M guanidine. Shown here are representative chromatograms from a variety of ages. These profiles represent pools of Bruch's membranes from donors with the indicated mean ages (77 years: five membranes; 45 years: two membranes; 31 years: three membranes; 9 years: two membranes). All age groups were run at least two times. Samples from intervening decades were also run with similar results (not shown). Profiles from donors under age 70 all had a similar appearance; over age 70, there was some variation primarily with relation to the tritium peak (see text). The arrow in the second panel indicates a small peak consistently found in profiles from donors over age 40. It is in this region that the differences become most apparent in aged eyes. Fractions between 50 and 100 were normally pooled for further characterization. For purposes of comparison, several well characterized proteoglycans elute at approximately the following positions: fraction 55—EHS-sarcoma basement membrane (heparan sulfate) proteoglycan (Mr = 5-600,000); fraction 78—corneal chondroitin sulfate proteoglycan (Mr = 150,000); V_2: fraction 43; V_1: fraction 120 (• = ^35SO_4; O = ^3H-glucosamine).

(highest density) fraction (Fig. 5, top panel). The A1 fraction was then adjusted to dissociative conditions (4 M guanidine-HCl) and recentrifuged. Under these conditions, approximately 30% of the tritium originally found in the A1 fraction was now in the fraction with the lowest buoyant density (A1-D4, Fig. 5, lower panel) while 75% of the ^35SO_4 remained in the most dense fractions (>1.5 gm/ml). This suggests that low buoyant density glycoproteins are associating with the high buoyant density proteoglycans. Similar findings were observed in four different series using pools of various ages ranging from infancy through the 9th decade.

Bruch's Membrane Proteoglycans and Pathology

During the course of the studies, a few eyes with retinal pathology were obtained and studied. We have already reported our findings on several cases of dominant RP. In these cases, there is a shift toward a higher molecular weight and an altered proportion of glycosaminoglycans (Table 2). On the other hand,
in eyes with large macular drusen and pigmentary changes, conditions often associated with age-related maculopathy, there is only a slight shift in size to a higher molecular weight similar to that found in aging (not shown). However, as with RP, there was also a change in the proportion of glycosaminoglycans (Table 2). In diabetic donors with little or no retinopathy as assessed by visual examination, the elution position of the $^{35}S$SO$_4$ was similar to that of proteoglycans from normal donors, although the tritium appeared shifted slightly towards a lower molecular weight (Fig. 6). However, in diabetic eyes with neovascularization, tortuous vessels, evidence of hemorrhage, and hard and soft exudates, the shift in the tritium peak is more apparent and both the tritium and $^{35}S$SO$_4$ peaks are much broader than in the diabetic samples without retinopathy. There is also an increased proportion of material eluting at the void volume (Fig. 6). The proportion of heparan sulfate in diabetic eyes with retinopathy is slightly elevated over that of the diabetic eyes without retinopathy (Table 2), although this is probably not significant. These findings suggest that the normal complement of proteoglycans is altered in the cases of retinal pathology examined here.

**Discussion**

The strategic location of Bruch's membrane between the outer retina-RPE complex and their primary source of nutrition, the choriocapillaris, has suggested that this structure provides a filtration barrier for nutrients reaching the retina via the choriocapillaris. It has been demonstrated that Bruch's membrane/choroid preparations have a net negative charge, which would allow them to act as a molecular barrier. This charge is likely due to the presence of proteoglycans throughout Bruch's membrane. While the idea of Bruch's membrane as a filtration unit is not a new one, only recently have studies been initiated to characterize the molecules that may be responsible for this function.

**Table 1. Accumulated and newly synthesized Bruch's membrane glycosaminoglycans**

<table>
<thead>
<tr>
<th>Glycosaminoglycan</th>
<th>Accumulated*</th>
<th>Newly synthesized†</th>
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<tbody>
<tr>
<td>Chondroitin/dermatan</td>
<td>42%</td>
<td>75-80%</td>
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<tr>
<td>Heparan sulfate</td>
<td>58%</td>
<td>20-25%</td>
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* Because of availability, this determination was made on pooled Bruch's membranes (10) from donors aged 60-75. Glycosaminoglycans from unlabeled tissue were determined by cellulose acetate electrophoresis before and after treatment with chondroitinase ABC and nitrous acid.

† Analysis of labeled material was by Sephadex G-50 column chromatography before and after chondroitinase ABC and nitrous acid treatment. Represents the range of results from eight determinations of pools of different age groups (see Fig. 3). Results between groups, regardless of age, did not vary by more than 10%.

In this study, we have demonstrated the biosynthesis and deposition of proteoglycans into human Bruch's membrane. The newly synthesized proteoglycans are predominantly of high buoyant density and eluted essentially as one major peak from a column of Sepharose CL-4B. Compared to the elution positions of other proteoglycans, this material contains molecules having an estimated molecular weight range of between 150,000 and 200,000, which is slightly larger than that found for similar preparations from cynomolgus monkeys. There appears to be an increase in the overall size of the proteoglycan with advanced age. Since the size of the GAG chains released by digestion with papain does not appear to be altered with age, the increase in proteoglycan size may reflect an inability of the cells to normally process the core protein. In some cases, but primarily in
Table 2. Altered glycosaminoglycan synthesis associated with retinal pathology

<table>
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<tr>
<th>Sample</th>
<th>Chondroitin sulfate/dermatan sulfate</th>
<th>Heparan sulfate</th>
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<tbody>
<tr>
<td>Normal*</td>
<td>77%</td>
<td>23%</td>
</tr>
<tr>
<td>Retinitis pigmentosa†</td>
<td>45%</td>
<td>55%</td>
</tr>
<tr>
<td>Macular drusen/pigmentary change‡</td>
<td>45%</td>
<td>55%</td>
</tr>
<tr>
<td>Diabetes§ without retinopathy</td>
<td>52%</td>
<td>48%</td>
</tr>
<tr>
<td>with retinopathy</td>
<td>36%</td>
<td>64%</td>
</tr>
</tbody>
</table>

* Mean from three sets of pooled, labeled Bruch's membranes which did not vary by more than 10%. Ages of donors correspond to the ages of the donors with the pathologies listed below.
† From two donors with dominant RP, ages 64 and 69; adapted from Hewitt and Newsome, 1985 (with permission).
‡ From pooled Bruch's membranes from donors aged 72, 73 and 78.
§ From pooled Bruch's membranes from donors aged 65, 77, 78 and 82 and aged 63 and 65 (diabetic without retinopathy) and from donors aged 62, 66, 72 and 77 (diabetic with retinopathy).

eyes from older donors and from samples with increased postmortem time, some material also eluted at the void volume. It is possible that the material in this peak represents precursor molecules, and that processing to the normal size also is slowed as a function of age or, perhaps, postmortem time.

Two types of glycosaminoglycans are found associated with the proteoglycans. Chondroitin sulfate/dermatan sulfate represents approximately 75–80% of the newly synthesized Bruch's membrane proteoglycans. This is mostly in the form of chondroitin sulfate since only slightly less material is digested with chondroitinase AC. The remainder of the newly synthesized proteoglycans is heparan sulfate. Whether these are linked to the same core protein, as has been shown for a mammary epithelium plasma membrane proteoglycan, or exist as distinct heparan sulfate and chondroitin sulfate/dermatan sulfate proteoglycans remains to be clarified. However, it is likely that they are on separate molecules because of the differences in distribution detected by EM histochemistry (see below). The proportions of glycosaminoglycans found in the newly synthesized proteoglycans are in contrast to results found with other types of basement membrane-synthesizing cells, which, under similar culture conditions, synthesize more heparan sulfate than chondroitin sulfate/dermatan sulfate. However, analyses of proteoglycans that have accumulated with age in Bruch's membrane and were extracted from pooled, unlabeled human (this study) and monkey material are more in keeping with that expected for basement membranes in that heparan sulfate is predominant.

The reasons for the differences between the results using radiolabeled and unlabeled material is not clear. This could be an artifact of the culture system. For example, the labeled precursors might not be gaining access to the RPE cells through the choroid–Bruch's membrane complex, so that the contribution to Bruch's membrane by the RPE is not detectable. This is unlikely, however, since macromolecules within the retina are also labeled in these cultures (unpublished observation). Alternatively, alterations in glycosaminoglycan content could be related to postmortem time. This would not appear to be the case since similar results were obtained in studies on cynomolgus monkey Bruch's membrane in which the eyes were cultured shortly after enucleation. Also, in the current study, similar results were obtained when fellow eyes, which had been enucleated less than 1 hr postmortem, were cultured starting at 2 hours and 24 hr postmortem. A more likely possibility is that the heparan sulfate proteoglycans turn over at a slower rate than the chondroitin sulfate/derma-
It is thought that basal lamina components have a longer half-life than components of other connective tissues. Consequently, the heparan sulfate proteoglycan, which one might expect to be within the basal lamina portions of Bruch's membrane, could turn over more slowly than the chondroitin sulfate proteoglycan, which one might expect to predominate within the inner and outer collagenous zones of Bruch's membrane. However, while this localization appears to be the case, the questions of both turnover and precursor-product relationship will be difficult to test since extended pulse-chase studies are precluded by time contraints on the whole-eye organ culture system.

An additional drawback to this culture system is the inability to determine the cellular origin of each proteoglycan. In this regard, it is likely that cell culture will be beneficial. For example, findings in this laboratory on RPE cell cultures have shown that the proteoglycan deposited on the culture dish by RPE cells is predominantly (greater than 85%) heparan sulfate. This would suggest that the RPE cells contribute predominantly heparan sulfate to Bruch's membrane. Several elegant electron microscope histochemical studies would support this contention. In these studies, anionic sites were visualized along both of Bruch's membrane's basal laminae and also as a network within the collagenous zones. Differential treatments with enzymes demonstrated that chondroitin sulfate/dermatan sulfate was localized within the collagenous layers and along the basal lamina of the choriocapillaris. Heparan sulfate was associated primarily with the RPE basement membrane. Similarly, immunofluorescence studies using antibodies directed against a basement membrane heparan sulfate proteoglycan react with the basal lamina portions of human and chick Bruch's membrane. Antibodies against the brain-derived chondroitin sulfate proteoglycan will also react with murine Bruch's membrane, being primarily associated with the collagenous zones.

There is little doubt that Bruch's membrane is composed of molecules that are synthesized by both the RPE and cells in the choriocapillaris. The complex nature of Bruch's membrane as well as its dual source, make the results obtained from pathological Bruch's membranes difficult to interpret (ref. 18 and current study), since an alteration in production of proteoglycans could reflect an abnormality in either the RPE or the choriocapillaris. However, the situation with Bruch's membrane is still more complicated because of evidence that the RPE exerts a supportive influence on the choriocapillaris. Destruction of RPE cells in vivo by either chemical means or patholgy, such as in retinitis pigmentosa, leads to atrophy and, perhaps, altered proteoglycan synthesis in the adjacent choriocapillaris. Consequently, defects in either or both the choriocapillaris and RPE could lead to the results found in the pathologies discussed in this paper.

However, it is interesting that all of the retinal pathologies studied exhibited an increased proportion of heparan sulfate. While the number of each type of pathologic sample was limited, these altered GAG proportions compared to the consistently reproducible findings for the large number of normal eyes would suggest that the differences are not due simply to chance. Considering the similar results from eyes having pathologies with different manifestations (eg, RP, macular drusen, diabetic retinopathy) and with varying degrees of retinopathy (eg, diabetic with unremarkable fundus appearance vis-à-vis with neovascularization or other signs of proliferative retinopathy), these differences could reflect a common underlying secondary affect on proteoglycan synthesis rather than a primary defect. It is possible that each condition could trigger an alteration in the turnover of either basement membrane or collagenous zone proteoglycans. Acute metabolic labeling would detect such differences that might be lost if only accumulated materials were analyzed. For example, a recent study using spontaneous and drug-induced diabetic rats indicated a reduction of anionic sites in Bruch's membrane. Such a result could even occur under conditions of increased rate of synthesis if accompanied by a net increase in degradation. The apparently lower molecular weight in diabetic samples might support such a mechanism. On the other hand, the higher apparent molecular weight seen in RP and age-related maculopathy would suggest an alternate mechanism, perhaps an inability to process precursor molecules normally.

Regardless of the etiology, it is clear that proteoglycan synthesis is altered in certain retinal pathologies. Bruch's membrane is unique, because of both its complex structural organization and the role that the chondroitin sulfate/dermatan sulfate proteoglycans are likely to have in filtration, a function normally associated with heparan sulfate proteoglycans. Consequently, alterations in proteoglycan synthesis and deposition into Bruch's membrane as well as in their supramolecular interactions could adversely influence the health of the photoreceptors by affecting the flow of nutrients from the choriocapillaris to the outer retina.

Key words: Bruch's membrane, proteoglycans, glycosaminoglycans, extracellular matrix, retinal pathology
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