The Effect of Age on the Macromolecular Permeability of Human Bruch's Membrane

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Purpose. To determine whether age-related changes in Bruch's membrane affect its permeability to macromolecules. Such changes have been postulated to underlie some pathologic manifestations of age-related macular degeneration.

Methods. Bruch's membrane preparations were isolated from the macular region of donated human eyes of differing age and mounted in a modified Ussing chamber. Permeability to macromolecules was assessed by simultaneously placing a physiological concentration of serum proteins adjacent to the choroidal margin of the membrane preparation and a saline solution adjacent to the retinal pigment epithelial basement membrane. After 24 hours, the protein content of the saline solution was measured by standard assay and permeability calculated as the quantity of protein traversing the membrane preparation per unit area. The spectrum of proteins able to cross the membrane was assessed by subjecting the diffusate proteins to electrophoretic separation and the resultant gel to scanning densitometry.

Results. The permeability of Bruch's membrane to serum proteins decreased 10-fold from the first to the ninth decade of life, and on regression analysis this decline exhibited a linear relationship with donor age ($P < 0.0005$). Membrane preparations from young donors were permeable to proteins with a molecular weight in excess of 200 kDa, but with increasing age, the membrane progressively impeded the passage of high-molecular-weight entities. Even so, elderly membranes were still permeable to macromolecules with molecular weights in excess of 100 kDa. Results from the oldest preparation studied suggest that by the ninth decade, the membrane may selectively impede the flux of specific proteins, based on a criterion other than molecular weight.

Conclusions. The results imply that, with increasing age, the capacity of Bruch's membrane to facilitate macromolecular exchange between the choroidal and the retinal pigment epithelial compartments is reduced. (*Invest Ophthalmol Vis Sci.* 2001;42:2970–2975)

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Age-related macular degeneration (ARMD) is the leading cause of irreversible blindness in Europe, North America, and other industrialized regions.[1–3] Evidence suggests that the pathologic manifestations of ARMD are the end stage of a lifelong continuum of change, influenced by both genetic predisposition and environmental factors, and focused at the level of Bruch's membrane.[4–7] The study of age-related changes are likely to be helpful in understanding ARMD. Morphologic and biochemical studies have demonstrated considerable senescent alteration to Bruch's membrane, typified by increased thickness and the progressive accumulation of deposits within the inner layers. A number of hypotheses, each with appropriate supporting evidence, have been proposed to explain these changes. These may not be mutually exclusive and include altered remodeling of the membrane, an immune response, dysfunctional choriocapillary endothelium and the deposition of incompletely digested waste material emanating from a dysfunctional retinal pigment epithelium (RPE).[8–10] It has been suggested that changes to Bruch's membrane progressively impede the net outflow of water from the retina and metabolic exchange between the choriocapillaris and the RPE.[4,11,12] We, and others, have demonstrated a senescent decline in the hydraulic conductivity (permeability) of both macular and peripheral Bruch's membrane.[13,14] In addition, we have ascertained that the presence of lipid deposits plays a small, yet statistically significant, role in this reduction in hydraulic conductivity.[14] These findings have been confirmed by others.[15] Lipids are but one component of the complex composition of deposits found in Bruch's membrane. Other components include collagens, adhesion molecules, lipoproteins, and advanced glycosylated end products.[4–6,8,10]

The effects of senescence on metabolic flux across Bruch's membrane have yet to be ascertained. However, evidence from two groups of studies suggests that macromolecular flux may be impaired with increasing age. First, fluorescein angiography has revealed that elderly patients with good visual acuity may have areas of the fundus that exhibit delayed choroidal perfusion.[16] Functional assessment showed that such areas exhibit elevated dark adaptation thresholds and a pattern of change similar to that observed in vitamin A deficiency.[17,18] Second, similar findings were found in mildly affected persons with the inherited disease Sorsby fundus dystrophy.[19] This disease is typified by midlife onset of loss of central vision, subretinal disease with similarities to ARMD, and the presence of a 30-μm thick lipid-rich deposit within Bruch's membrane.[20,21] The conclusion from both sets of studies was that functional loss is due to diffuse deposits in Bruch's membrane that act as a barrier to metabolic exchange.

This concept has been further supported by clinical trials that have demonstrated that dietary supplementation with vitamin A can reverse the elevation in scotopic thresholds in the early stages of Sorsby fundus dystrophy.[22] It is assumed that this occurs by raising the systemic concentration of vitamin A and thus the gradient that drives flux, leading to an increase in vitamin A transport across Bruch's membrane and improvement in photoreceptor function. Unfortunately, the high doses of vitamin A required to alleviate blindness in these studies were too toxic for long-term use. Vitamin A (retinol) moves

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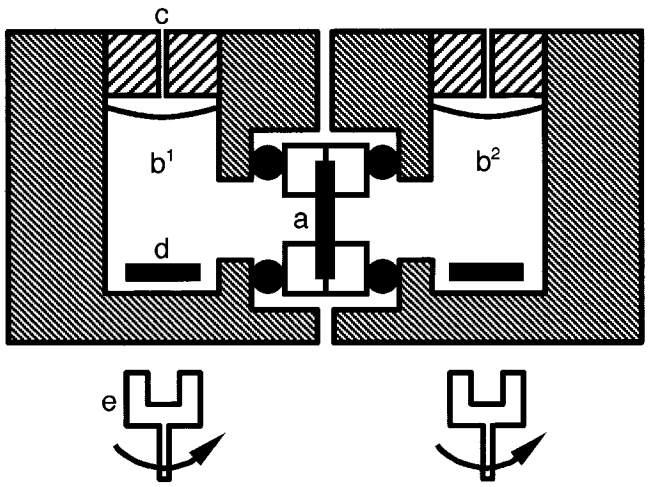

FIGURE 1. The modified Ussing chamber used in the determination of permeability. A Bruch's membrane preparation (a) was mounted so that it formed the only barrier between two compartments (b^1, b^2). A solution of serum proteins was placed in b^1 and an equal volume of saline in b^2. The compartments were covered with vented caps (c) and the contents agitated by magnetic bars (d) rotated by an external magnetic stirrer (e). After 24 hours, the protein content of the saline solution was measured.

across Bruch's membrane bound to a carrier, retinoid-binding protein (RBP), and an associated protein, transthyretin.[23] This complex has a combined molecular weight of 75 kDa. Therefore, for good vision to be maintained, Bruch's membrane has to be readily permeable to entities of considerable mass and size. There is no information on the macromolecular permeability of human Bruch's membrane or its variation throughout life.

Using human serum as a tracer, the purpose of this study was to measure the macromolecular permeability of human macular Bruch's membrane, the magnitude of the molecular weight limit imposed on macromolecular transport, and the effect of age on these parameters.

METHODS

Donor Eyes

The University of Auckland Human Subjects Ethics Committee granted ethical approval for this study, and all procedures were in accordance with the Declaration of Helsinki. Seventeen human eyes with no known ophthalmic disease were obtained from the New Zealand National Eye Bank, where the next of kin had given permission for donor tissue to be used for research. Corneas had previously been removed from all eyes for use in transplantation surgery. Donor age ranged from 9 to 85 years, and eyes reached the laboratory with a mean delay of 36.45 ± 23.49 hours from time of death. Little information was available concerning the eye history of the donors. None of the eyes used in this study had undergone cataract surgery or other invasive procedures, and on macroscopic examination of the retina during dissection, no manifestations of overt senescence or other disease was discernible.

Permeability Measurement

A Bruch's membrane preparation was isolated from the macular region of each eye and mounted in a modified Ussing chamber (Fig. 1), in a manner identical with that described for measuring hydraulic conductivity.[13]

Once mounted in the chamber, a 4-mm-diameter area of the preparation (Fig. 1, a) formed the only barrier between two identical compartments (b^1 and b^2). Human serum (1.25 ml) of concentration 90.17 ± 1.81 mg/ml was placed in the compartment adjacent to the choroidal surface of the preparation (b^1). Simultaneously, the same volume of 0.855% sodium chloride solution was placed in the compartment adjacent to the RPE basement membrane (b^2). This concentration of sodium chloride was of equal osmolarity to the serum. The compartments were covered with caps (c) that contained vents to ensure no pressure gradients were introduced during fitting. The contents of each compartment were agitated by a small magnetic bar (d) rotated at 150 rpm by a magnetic stirrer (e). Both the chamber and the solutions were pre-equilibrated to 22 ± 1°C and maintained at this temperature. After 24 hours, the amount of serum flux through the membrane preparation was determined by assaying the protein content of the sodium chloride solution in compartment b^2. This was accomplished with a commercially available protein assay kit (Sigma, St. Louis, MO). Briefly, this kit exploits the fact that proteins reduce alkaline Cu (II) to Cu (I) in a concentration-dependent manner, by using a chromogenic reagent, bicinchoninic acid, that combines with Cu (I) to form a purple complex. This complex was detected and quantified using a spectrophotometer, as it possesses an absorbance maximum at 562 nm. Bovine serum albumin was used as a standard. Permeability was calculated as the quantity of serum protein traversing the membrane preparation per unit area in 24 hours.

Serum

Strict control was undertaken to ensure that all the serum used in this study was, as far as possible, identical. A single frozen batch was purchased, thawed on arrival at the laboratory, and then divided into aliquots before being refrozen and stored at −40°C. Individual aliquots were thawed and used as required, with any excess being discarded. Thus, all serum underwent the same minimal freeze–thaw regimen. No storage-related changes were observed in the composition of the serum during the duration of this study.

Protein Separation

To ascertain the molecular weight of macromolecules capable of traversing selected Bruch's membrane preparations, diffusate proteins in the sodium chloride solution underwent a fourfold concentration by microcentrifugation and subsequent separation by polyacrylamide gel electrophoresis. Some of the solution in compartment b^2 (400 μl) was added to a microconcentrator tube (Millipore, Bedford, MA) and centrifuged at 2000*g* at 4°C for 30 minutes. The microconcentrator tubes possessed low protein-binding characteristics and retained entities with a molecular weight higher than 5 kDa. The filtrate containing proteins of weight lower than this mass was discarded. The separation of proteins in the filtrate was undertaken by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, according to the method of Laemmli.[24] Filtrate protein (40 μg) was separated on a 7.5% acrylamide gel, along with a range of molecular weight marker proteins and a serum sample as a standard. Gels were initially stained with Coomassie brilliant blue R-250 and visualization subsequently enhanced by silver staining using a commercially available kit (Sigma). Gel images were digitally scanned and subjected to densitometric analysis on computer (NIH Image, ver. 1.62; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD; available at http://www.ncbi.nlm.nih.gov).

Statistical Analysis

Because of the nature and duration of the testing, it was not possible to repeat measures of permeability. Through pilot studies we were aware that prolonged incubation (72 hours) with physiological solutions could result in microbial growth on the inner and outer surfaces of an isolated Bruch's membrane preparation. We wanted to avoid this situation and therefore used the shortest incubation period that would allow the study to be successfully undertaken. All assays were performed in duplicate or triplicate. Data are expressed as mean ± SD. Regression analysis on the senescent profile of permeability was un-

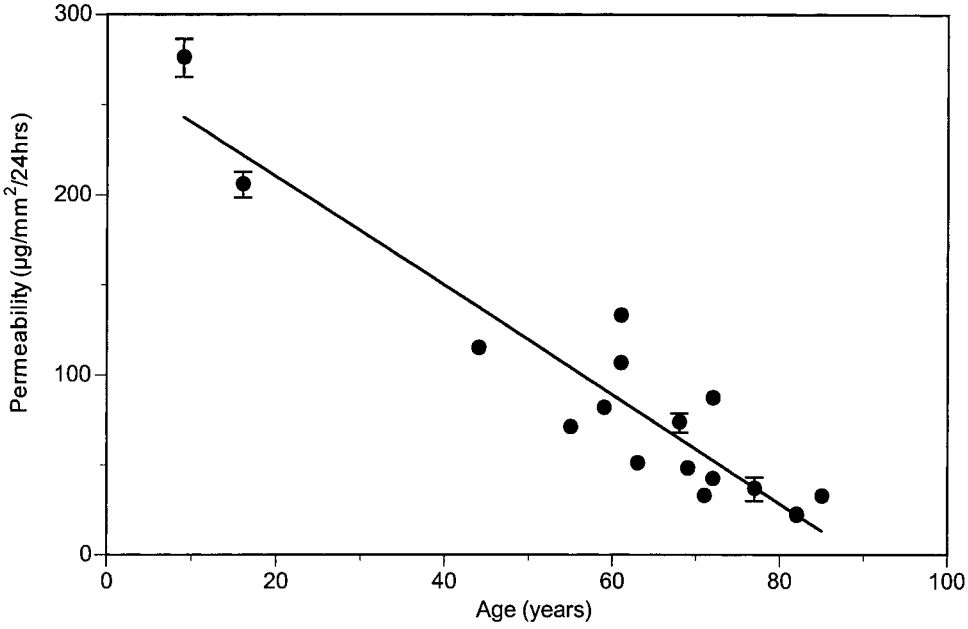

FIGURE 2. The correlation between donor age and the macromolecular permeability of Bruch's membrane. Each point represents the mean ± SD of duplicate assays to determine the quantity of serum protein able to diffuse through a unit area of isolated Bruch's membrane preparation in 24 hours. Error bars are shown only where the SD falls outside the area encompassed by the symbol. On first-order regression analysis, permeability = $-3.027 \times$ age + 270.545 ($R^2 = 0.881$, $P < 0.0005$).

dertaken on computer (Deltagraph, ver. 4.01; Deltapoint/SPSS, Chicago, IL). The significance of correlation analysis was determined by Student's *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

Permeability

The permeability of Bruch's membrane for serum proteins was found to decrease throughout life (Fig. 2). The magnitude of the decrease was 10-fold, from approximately 250 $\mu g/mm^2$ per 24 hours in the first two decades of life to 25 $\mu g/mm^2$ per 24 hours in the ninth decade. Despite the lack of data from the third and fourth decades, regression analysis demonstrated a significant linear relationship between permeability and age ($R^2 = 0.881$, $P < 0.0005$). There was no correlation between the postmortem delay before testing and either permeability ($P > 0.1$) or donor age ($P > 0.1$).

The quantity of protein to traverse a Bruch's membrane preparation during the 24-hour incubation period was never more than 3.5% of the total protein available. For most of the membranes, this value was below 1.5%. Therefore, there was only negligible reduction in the concentration gradient across the membrane during testing. However, this reduction was largest in preparations from younger donors because of their greater permeability. As such, measures of permeability in these specimens may be marginally underestimated when compared with those from older donors.

Protein Separation

Typical electrophoretic profiles of the proteins able to traverse Bruch's membranes of differing age are shown in Figure 3. Up to 100 kDa, all profiles appeared similar and comparable to the serum standard, indicating that the membrane presented only a nonselective impediment to the flux of macromolecules less than this mass. This range encompassed most of the serum proteins.

Above this range, it was evident that serum proteins with a molecular weight up to and higher than 200 kDa were able to traverse the membrane. However, when compared with the serum control, it became manifest that the flux of proteins of very high molecular weight was inhibited by the membrane preparation.

Age-related differences in profiles, most readily discernible on densitometric analysis, were apparent for proteins higher than 100 kDa (Fig. 4). The profiles from young Bruch's membrane contained four distinct protein bands with molecular weight greater than 120 kDa (Fig. 4, asterisks). With increasing age, the quantity of each of these proteins gradually decreased, indicating a progressive senescent impediment of their flux by the membrane. However, these proteins constitute only a small fraction of the total serum compliment, and their restriction by the membrane cannot account for the massive reduction in permeability seen in Figure 2.

In the membrane preparation from an 85-year-old donor, there appeared to be selective restriction of flux (Fig. 4). In the density profile of the diffusate of this preparation, the quantity of the protein represented by the peak labeled x, was considerably lower than the protein of greater mass, labeled y. However, both proteins were equally represented in the diffusates of younger specimens. This suggests that the flux of the smaller protein (y) was preferentially impeded by the membrane in this elderly specimen.

DISCUSSION

This is the first study to measure the permeability of human Bruch's membrane to macromolecules,[25] the first to attempt to determine the size exclusion limit of the membrane, and the first to ascertain the effects of senescence on the barrier the membrane presents to the flux of entities other than water.

There are a number of important areas regarding the method used in this study that warrant discussion. The number of specimens used in this study could be considered small; however, it is in keeping with other studies measuring permeability of human Bruch's membrane.[13,14,26,27] The primary reasons for these limitations were a lack of donor eyes for which research permission had been given by the next of kin, competition for donor material from other studies, and the duration of each measure of permeability. With prior knowledge of these limitations, we did not set out to investigate topographical or bilateral variation in macromolecular permeability, or to correlate histologic features with permeability. These areas still require investigation.

A criticism of this study is that we used a Bruch's membrane preparation that contained an adherent portion of the under-

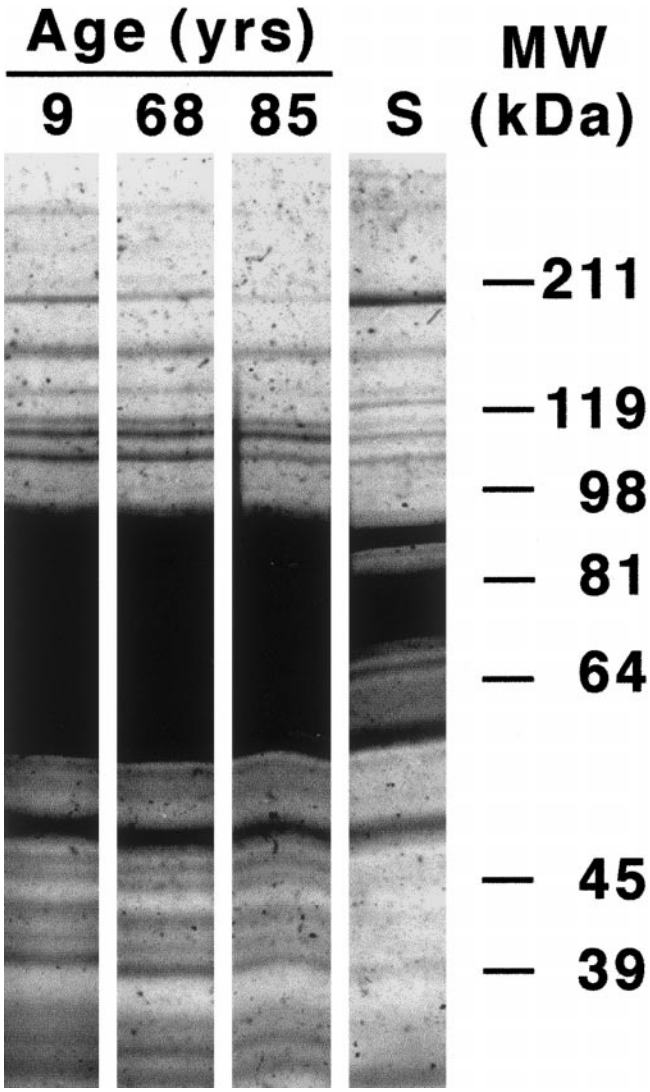

FIGURE 3. Typical electrophoretic profiles of serum proteins able to diffuse through Bruch's membrane preparations of differing age. *Lane S*: whole serum for comparison.

lying choroid. Since we first reported the isolation of such a preparation and discussed its relative merits,[13] it has become the standard for use when measuring the hydraulic permeability of Bruch's membrane.[13–15,26,27] In our experience, attempts to remove choroidal elements result in deformation and damage to Bruch's membrane, rendering it unfit for further study. The attached choroid can be viewed as two components, the choriocapillaris and the stroma, with both having different effects on permeability. First, the border between the choriocapillaris and the outer limit of Bruch's membrane is difficult to define, because the outer collagenous layer extends to form the intercapillary columns of the choriocapillaris. Similar to Bruch's membrane, these columns undergo age-related alteration, becoming broader and accumulating deposits.[28,29] As such, they may also influence metabolic exchange between the choroid and RPE. Second, extending from the choriocapillaris to the outer edge of the preparation was a large portion of stroma. Hydraulic conductivity studies have demonstrated that the barrier presented by this stromal component is infinitesimal when compared with that of Bruch's membrane.[26]

Our previous studies suggest that the total thickness of isolated membrane preparations decreases with increasing age, primarily because of age-related choroidal atrophy within the stroma.[30] If such a trend were to have a biophysical effect it would be to increase permeability, and it therefore cannot explain the age-related decrease in the macromolecular permeability observed in the present study. The only effect of the stromal component of preparations would be to mask an even greater senescent decline in permeability. Therefore, the senescent decrease in macromolecular permeability must result from alteration to factors intrinsic to Bruch's membrane.

In planning this study, we assumed that some of the serum protein molecules might be too large to traverse the membrane. Therefore, there was a need to reduce any osmotic fluid movement that could have resulted in the development of a hydrostatic gradient and a reduction in the concentration gradient across the tissue. We attempted to lessen the osmotic potential by placing a sodium chloride solution of equal osmolarity to the serum in the adjacent compartment of the modified Ussing chamber. Theoretically, this approach is flawed, in that the diffusion coefficient of Na^+Cl^- in both free solution and Bruch's membrane would be expected to be greater than that of any given protein component of serum. Thus the Na^+Cl^- would more rapidly distribute between the compartments and would not act to reduce the development of a hydrostatic gradient or of a reduction in the serum concentration gradient. However, we observed no differential in the volume of fluid between the Ussing chamber compartments after a 24-hour incubation period. In pilot studies in which water was used instead of a sodium chloride solution, an increase in volume of approximately 10% to 15% was measured in the serum compartment over the same incubation period.

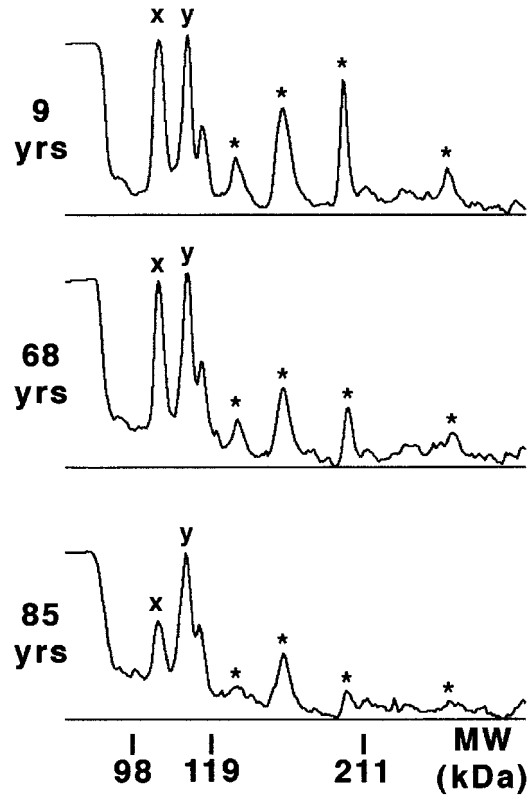

FIGURE 4. Density plots of the higher molecular weight protein bands contained within the electrophoretic profiles shown in Figure 3. There was a gradual age-related reduction in the presence of the highest molecular weight proteins (*). In addition, the flux of the protein represented by peak x appeared to be selectively restricted by the Bruch's membrane of an 85-year-old donor eye when compared with the protein of a slightly larger mass represented by peak y.

Thus, the sodium chloride solution substantially neutralized the development of a hydrostatic gradient.

The electrophoretic separation of diffusate protein was undertaken using a denaturing buffer system, and therefore the actual molecular weight of proteins capable of traversing Bruch's membrane may be greater than those measured in this study.

We possessed minimal clinical information about the donors, and it is possible that some donors had diseases or had undergone treatments that influenced our results. However, macroscopic examination during dissection did not identify any eyes with features of overt senescence, other disease, or laser treatment. Combined with the consistency of the data, this indicates that the results represent the normal aging profile of the macromolecular permeability of Bruch's membrane.

Our results indicate that senescence has three effects on the membrane's macromolecular permeability. First, with increasing age, the membrane progressively impedes the flux of all macromolecules. Second, the molecular weight exclusion limit of the membrane gradually reduces with age, with the large macromolecules that were able to pass through the membrane in youth being excluded or restricted in old age. Third, by the ninth decade the membrane may selectively impede the passage of specific macromolecules, with this restriction based on criteria other than molecular weight.

This senescent decline in permeability lends considerable support to the current hypothesis of the ageing Bruch's membrane's presenting an increasing barrier to metabolic exchange between the choriocapillaris and the RPE.[4,11,12] A recent pilot study using similar methods also supports this concept.[31] The decline in permeability, in the absence of obvious disease, indicates either that there is a large excess capacity for flux within Bruch's membrane and/or that there is a tolerance to reduced flux in adjacent tissues. However, a permeability threshold can be envisaged below which rates of metabolic exchange become limiting for maintenance of the overlying retina, which may contribute to pathologic manifestations of outer retinal senescence. An investigation comparing the permeability of the membrane from eyes with and without early signs of ARMD would be useful in this respect.

The current experimental procedures did not allow precise determination of the size-exclusion limit of the membrane preparations. However, we were able to ascertain that this limit was greater than 200 kDa in the young and reduced to between 100 and 200 kDa in the elderly. A barrier of this magnitude would be insufficient to exclude the vitamin A-RBP-transthyretin complex. However, the discovery of choriocapillary-derived fibrinogen (340 kDa) and compliment components C_{1q} (400 kDa) and C_{3c} (≈185 kDa) at the outer limit of the outer collagenous layer support our findings of Bruch's membrane as a barrier to larger macromolecules.[32]

Assessment of the physiological impact of a decline in the size exclusion limit of the membrane is limited. One approach would be to draw comparisons with the exclusion limits of the routes of flux across the choriocapillary endothelium. Unfortunately, most studies on the latter have been undertaken on animal models, and the application of findings to the present human study is precluded by species variation in ocular architecture and the resultant differing metabolic demands of the retinas.[33–37]

The mechanism that brings about a reduction in permeability is unknown. That the decline appears to begin early in life before major accumulation of deposits within the membrane suggests that a combination of factors are involved. For the most part permeability depends on the thickness and the porosity of the membrane, dictated by the state of the fibers, the interfiber matrix, and the deposition of lipid-rich debris. Any mechanism acting to alter one or more of these components could alter permeability. A combination of mechanisms is envisaged. For example, altered remodeling of the membrane could reduce porosity, leading to a decrease in permeability, precipitating the deposition of debris, which further decreases permeability. Support for such concepts comes from a recent pilot study in which reduced dimensions of pores were reported in the fibrous layers of elderly membranes.[38]

The specific restriction by an elderly membrane of one macromolecule compared with another of similar molecular weight could be due to a combination of two factors. First, the restricted protein may possess a more globular structure giving it greater dimensions and the inability to pass through narrowed flux routes. Second, selectivity may occur based on the electrostatic charge carried by the molecule and the membrane at physiological pH. Similar to all basement membrane systems, Bruch's membrane is rich in proteoglycans, the glycosaminoglycan moieties of which are some of the most highly negatively charged molecules in the body and have been shown to endow extracellular matrices with selective filtration properties.[39–41] Alteration in glycosaminoglycan content has been shown to change the macromolecular permeability of extracellular matrices.[41] No study has directly assessed senescent variation in the proteoglycan content of Bruch's membrane. However, it has been determined that glycosaminoglycans are synthesized in the same ratio throughout life, but after the age of 70 there is an increase in the molecular weight of proteoglycans in Bruch's membrane.[42] Alteration in proteoglycan content and structure may give rise to a change in the charge selectivity of the membrane and differential exclusion of molecules in the elderly membrane, as observed in this study.

From the vitamin A supplementation studies in persons with scotopic threshold defects due to Sorsby fundus dystrophy, we know that it is possible to overcome presumed flux inhibition by Bruch's membrane and reverse visual function loss. For supplementation to be a viable therapy, a diet rich in or supplemented with all the metabolites needed by the outer retina would be required. Unfortunately, the spectrum of metabolites remains unknown. Another strategy would be to elevate permeability by removing the barrier within the membrane. Pilot studies on the feasibility of such an approach are needed.

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References

1. Evans J. Causes of blindness and partial sight in England and Wales. *Studies on Medical and Population Subjects.* No. 57. London: Her Majesty's Stationary Office; 1995:1990–1991.
2. Klein R, Klein BEK, Linton KLP. Prevalence of age-related maculopathy: The Beaver Dam Eye Study. *Ophthalmology.* 1992; 99:933–943.
3. Mitchell P, Smith W, Attebo K, Wang JJ. The prevalence of age-related maculopathy in Australia: The Blue Mountains Eye Study. *Ophthalmology.* 1995;102:1450–1460.
4. Sarks SH. Aging and degeneration in the macular region: a clinico-pathological study. *Br J Ophthalmol.* 1976;60:324–341.
5. Feeney-Burns L, Ellersieck MR. Age related changes in the ultrastructure of Bruch's membrane. *Am J Ophthalmol.* 1985;100:686–697.
6. Green WR, Enger C. The 1992 Lorenz E Zimmerman Lecture. Age related macular degeneration histopathologic studies. *Ophthalmology.* 1993;100:1519–1535.
7. Ramrattan RS, van der Schaft TL, Mooy CM, de Bruijn WC, Mulder PGH, de Jong PTVM. Morphometric analysis of Bruch's membrane,

the choriocapillaris and the choroid in aging. *Invest Ophthalmol Vis Sci.* 1994;35:2857–2864.

8. Marshall J, Hussain AA, Starita C, Moore DJ, Patmore AL. Aging and Bruch's membrane. In: Marmor MF, Wolfensberger TJ, eds. *The Retinal Pigment Epithelium.* Oxford: Oxford University Press; 1998:669–692.
9. Johnson LV, Staples MK, Nealon MR, Anderson DH. Age-related maculopathy: immune complex pathogenesis in drusen formation. *Invest Ophthalmol Vis Sci.* 2000;41(4):**S**24. Abstract nr 121.
10. Karwatowski WSS, Jefferies TE, Duance VC, Albon J, Bailey AJ, Easty DL. Preparation of Bruch's membrane and analysis of age-related changes in the structural collagens. *Br J Ophthalmol.* 1995;79:944–952.
11. Bird AC, Marshall J. Retinal pigment epithelial detachments in the elderly. *Trans Soc Ophthalmol UK.* 1986;105:674–682.
12. Grindle CFJ, Marshall J. Aging changes in Bruch's membrane and their functional implications. *Trans Soc Ophthalmol UK.* 1978;98: 172–175.
13. Moore D, Hussain AA, Marshall J. Age-related variation in the hydraulic conductivity of Bruch's membrane. *Invest Ophthalmol Vis Sci.* 1995;36:1290–1297.
14. Moore DJ, Clover GM. The effect of extracting lipid deposits on the hydraulic conductivity of Bruch's membrane. *Invest Ophthalmol Vis Sci.* In press.
15. Hodgetts A, Hussain AA, Marshall J. Effect of enzymatic modulation on the hydrodynamics of ageing human Bruch's membrane [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 1998;39(4):**S**369. Abstract nr 1724.
16. Pauleikhoff D, Chen J, Chisholm I, Bird AC. Choroidal perfusion abnormality with age related Bruch's membrane change. *Am J Ophthalmol.* 1990;109:211–217.
17. Chen J, Fitzke F, Pauleikhoff D, Bird A. Functional loss in age related Bruch's membrane change with a choroidal perfusion defect. *Invest Ophthalmol Vis Sci.* 1992;33:334–340.
18. Steinmetz RL, Haimovichi R, Jubb C, Fitzke RW, Bird AC. Symptomatic abnormalities of dark adaptation in patients with age related Bruch's membrane change. *Br J Ophthalmol.* 1993;77: 549–554.
19. Steinmetz RL, Polkinghorne PC, Fitzke FW, Kemp CM, Bird AC. Abnormal dark adaptation and rhodopsin kinetics in Sorsby's fundus dystrophy. *Invest Ophthalmol Vis Sci.* 1992;33:1633–1636.
20. Sorsby A, Mason MEJ, Gardener N. A fundus dystrophy with unusual features. *Br J Ophthalmol.* 1949;33:67–97.
21. Capon MR, Marshall J, Kraft J, Alexander RA, Hiscott PS, Bird AC. Sorsby's fundus dystrophy: a light and electron microscopic study. *Ophthalmology.* 1989;96:1769–1777.
22. Jacobson SG, Cideciyan AV, Regunath G, et al. Night blindness in Sorsby's fundus dystrophy reversed by vitamin A. *Nat Genet.* 1995;11:27–32.
23. Bok D. Retinal photoreceptor-pigment epithelium interactions. Friedenwald Lecture. *Invest Ophthalmol Vis Sci.* 1985;26:1659–1694.
24. Laemmli VK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 1970;227:680–685.
25. Moore DJ, Hussain AA, Marshall J. Senescent reduction in the macromolecular permeability of Bruch's membrane [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 1997;38(4):**S**353. Abstract nr 1647.
26. Starita C, Hussain AA, Patmore A, Marshall J. Localisation of the site of major resistance to fluid transport in Bruch's membrane. *Invest Ophthalmol Vis Sci.* 1997;38:762–767.
27. Starita C, Hussain AA, Pagliarini S, Marshall J. Hydrodynamics of ageing Bruch's membrane: implications for macular disease. *Exp Eye Res.* 1996;62:565–572.
28. Pauleikhoff D, Harper CA, Marshall J, Bird AC. Aging changes in Bruch's membrane: a histochemical and morphological study. *Ophthalmology.* 1990;97:171–178.
29. Olver J, Pauleikhoff D, Bird AC. Morphometric analysis of age changes in the choriocapillaris [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 1990;31(4):**S**229. Abstract nr 47.
30. Moore D. An investigation of the permeability of Bruch's membrane and its variation with age. Doctoral thesis. London: University of London Library; 1996.
31. Hussain AA, Starita C, Marshall J. Molecular weight size exclusion limit and diffusional status of aging human Bruch's membrane [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 1999;40(4):**S**920. Abstract nr 4852.
32. van der Schaft TL, Mooy CM, de Bruijn WC, de Jong PT. Early stages of AMD an immunofluorescence and electron microscopic study. *Br J Ophthalmol.* 1993;77:657–661.
33. Bernstein MH, Hollenberg MJ. Fine structure of the choriocapillaris and the retinal capillaries. *Invest Ophthalmol.* 1965;4:1016–1025.
34. Okuma M, Uyama M. Electron microscopic studies of permeability of the choroidal vessels. I: ferritin transfer across the choriocapillary wall. *Acta Soc Ophthalmol Jpn.* 1972;76:323–330.
35. Pino RM, Essner E. Permeability of the rat choriocapillaris to haeme proteins: restriction of tracers by a fenestrated endothelium. *J Histochem Cytochem.* 1981;29:281–290.
36. Pino RM, Thuron CC. Vascular permeability in the rat eye to endogenous albumin and IgG examined by immunohistochemical methods. *J Histochem Cytochem.* 1983;31:411–416.
37. Pino RM. Restriction of endogenous plasma proteins by a fenestrated capillary endothelium: an ultrastructural immunocytochemical study of choriocapillary endothelium. *Am J Anat.* 1985;172: 279–289.
38. Hussain AA, Starita C, Patmore A, Marshall J. Ultrastructural evaluation of porosity of individual layers of Bruch's membrane and correlation with the rate limiting site for fluid transport [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 1997;38(4):**S**353. Abstract nr 1648.
39. Pino RM, Essner, Pino LC. Localisation and chemical composition of anionic sites in Bruch's membrane of the rat. *J Histochem Cytochem.* 1982;30:245–252.
40. Call T, Hollyfield J. Sulphated proteoglycans in Bruch's membrane of the human eye: localisation and characterisation using cupromeronic blue. *Exp Eye Res.* 1990;51:451–462.
41. Kanwar YS, Linker A, Farquar MG. Increased permeability of the glomerular basement membrane to ferritin after removal of glycosaminoglycans (heparan sulphate) by enzyme digestion. *J Cell Biol.* 1980;86:688–693.
42. Hewitt AT, Nakazawa K, Newsome DA. Analysis of newly synthesized Bruch's membrane proteoglycans. *Invest Ophthalmol Vis Sci.* 1989;30:478–486.