The Influence of Path Length and Matrix Components on Ageing Characteristics of Transport between the Choroid and the Outer Retina

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Purpose. To determine the relative influence of path length and matrix components on the movement of small solutes and water between the choroid and the outer retina.

Methods. Human and bovine Bruch's membrane-choroid (BC) tissue samples were mounted in modified Ussing chambers, and the diffusion of taurine and hydraulic conductivity (Lp) was determined. In humans, diffusion of taurine was determined as a function of age of the donor. The relative contribution of Bruch's membrane in the BC complex to transport processes was measured after its removal by laser ablation. Similarly, the effect of choroidal path length was determined. In humans, tracking the trend of age-related thinning provided samples of various path lengths. In young bovine animals (≤2 years old), choroidal thickness was adjusted by laser ablation.

Results. Diffusion of taurine across human BC decreased linearly from 162.7 to 105.9 nanomoles/h per 3 mm between 10 and 90 years of age ($P < 0.05$). Ablation of Bruch's membrane increased diffusion of taurine from 129 to 287.9 nanomoles/h per 4 mm in human (donor age 55, 74, and 82 years; $P < 0.005$) but caused no statistically significant change in bovine BC. Diffusion of taurine across bovine BC was greater in samples with partially ablated choroid (218 nanomoles/h per 4 mm) than in normal control samples (128.75 nanomoles/h per 4 mm). Lp was not measurable in bovine samples after complete ablation of Bruch's membrane, but did not change significantly as the choroid thinned.

Conclusions. The data suggest that both path length and matrix components contribute to the decline of diffusion of small solutes across BC with age. The importance of matrix components was also demonstrated in restricting the movement of water while choroidal thickness played little if any role. (*Invest Ophthalmol Vis Sci.* 2004;45:1493–1498) DOI:10.1167/iovs.03-0765

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Presented in part at the annual meeting of the German Ophthalmological Society (DOG), Berlin, Germany, September 2002.

Supported by Deutsche Forschungsgemeinschaft Grant Hi 758/1-1 (JH); The PPP Foundation and The Iris Fund, United Kingdom (AAH); and the Special Trustees of Guys and St. Thomas' Hospital and The Allerton Trust (TLJ).

Submitted for publication July 20, 2003; revised November 11, 2003, and January 24, 2004; accepted January 29, 2004.

Disclosure: **J. Hillenkamp**, None; **A.A. Hussain**, None; **T.L. Jackson**, None; **J.R. Cunningham**, None; **J. Marshall**, None

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Age-related macular degeneration (AMD) is the leading cause of untreatable legal blindness in the Western world. At present the etiology and genetic predisposition are unknown, but the risk of development of some form of visual loss due to age-related processes in the macula is 30% at the age of 80 years.[1,2]

In the macular region of the human fundus, maintenance of photoreceptor function and viability are inherently dependent on an adequate transport capacity for nutrients between the outer retina and the choroidal circulation.[3,4] An understanding of the relationship between structural ageing changes of Bruch's membrane and the choroid and its transport characteristics is essential for predicting the physiological and pathologic effects of the ageing process.

In the ageing eye, Bruch's membrane progressively increases in thickness by 135% from 2 μm in the first decade to 4.7 μm in the 10th decade.[5] Simultaneously, choroidal thickness decreases by 57% from 193.5 to 83.5 μm, including a decrease of diameter of the choriocapillaris by 34% from 9.8 to 6.5 μm.[5] Choriocapillary density decreases by 45%.[5] In eyes with AMD, the density of the choriocapillaris and the choriocapillary diameter are even lower, whereas choroidal thickness remains unchanged compared with that in normal eyes of the same age group.[5]

The increase in thickness of Bruch's membrane is accompanied by a progressive increase in lipid-rich membranous debris after the first decade and in many individuals, an increase in basal laminar deposits after the sixth decade.[5–9] The latter is claimed by some authorities to be a precursor of AMD.[10,11] Deposition of lipid-rich material within Bruch's membrane has been suggested to cause an increase in resistance to solute and water flux across the system.[12] This concept has been supported by experimental work determining hydraulic conductivity of isolated Bruch's membrane-choroid preparations as a function of age, in which it has been shown that there is an exponential increase in resistance to water movement.[13,14] However, these studies have clearly demonstrated that the largest increase in resistance occurs before the significant increase in membranous debris or lipids. This observation suggests that other changes that begin earlier in life, such as increased fiber cross-linking[15] and accumulation of advanced glycation end products (AGEs)[16] remodel Bruch's membrane, thereby diminishing membrane porosity, which has been suggested to be a cause of the observed early increase in resistance to the movement of water in Bruch's-choroid preparations.[17] The movement of water across Bruch's-choroid and solute permeability are thermodynamically independent processes in a passive barrier and thus cannot be compared directly. Experimentally, the hydraulic conductivity of human Bruch's-choroid has been shown to decline exponentially, whereas permeability to small solutes and macromolecules shows a linear decline as a function of age.[18,19]

All the in vitro studies cited above investigated transport functions across the isolated Bruch's-choroid complex and thus invariably incorporated the choroidal mass as an addi-

tional barrier. Physiologically, the proximity of the choriocapillaris to Bruch's membrane means that the large choroidal mass does not constitute a barrier to fluid and metabolite transport. Thus, on fluorescein angiography, the label diffuses out profusely from the choriocapillaris and over time slowly stains the remaining choroidal mass.[20]

To our knowledge, changes in diffusional transport and water transport across the human choroid in relation to that across Bruch's membrane have not yet been investigated. It was the purpose of this study to determine the relative influence of age-related path-length changes caused by atrophy of the choroid, together with the increase in thickness of Bruch's membrane, and age-related changes of matrix components of Bruch's membrane on movement of water and small solutes across Bruch's-choroid in vitro.

Materials and Methods

Human Bruch's Membrane–Choroid Preparation

We used 29 human eyes from donors aged 13 to 88 years (UK Transplant Support Service, Bristol Eye Bank, Bristol, UK). Donor eyes were obtained and used in accordance with the provisions of the Declaration of Helsinki for research involving human tissue. Whole globes were dissected in a Petri dish lined with filter paper (Grade 50; Whatman, Maidstone, UK), moistened with phosphate-buffered saline, (PBS, composition: NaCl, 90 g; NaH_2PO_4, 13.65 g; and $KH_2PO_4.2H_2O$, 2.43 g dissolved in distilled water with final volume adjusted to 1 L [pH 7.4]; Sigma-Aldrich, Poole, UK) containing 100 U/mL penicillin and 100 μg/mL streptomycin. The anterior portion of the eye was carefully removed by a circumferential incision at the pars plana, and the cornea, together with the lens, iris, and vitreous, were discarded. The posterior globe was inspected for any evidence of subretinal blood, extensive drusen, or irregular pigmentation of the RPE or any gross disease of the retina, and those exhibiting any abnormal appearance were discarded. The neural retina was gently peeled away from the underlying RPE and cut at the optic nerve head. A series of four samples were obtained from the midperiphery of each eyecup with an 8-mm trephine (Stiefel Laboratories, Buckinghamshire, UK). The RPE was carefully brushed away with a fine sable-hair brush, and the Bruch's membrane-choroid complex was gently teased away from the underlying sclera.

The isolated preparation was mounted between the two halves of a Perspex modified Ussing chamber. Both surfaces were rinsed several times with PBS.

Bovine Bruch's Membrane–Choroid Preparation

Fresh bovine eyes of Friesian cows aged 18 to 24 months were obtained from a local abattoir and experiments initiated within 6 hours of death. We prepared the eyes in the same way as the human eyes. Samples were obtained from the midperipheral nontapetal fundus area with an 8-mm trephine.

Excimer Laser Ablation

Full-thickness human or bovine Bruch's-choroid tissue samples were placed on a PBS-soaked piece of filter paper in a covered Petri dish with either the Bruch's membrane side or the choroidal side facing up. The Petri dish was then placed on a laboratory jack under the exit port of an excimer laser (Apex Plus; Summit Technology, Boston, MA). The Petri dish was manipulated so that the specimen was centered using the helium-neon laser-aiming beams. After the Petri dish was uncovered, the surface fluid on the tissue preparation was removed by using the capillary action of a Wechsel sponge, and the helium-neon laser-aiming beams were then refocused on the surface of the tissue. Ablations were performed at a radiant exposure of 180 mJ/cm^2 per pulse, with the machine operating in the phototherapeutic keratectomy mode. The laser beam diameter was 6.5 mm, and 20 to 30 pulses were applied per ablation zone for both the bovine and human specimens to ablate Bruch's membrane and 100 pulses to partially ablate the choroid in bovine samples. The lid of the Petri dish was removed only during the periods of excimer ablation in an attempt to minimize drying of the tissues. A typical procedure took between 5 and 10 minutes. Immediately after the ablation process, the specimens were reimmersed in PBS. The sample was then used for measurement of taurine diffusion or hydraulic conductivity.

We processed one bovine and one human Bruch's-choroid sample treated with 20 excimer laser pulses to Bruch's membrane for histology to confirm that Bruch's membrane was fully removed after laser application.

Taurine Diffusion Studies

Human Tissue. Twenty-six Bruch's-choroid samples from 26 donors aged 13 to 88 years were used to assess taurine diffusion as a function of age. Samples from three of these donors (aged 55, 74, and 82 years) were then subjected to laser ablation to remove Bruch's membrane, and the corresponding effect on diffusion rate was quantified.

Bovine Tissue. We compared the diffusion of taurine across 18 intact bovine Bruch's-choroid samples with five samples after ablation of Bruch's membrane. Furthermore, we compared the diffusion rate of taurine across bovine Bruch's-choroid as a function of choroidal thickness by partially ablating the choroid with an excimer laser in four samples. The diffusion rate of these four samples was compared with that in four nonablated samples. The tissue thickness of these eight samples was measured by optical coherence tomography (OCT), a technique that allows more rapid estimation than laborious histologic methods.

Assessment of Tissue Integrity. Manipulation of the preparation during isolation and edge compression during mounting in the Ussing chamber were the two most likely procedures that could cause tissue damage. These manipulations could result in holes or destruction of the exclusion limit of Bruch's membrane, previously determined to be 66 ± 10 kDa (Hussain AA, et al. *IOVS* 1999;40:ARVO Abstract 4852). An osmotic method was devised to confirm the integrity of each Bruch's membrane–choroid preparation before the experimental studies. Briefly, a 0.412-mM solution of a 162-kDa dextran (Sigma-Aldrich) constituted in PBS was placed in one half-chamber, with an equal volume of PBS in the other—both solutions initially reaching the same height in the reservoir columns of both compartments. In an intact preparation, the compartment containing the impermeable dextran solution would exert an osmotic pressure, drawing fluid into it from the other compartment, leading to a discrepancy in heights of the two solutions in the reservoir columns. Tissue damage, in contrast, would lead to mixing of the dextran between the two compartments, thereby abolishing the osmotic response. Thus, the integrity of the preparations was assessed after an incubation of 3 hours and, if suitable, the preparations were rinsed several times with PBS over the next 3 hours and then used in the experimental studies.

Measurement of Taurine Diffusion. The diffusion of taurine across human and bovine Bruch's-choroid samples was assessed as described previously.[18] Briefly, Ussing chambers with a central aperture exposing a 0.071 cm^2 (3 mm disc diameter) or 0.126 cm^2 disc (4 mm disk diameter) of Bruch's membrane or choroid, respectively, to one half compartment were used. After confirmation of tissue integrity, an aliquot of 5 mL of taurine at a concentration of 10 mM in PBS was placed in one half-chamber (with exposed choroidal surface) and an equal volume of PBS in the other half-chamber. Both solutions were added simultaneously to the Ussing chamber with a dual syringe system of delivery so as to avoid undue pressure differentials to the membrane preparations. Stirring bars were placed in each half-chamber and the assembly transferred to a magnetic stirring plate for continuous mixing in each compartment. At timed intervals of 15, 30, 60, 120, and 180 minutes, a 60-μL aliquot was withdrawn from each half-chamber for quantification of taurine.

High-Performance Liquid Chromatography. Taurine was measured using precolumn derivatization by reversed-phase liquid chromatography and fluorescence detection.[21] Samples (25 μL) were derivatized for 1 minute with *o*-phthalaldehyde (OPA; 50 μL) and then injected through an autosampler onto a chromatography column (Spherisorb ODS2, 25 cm × 4.6 mm; Waters Corp., Milford, MA). Amino acids were eluted with a gradient generated with a pump (model PU980; Jasco, Great Dunmow, UK) and gradient unit (model PU980–02; Jasco). The flow rate was 1 mL/min. The fluorescence detector (Jasco) was set at an excitation of 360 nm and emission of 430 nm. Results were analyzed on computer (Borwin Chromatography software; Jasco).

Hydraulic Conductivity Studies

We measured the Lp in nine bovine Bruch's-choroid samples. In three samples, we ablated Bruch's membrane with 20 pulses of an excimer laser; in three samples we applied 20 pulses to the choroid, and we used three untreated samples as the control.

Bovine Bruch's membrane-choroid samples flattened on a piece of filter paper were clamped into a modified Ussing chamber with a 0.283-cm^2 aperture. Both compartments were flushed several times with PBS before being slowly filled to avoid any air bubbles. The compartment appositional to the Bruch's membrane surface was then coupled to a constant-pressure reservoir of PBS, and the choroidal compartment was linked to a horizontal capillary tube, allowing the meniscus to be tracked by a traveling microscope. The digital micrometer on the traveling microscope had a resolution (b) of ± 0.01 mm and the radius of the capillary (r) was 0.05 mm. Thus, the minimum change in volume before its detection was $\pi r^2 b = 7.85 \times 10^{-5}$ mm^3.

The Lp was determined as described by Starita et al.[14,22] Briefly, pressure was applied to the Bruch's membrane surface by the PBS reservoir at a fixed height of 220 mm, which applied a constant pressure of 2156 Pa to the preparation. Therefore, as the flow of buffer through the preparation occurred, the meniscus movement in the capillary tube was observable over time, allowing the Lp to be calculated.[13,14,22]

Once the tissue was under pressure, if a hole was present in Bruch's membrane, it became readily apparent because of the speed of movement of the meniscus in the capillary tube, and such samples were discarded. Intact samples were allowed to equilibrate for 30 to 60 minutes under constant pressure. Measurements of the movement of the meniscus were taken every 10 minutes for a period of up to 1 hour.

Thickness Measurement by OCT

In vitro examination of Bruch's-choroid samples was performed as described by Chauhan and Marshall.[23] Briefly, the OCT scanner was modified by placing a plane mirror above and at 45° to the objective lens, thus allowing the acquisition of images of horizontally oriented tissues. The specimens, originally clamped in a tissue cassette for measurement of diffusion, were carefully removed and placed in a Petri dish containing PBS, to a depth of 5 mm. Multiple scans (of length 1.7 mm) were obtained along the central axis of the region used in the diffusion experiments. All scans on each specimen were performed at the same focal plane, using the same power and polarization settings, and were repeated three times.

For thickness measurement of the colored image, the measuring facility of the image-processing program was used. The mean of five readings at various locations was taken as the thickness of the tissue sample. These measurements were converted into micrometers by using the manufacturer's conversion factor of 4 μm per z-axis pixel (Humphrey Instruments; Carl Zeiss Meditec, Oberkochen, Germany).

Pilot experiments were undertaken beforehand to validate the technique (Hillenkamp J, et al. *IOVS* 2001;42:ARVO Abstract 2712). Five bovine samples were scanned by OCT and then processed for histologic quantification of tissue thickness. The mean ± SD thicknesses by OCT and light microscopy were determined to be 153.8 ± 27.9 and 161.8 ± 20.2 μm respectively, demonstrating the usefulness of the rapid OCT technique.

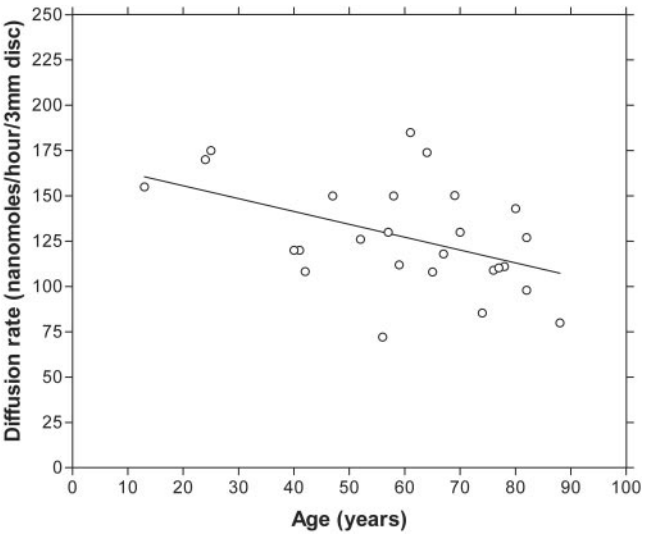

Figure 1. Diffusion of taurine across human Bruch's-choroid preparations. Intact human Bruch's-choroid preparations were mounted in Ussing chambers, and transport studies were initiated by addition of taurine (final concentration, 10 mM) to the choroid-facing half-compartment in 26 samples from 26 donors. Aliquots were removed at timed intervals, and the amount of taurine crossing the preparation was quantified by HPLC. Linear regression, $P < 0.05$, $r^2 = 0.22$.

Statistical Analyses

Linear regression and significance analyses were undertaken on computer (Prism, ver. 3.02; Graph Pad Software, Inc., San Diego, CA, and Fig.P; Fig.P Software Co., Durham, NC).

Results

Diffusion of Taurine

The rate of diffusion of taurine across human Bruch's-choroid at a concentration gradient of 10 mM declined linearly with age at 7.1 nanomoles/3 mm per hour per 10 years from a rate of 162.7 nanomoles/3 mm per hour at age 10 years to 105.9 nanomoles/3 mm per hour at age 90 ($N = 26$ donors, $P < 0.05$; Fig. 1). In three of these donors, aged 55, 74, and 82 years, the average rate of diffusion of taurine of 129 nanomoles/4 mm per hour increased to 287.9 nanomoles/4 mm per hour after laser ablation to remove Bruch's membrane (Fig. 2).

The rate of diffusion of taurine across bovine Bruch's-choroid was 113.04 nanomoles/4 mm per hour ($n = 18$ animals), and in five samples, removal of Bruch's membrane resulted in a slight increase to 128.7 nanomoles/4 mm per hour, which was statistically insignificant (Fig. 3). Alterations in the thickness of the choroid, mediated by laser ablation, resulted in increased diffusion of taurine. Using a transpreparation concentration gradient of 10 mM taurine, an average thickness of 239 μm for the unablated samples gave an average diffusion rate of 128.75 ± 16.74 (SEM) nanomoles/4 mm per hour, whereas an average thickness of 103 μm for the ablated samples yielded an average diffusion rate of 218 ±16.005 nanomoles/4 mm per hour (Fig. 4). Thus, despite the limited data set in Figure 4, the linear relationship between diffusion rate and thickness of preparation was significant ($P < 0.005$). The results therefore show that at a transtissue gradient of 10 mM, taurine diffusion increased by 6.757 nanomoles/4 mm per hour for a 10-μm reduction in choroidal thickness. The laser exper-

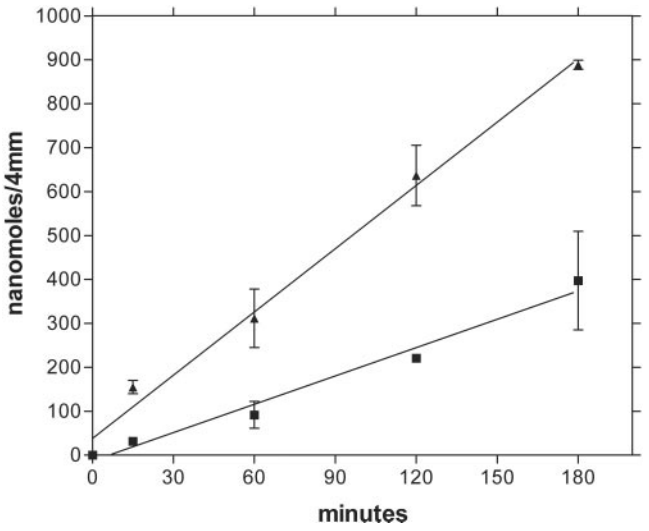

FIGURE 2. Diffusion of taurine across human Bruch's-choroid preparations, with and without ablation of Bruch's membrane. Human Bruch's-choroid preparations from three donors, aged 55, 74, and 82 years with intact Bruch's membrane and after laser ablation of Bruch's membrane from the same eye, were mounted in Ussing chambers, and transport studies were initiated by addition of taurine (final concentration, 10 mM) to the choroid-facing half-compartment. Aliquots were removed at timed intervals, and the amount of taurine crossing the preparation was quantified by HPLC. Data are expressed as the mean ± SEM. The linear gradients were significantly different ($P < 0.005$). (■) Bruch's membrane intact; (▲) Bruch's membrane ablated by laser.

iments also provided an ablation rate for the choroidal mass of 1.36 μm per pulse.

Hydraulic Conductivity

Hydraulic conductivity (Lp) in three bovine Bruch's-choroid samples was determined as $3.45 \times 10^{-11} \pm 0.39$ (SEM) m s^{-1} Pa^{-1}. Removal of Bruch's membrane in three samples after exposure to 20 excimer pulses resulted in total loss of the hydraulic barrier, so that the rapid movement of the capillary meniscus was unrecordable. Laser ablation of the choroidal surface with 20 pulses resulted in removal of 27 μm of tissue layer (from the above ablation rate of 1.36 μm/pulse), which was without significant effect on the hydraulic conductivity of the preparation, measured as $3.143 \times 10^{-11} \pm 0.113$ m s^{-1} Pa^{-1}.

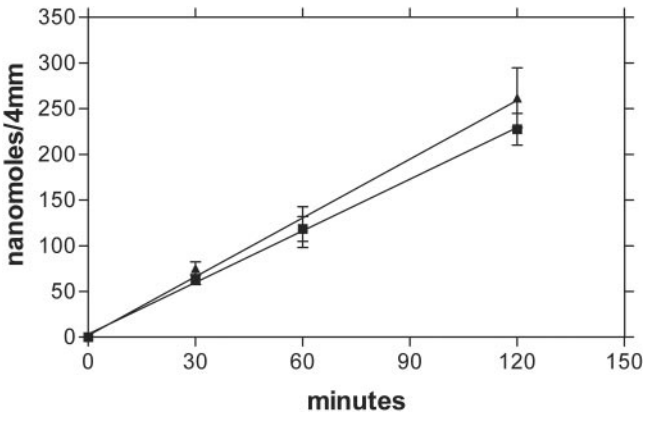

FIGURE 3. Diffusion of taurine across bovine Bruch's-choroid preparations, with and without ablation of Bruch's membrane. Bovine Bruch's-choroid preparations from 18 animals with intact Bruch's membrane and from five animals after laser ablation of Bruch's membrane were mounted in Ussing chambers and transport studies were initiated by addition of taurine (final concentration, 10 mM) to the choroid-facing half-compartment. Aliquots were removed at timed intervals, and the amount of taurine crossing the preparation was quantified by HPLC. Data are expressed as the mean ± SEM. The linear gradients were not significantly different. (■) Bruch's membrane intact ($n = 18$); (▲) Bruch's membrane ablated by laser ($n = 5$).

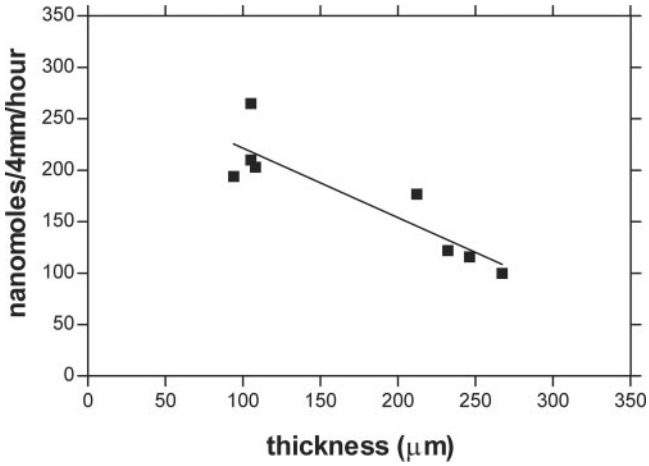

FIGURE 4. Diffusion of taurine across bovine Bruch's-choroid preparations as a function of tissue thickness. Bovine Bruch's-choroid preparations from four animals with intact Bruch's membrane and from four animals after partial laser ablation of the choroid (thin specimens) were mounted in Ussing chambers, and transport studies were initiated by addition of taurine (final concentration, 10 mM) to the choroid-facing half-compartment. Linear regression, $P < 0.005$, $r^2 = 0.79$.

DISCUSSION

Several research groups have investigated the effect of age on the permeability of human Bruch's membrane.[13,18,19,22,24] Ideally, such studies would be undertaken using Bruch's membrane isolated from the choroid. However, the isolation of Bruch's membrane without causing damage to its structure is technically difficult if not impossible. Pilot studies undertaken to strip the choroidal vessels and fibers by microdissection invariably led to holes, tearing, and permanent deformation of our specimens.[13] Furthermore, the posterior border of Bruch's membrane is difficult to identify, because the membrane expands to form the intercapillary pillars of the choriocapillaris (Olver J, et al. *IOVS* 1990;31:ARVO Abstract 229). Schütt and Holz[25] described the ablation of choroidal tissue from Bruch's membrane using an excimer laser. It was possible to debulk choroidal tissue but complete denuding of Bruch's membrane could not be achieved without damaging its structure. Therefore, Bruch's membrane was never isolated from the underlying choroid but was investigated as part of the Bruch's-choroid complex.

The purpose of the present study was to investigate the relative influence of ageing changes in Bruch's membrane and the choroid on the overall experimental characterization of transport processes for water and small solutes across the intact Bruch's-choroid preparation in vitro. We found a linear decline of taurine diffusion as a function of age, which is in accordance with our own pilot studies on taurine and other amino acids[18] and the decline of the diffusion of macromolecules.[19]

We know from the work of Ramrattan et al.[5] that the human choroid thins linearly with age by 11 μm per 10 years. Our data on taurine diffusion as a function of age showed a linear decline of 4.2% of the permeability of Bruch's-choroid at age 10

years per 10 years of age. Our diffusion experiments conducted in tissue of young cows of different choroidal thicknesses showed an increase in diffusion of 5.2% per 10 μm, as the choroidal mass was thinned in the ablation experiments. Therefore, in the human, based on the effect of age-related thinning of the choroid alone, the permeability should *increase* with age in our in vitro experiments. Because permeability was actually observed to decline linearly between the ages of 10 and 90 years by 37.6%, this decline is most likely to be related to changes in Bruch's membrane.

The bovine samples in the study from 18- to 24-month-old animals belong to a young population and are devoid of the gross ageing alterations associated with the elderly human population. In these animals, laser-mediated removal of Bruch's membrane produced no statistically significant effect on the diffusional process through the Bruch's-choroid complex. In these specimens, resistance to solute transport was provided by the choroidal mass. Furthermore, taurine diffusion was observed to be directly proportional to the concentration difference across the preparation divided by the path length, as predicted by Fick's first law of diffusion ($F = D \cdot dC/dx$). This was shown by the choroidal ablation experiments in which a rough halving in choroidal thickness resulted in virtual doubling of diffusion. By contrast, in the older human subset, laser ablation to remove Bruch's membrane resulted in a massive increase in taurine diffusion across the remaining preparation. Thus, despite its relative thinness in the Bruch's-choroid complex, in the human, Bruch's membrane per se would be assigned the role of a major resistance barrier to the movement of small solutes. We can therefore conclude that Bruch's has only a relatively small if any restricting effect in the young bovine eye, but it significantly restricts movement of small solutes in the human older age group.

Ageing of Bruch's membrane results in increased thickening and accumulation of abnormal matrix components,[5–11] both of which are processes capable of modulating the transport of fluids and solutes across the preparation. If taurine diffusion across the complex was dictated by the path length across Bruch's alone, then the 2.35-fold increase in its thickness over a 10-decade period would predict that solute concentration gradients would be reduced by a factor of 0.425, resulting in similar alterations in diffusion. Because the experimentally determined change in diffusion was of the magnitude predicted, it could be argued that transport in the elderly was very much a result of the age-related increase in thickness of Bruch's membrane. However, age-related thinning of the choroid counteracted this effect in our in vitro experiments; therefore, changes in matrix components of Bruch's membrane are likely to contribute to the overall age-related decline of diffusion.

Ageing of Bruch's is associated with major morphologic alterations and, at the molecular level, with accumulation of denatured and highly cross-linked collagens, deposition and entrapment of large molecular aggregates of proteins and lipid-rich entities, and increased cross-linking of proteins due to enhanced presence of AGEs and loss of free thiol groups.[15–17] Thus, the overall reduction of diffusion may be related to both the increase in thickness and the age-related alterations in the matrix components of Bruch's membrane.

Our calculations are based on in vitro experiments. They do not take other age-related choroidal changes into account so that the actual restriction caused by the changes in Bruch's membrane in vivo may differ from our calculations. Other investigators have described a decrease in choriocapillary density (Olver J, et al. *IOVS* 1990;31:ARVO Abstract S47),[5] a decrease in the diameter of the choriocapillaris,[5] and the age-related accumulation of lipids in the intercapillary columns of the choriocapillaris.[7] In vivo, these changes may also influence diffusion across Bruch's-choroid.

An indocyanine green angiography study of normal subjects showed a decrease in fluorescence intensity in the macular region and slowed filling of the choroidal vasculature with age.[26] This phenomenon is probably related to systemic age changes, with the fluorescence intensity being proportional to the density of choroidal blood vessels. It may reduce the transport across Bruch's-choroid in vivo, but it is not related to a tissue-inherent reduction of permeability as measured by our in vitro experiments.

Age-related choroidal thinning is unlikely to play an important role in ageing characteristics of transport between the choroidal circulation and the outer retina in vivo. In vivo, within the choroid, nutrients are released only from the fenestrated choriocapillaris to pool simultaneously in the loose connective tissue of the choroid situated between Bruch's membrane and the sclera[20] and to diffuse passively across Bruch's membrane along a concentration gradient. Our experiments were undertaken in vitro and therefore do not directly reflect the in vivo situation. They show, however, that diffusion of taurine across the choroid is inversely proportional to choroidal thickness. Furthermore, the influence of choroidal path length is relevant to the interpretation of all in vitro studies of diffusion of solutes across Bruch's membrane, because all these studies used intact Bruch's-choroid complex, since Bruch's membrane cannot be separated from the choroid without damage.[13]

The importance of matrix components was also demonstrated in restricting the movement of water, whereas choroidal thickness played little if any role. Fluid transport through the Bruch's-choroid preparations was driven by hydrostatic pressure gradients and in bovine samples, laser-mediated removal of Bruch's membrane led to a collapse in tissue resistance, so that the ensuing rapid fluid flow was difficult to quantify. These results are in accordance with those on human preparations used by Starita et al.[22] Conversely, partial removal of the choroidal compartment was without effect on the hydraulic conductivity of the remaining preparation. The age-related changes in structure of Bruch's membrane therefore determine the Lp of the preparation. The resistance barrier has previously been localized to a narrow region within the inner collagenous layer in juxtaposition to the elastin layer of Bruch's membrane.[22] The nature of ageing alterations at this site are presently unknown, but the heavy deposition of debris in the elderly may augment path lengths in both the individual layers of Bruch's and also at the barrier itself. Similarly, compositional alterations of the type discussed earlier may also modify properties of the barrier. Nonetheless, Lp measurements performed within the rather artificial setting of an in vitro preparation can reflect alterations at the resistance barrier within Bruch's membrane,[22] since this is the rate-limiting step in the transport process for fluids.

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