Serum Proteins and Aqueous Outflow Resistance in Bovine Eyes

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Purpose. Recent evidence shows that much of the protein in the anterior chamber aqueous humor enters diffusively through the root of the iris. The proximity of the protein entry point to the trabecular meshwork suggests that the protein content of the aqueous humor in the trabecular meshwork might be much higher than previously suggested. The authors were interested in investigating the possible hydrodynamic implications of these proteins.

Methods. Bovine eyes were perfused with concentrations of bovine serum in buffer ranging from 0% to 15% to determine the effect on outflow resistance. Immunohistochemical methods were used on these eyes and unperfused eyes to determine the distribution of albumin in the anterior segment.

Results. Preliminary perfusion studies suggested that increasing the concentration of serum in buffer from 0% to 15% decreased the rate of "wash-out" in bovine eyes, with a 15% solution essentially eliminating the wash-out phenomenon. Perfusion of a series of bovine eyes with a total of 5 ml of 15% serum in buffer showed a "wash-out" rate of 0.0498 ± 0.0428 ([μl/min/mm Hg]/[ml perfusate]), whereas control eyes perfused with buffer washed-out at a rate of 0.1677 ± 0.0271 ($P < 0.05$); a second series of eyes perfused with a total of 10 ml of 15% serum washed-out at a rate of 0.0533 ± 0.0294, whereas control eyes had a rate of 0.1813 ± 0.0342 ($P < 0.02$). Immunohistochemical investigations showed significant quantities of albumin in the outflow pathway of unperfused eyes, whereas perfusion with buffer eliminated this protein; perfusion with 10% to 15% serum in buffer maintained the level of albumin in the outflow pathway similar to that found in unperfused eyes. Use of cuprolinic blue in a critical electrolyte concentration confirmed previous findings that sulfated proteoglycans are not eliminated from the trabecular meshwork during wash-out.

Conclusions. Wash-out in nonhuman species may result from progressive depletion of an anterior segment depot of plasma-derived proteins entering the trabecular meshwork. Modeling studies confirm that plasma-derived proteins in the aqueous humor of the trabecular meshwork can generate a significant fraction of aqueous outflow resistance. The lack of wash-out in human eyes suggests that this system may maintain flow resistance in a fashion fundamentally different from other species. Invest Ophthalmol Vis Sci. 1993;34:3549–3557.

As aqueous humor flows through the aqueous outflow pathway (uveal and trabecular meshwork, juxtacanalicular connective tissue, inner wall of Schlemm's canal, Schlemm's canal itself, and the aqueous veins), its pressure decreases approximately 6 mm Hg in a normal eye. In chronic glaucoma, the pressure decrease can be as much as 40 mm Hg with nearly the same flow rate. The basis for normal outflow resistance and elevated resistance in chronic open-angle glaucoma has eluded researchers for more than a century. Hydrodynamic analyses of the size of the flow pathways have established that the apparently open (electron transparent) regions through which the aqueous humor passes are much too large to generate the measured aqueous outflow resistance,[1,2] and it has been suggested[2,3] that the open spaces in the juxtacanalicular connective tissue are filled with an extracellu-

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lar matrix gel that is responsible for the bulk of aqueous outflow resistance. It has long been established that enzymes (eg, hyaluronidase) that digest glycosaminoglycans and proteoglycans greatly decrease aqueous outflow resistance.[4]

Among the unexplained observations concerning aqueous outflow resistance is a phenomenon known as the "wash-out effect." When an in vivo or enucleated eye (bovine, primate, dog, cat, porcine, rabbit) is perfused experimentally, the flow resistance of the eye decreases progressively.[4–8] (It is interesting that this phenomenon does not occur when enucleated human eyes are perfused.[9]) This wash-out phenomenon occurs even if aqueous humor is used as the perfusion fluid.[4] Even though the wash-out phenomenon appears to mimic the effects of glycosaminoglycan-degrading enzymes on outflow resistance, wash-out does not appear to be associated with a decrease of glycosaminoglycans in the aqueous outflow pathway. Knepper, Farbman, and Tesler[8] biochemically analyzed the composition of the rabbit trabecular meshwork before and after wash-out and could not detect a significant change in the glycosaminoglycan composition. As part of our current study, we have used the cuprolinic blue technique[10] to investigate the sulfated proteoglycans ultrastructurally in the bovine trabecular meshwork before and after wash-out.

Aqueous humor removed from the anterior chamber of the eye contains a protein concentration approximately 1/100 that of serum.[11] Given the low concentration, it was widely presumed that aqueous humor proteins could not play a significant role in aqueous outflow resistance. It has been shown recently,[12,13] however, that much of this protein enters the aqueous humor of the anterior chamber directly by diffusion through the root of the iris, rather than by bulk flow through the posterior chamber as previously thought. This plasma-derived protein originates from the ciliary body stroma. Although the details of protein transport in the vicinity of the trabecular meshwork are not fully understood and presumably would be influenced by factors such as unconventional outflow, the proximity of the point of entry for this protein pathway to the aqueous outflow system strongly suggests that the protein concentration entering the trabecular meshwork may be much higher than previously thought. The possibility that additional protein might enter the aqueous humor just proximal to the outflow pathway led us to suspect that these plasma-derived proteins may be involved in the generation of aqueous outflow resistance, and that, during perfusion experiments, protein in the ciliary body is washed-out, thereby leading to the observed decrease in outflow resistance. Therefore, we conducted experiments perfusing bovine eyes with various concentrations of serum proteins to determine the effects on aqueous outflow resistance, wash-out, and distribution of albumin in the anterior segment of the eye before and after perfusion.

MATERIALS AND METHODS

Materials

Seventeen pairs of enucleated bovine eyes from 2-week-old calves were procured from a local abattoir (Arena & Sons, Hopkinton, MA), transported to the laboratory in ice–saline, and used within 6 hours postmortem. The fluid used in the perfusion experiments was Dulbecco's phosphate-buffered saline (PBS) (Life Science Technology, Chagrin Falls, OH) with 5.5 mM glucose, passed through a 0.2-μm filter, with bovine serum (Sigma Chemical Company, St. Louis, MO) added to the appropriate concentration for that experiment. For series A described below, the serum was defrosted and ultracentrifuged at 100,000g for 30 minutes to remove any precipitates that might have formed during the freezing process, and then it was added to the buffer. For series B, the serum was defrosted and added to the buffer; then the solution was filtered through a 0.1-μm filter.

Perfusion Procedure

Series A. A preliminary series of perfusions on six pairs of bovine eyes was conducted to determine whether serum proteins had an effect on aqueous outflow resistance and the wash-out phenomenon, and to establish the concentration of serum proteins at which effects on aqueous outflow dynamics became significant. The perfusion procedure followed that described in Johnson, Chen, Epstein, and Kamm[14]: in brief, a needle was placed through the cornea to introduce the perfusion fluid into the anterior chamber of the eye, anterior chamber deepening was prevented with an iridotomy, and the temperature was held at 34°C. For each experiment on a pair of eyes, two different concentrations of bovine serum were used (10% serum in buffer versus 1%, n = 1; 15% versus 5%, n = 1; 12% versus 0%, n = 3; 8% versus 4%, n = 1). The eye receiving the higher concentration was perfused at a constant pressure of 15 mm Hg, whereas the eye receiving the lower concentration was perfused at a flow rate identical to that received by the fellow eye. This scheme was selected to maintain both eyes at relatively low pressure while ensuring that both eyes received the same total volume of perfusion fluid. (We have shown previously[14] that the wash-out effect is a function of the total volume of perfusate passing through the aqueous outflow pathway.) After perfusion, an immunohistochemical investigation of the albumin distribution in the anterior segment was performed (see below).

Series B. After it was established that a concentration of 15% serum in buffer seemed to greatly reduce or eliminate the wash-out phenomenon, a second series of tests was conducted to established whether a statistically significant difference could be established between perfusion with 15% serum in buffer and perfusion with buffer alone. The experiments were conducted at a perfusion pressure of 15 mm Hg in both experimental and control eyes, with the perfusion needle placed in the posterior chamber (34°C). The eyes were randomized as to which perfusion system was used for each experiment. For the first six pairs of eyes, a total of 5 ml perfusion fluid was passed through the eyes, whereas for the next five pairs of eyes, 10 ml perfusion fluid passed through the eyes. The wash-out rate was calculated with a linear regression for outflow facility as a function of volume perfused (data were recorded every 10 seconds); the first 1 ml that entered the eye during the equilibration period was ignored.

Immunolight Microscopic Examination

Perfused and unperfused bovine eyes were dissected with an equatorial incision and immersed in Carnoy's fluid for 12 to 18 hours. Then they were processed for paraffin embedding. (One pair was fixed at the abattoir immediately postmortem.) Five-micron sections of bovine anterior chamber tissues then were deparaffinized, rehydrated in PBS (pH 7.3) for 10 minutes, and incubated with the primary antibody (monoclonal antibovine serum albumin, Sigma) for 2 hours at room temperature. Next, the sections were washed three times in PBS containing 0.01% Tween 20 (Sigma, St. Louis) and then incubated with the secondary antibody (5 nm gold-conjugated rabbit antimouse IgG, Amersham, Arlington Heights, IL) for 4 hours at room temperature. The sections again were washed three times in PBS containing 0.01% Tween 20, and a silver enhancement solution (Amersham) was applied to the sections for 8 to 15 minutes. After they were washed, the sections were counterstained with hematoxylin and coverslipped with Permount (Fisher Scientific, Fair Lawn, NJ). Photographs were taken with a Leitz (Wetzlar, Germany) photomicroscope. Control studies included preadsorption of antialbumin with bovine albumin and substitution of the primary antibody with PBS or mouse IgG.

Electron Microscopic Examination

Conventional Procedures. In several perfused eyes, half or all of the eye was fixed in 2% paraformaldehyde–2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 3 hours at room temperature. They were rinsed in the same buffer and cut radially into small pieces. The specimens were postfixed in 1% osmium tetroxide and 1.5% potassium ferrocyanide for 2 hours, dehydrated in a graded series of ethanols, and embedded in Epon-Araldite (Electron Microscopy Science, Washington, PA). Semithin sections for light microscopic examination were cut so that the orientation of the specimens could be examined. Ultrathin sections were cut, counterstained with uranyl acetate and lead citrate, and examined with a Philips-300 Electron Microscope (Eindhoven, The Netherlands).

Cuprolinic Blue Staining Procedures. The anterior segments of two unperfused eyes and two eyes perfused for 5 hours with Dulbecco's PBS (without protein added) were fixed in 2.5% glutaraldehyde in 25 mM sodium acetate buffer (pH 5.7) for 1 hour, cut radially into small pieces, and transferred into a mixture of 0.2% cuprolinic blue, 0.3 M $MgCl_2$, and 2.5% glutaraldehyde in 25 mM sodium acetate buffer (pH 5.7) and stained for 20 to 24 hours at room temperature. After they were rinsed with the same buffer, the specimens were washed in aqueous 0.5% sodium tungstate and then alcoholic (50% v/v) 0.5% sodium tungstate, dehydrated, and embedded in Epon-Araldite. Semithin sections for light microscopic examination were cut so that the orientation of the specimens could be examined. Ultrathin sections were cut, counterstained with uranyl acetate, and examined with a Philips-300 Electron Microscope (Eindhoven, The Netherlands).

RESULTS

Perfusion Studies

Figure 1 shows typical results from the perfusion studies in series A (only selected results are shown to avoid overlap of the curves; all results showed similar trends). The rate of increase of outflow facility with perfused volume (wash-out) decreased as the serum concentration used to perfuse the eyes was increased. In each of these six pairs of eyes, the wash-out rate was

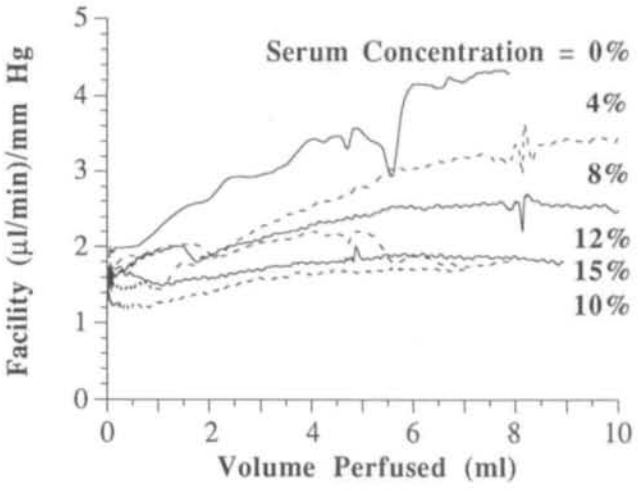

FIGURE 1. Outflow facility as a function of perfusion volume with variations in the concentration of bovine serum in Dulbecco's PBS from 0% to 15%.

higher in the eye perfused with the lower concentration of serum proteins.

These findings suggested that wash-out could be minimized (and in some eyes eliminated) by perfusing these eyes with a concentration of approximately 15% serum in buffer. A second series of studies (series B) was conducted to quantify this effect (Table 1). In six pairs of eyes perfused with 5 ml 15% serum in buffer, the wash-out rate was found to be not statistically significantly different than zero ($P > 0.3$) and significantly lower than that of the control eyes perfused with buffer alone ($P < 0.05$) (two-sided Student's t-test). In five separate pairs of eyes perfused with 10 ml 15% serum in buffer, the wash-out rate again was found to be significantly lower than that of the control eyes perfused with buffer ($P < 0.02$).

Immunohistochemical Studies

Preadsorption of the primary antibody with albumin (Fig. 2A), or substitution with mouse IgG or PBS, resulted in no staining in anterior chamber tissues. Four unperfused bovine eyes were investigated to determine the normal distribution of serum albumin in the bovine outflow pathway. Albumin was found consistently in the trabecular meshwork, cornea, iris, and ciliary body (Figs. 2B, C). A substantial quantity of albumin was found in the outflow pathways that appeared to originate in the ciliary body region. The distribution of albumin was the same in a pair of eyes fixed immediately postmortem at the abattoir. There was also no difference in the distribution of albumin in the outflow pathway if the eye was allowed to sit, unperfused, in buffer for 5 hours (data not shown).

The albumin distribution also was examined in three pairs of eyes after perfusion. Compared with that in unperfused calf eyes (Fig. 2), the amount of serum albumin in the trabecular meshwork, iris, and ciliary body was decreased substantially after prolonged perfusion with protein-free buffer (Fig. 3A). After perfusion with 5%, 12%, and 15% bovine serum in buffer, however, the albumin concentration in the trabecular meshwork, iris, and ciliary body (Fig. 3B) was similar to that in unperfused eyes.

Electron Microscopic Examination

With conventional tissue-preparation methods, ultrastructural examination of the aqueous outflow pathway showed no obvious differences between eyes perfused with protein-free buffer for 5 hours (Fig. 4A) and those perfused with 12% serum in buffer for 5 hours (Fig. 4B); no evidence of protein accumulation or blockage was found.

We have shown previously[10] that the cuprolinic blue technique can be used to investigate the distribution of sulfated proteoglycans in the trabecular meshwork. Using this technique to investigate differences in sulfated proteoglycans in the bovine trabecular meshwork between unperfused eyes and eyes perfused for 5 hours with buffer, we could not detect a change in the sulfated proteoglycans (Figs. 5A, B).

DISCUSSION

Recent evidence[12,13] indicates that an appreciable fraction of the protein in the aqueous humor enters the anterior chamber by diffusion through the root of the iris. Additional confirmation of this pathway has been obtained recently with magnetic resonance imaging[15] and modeling techniques.[16] An important implication of this pathway is that the protein concentration of the aqueous humor within the trabecular meshwork may be much higher than the concentration found in the anterior chamber. From the previous work of Epstein, Hashimoto, and Grant,[17] we know that perfusion of serum proteins, in high concentrations, can increase aqueous outflow resistance significantly. Until now, it generally was assumed that soluble protein levels greater than 1% of plasma levels in the trabecular meshwork would be nonphysiological. Because the exit portal of this newly described pathway is immediately proximal to the entrance to the trabecular meshwork, it would be expected that a substantially higher concentration of the plasma-derived proteins normally would be entering the trabecular meshwork under physiological conditions.

We recently described a model[18] that shows how mobile serum proteins in a perfusion fluid might generate enhanced flow resistance as they pass through the extracellular matrix. As the proteins in the aqueous humor pass through the trabecular meshwork, they will be hindered in their transport by interactions and entanglements with the resident extracellular matrix.[19] These proteins tend to move more

TABLE 1. Effect on Wash-Out Rate of 15% Serum in Buffer Compared with Buffer Alone

Total Amount Perfused (ml)	N *(pairs)*	*dC/dV (μl/min/mmHg)/(ml perfusate)*		*Statistical Significance (P value)*
		15% Serum in Buffer	*Buffer Alone*	
5	6	0.0498 ± 0.0428	0.1677 ± 0.0271	<0.05
10	5	0.0533 ± 0.0294	0.1813 ± 0.0342	<0.02

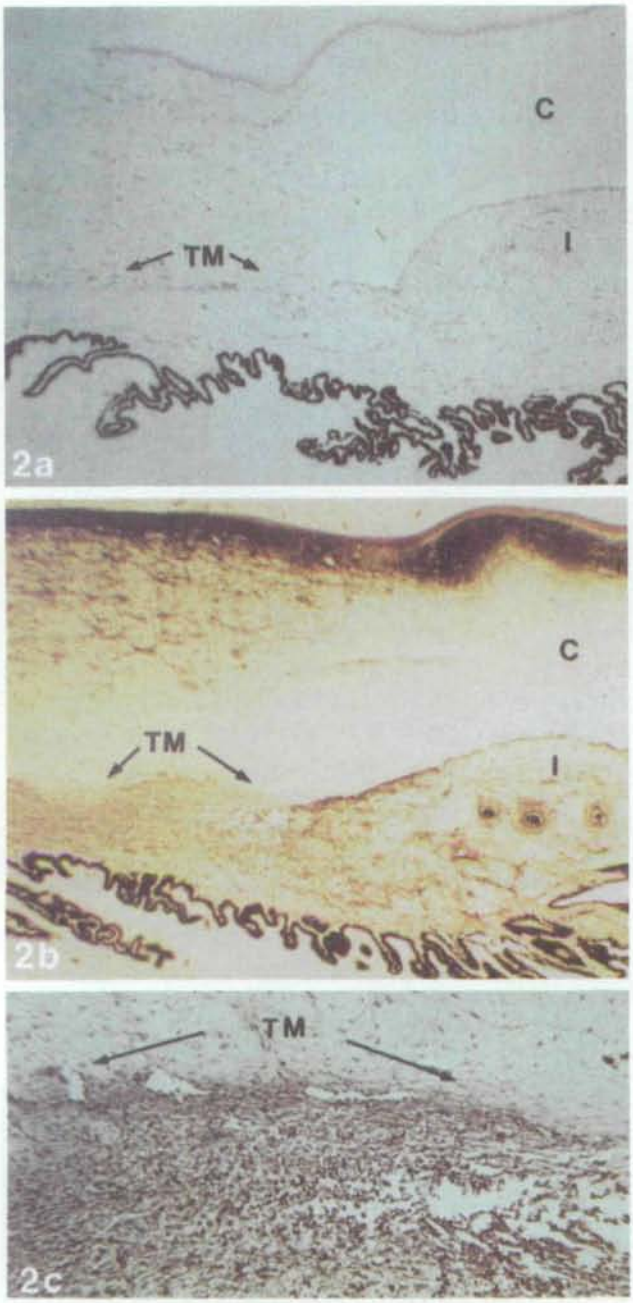

FIGURE 2. Distribution of albumin in an unperfused bovine eye fixed immediately on arrival from the abattoir. (**A**) Control showing primary antibody preadsorbed with albumin. (**B**) Anterior segment. (**C**) Trabecular meshwork. Brown areas are immunostained for albumin (TM: trabecular meshwork; C: cornea; I: iris). (**A**, ×23.4; **B**, ×23.4; **C**, ×107.25.)

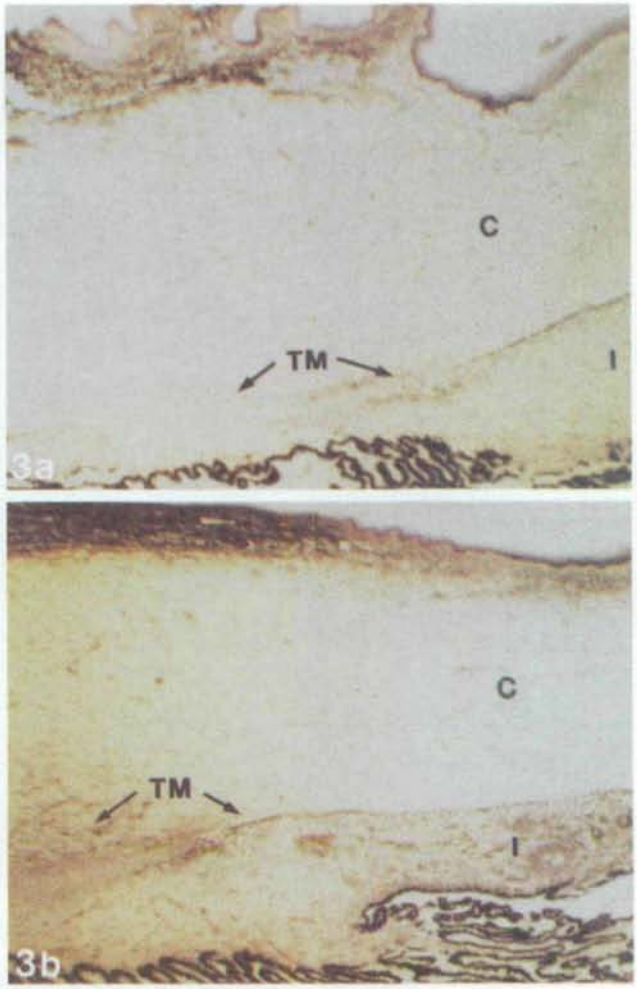

FIGURE 3. Distribution of albumin in the anterior segment of a bovine eye (**A**) perfused for 5 hours with PBS or (**B**) perfused for 5 hours with 12% serum in PBS. (TM: trabecular meshwork; C: cornea; I: iris.) Magnification ×23.4.

slowly than the saline in which they are carried; they also tend to accumulate locally. The difference in speed between the saline and protein will generate a flow resistance that augments the local flow resistance caused by viscous interactions with the stationary components of the extracellular matrix, and this effect is increased by the local accumulation of protein.

The protein delivered by the pathway from the ciliary body stroma offers a plausible explanation of the wash-out effect. Among the puzzling aspects of the wash-out phenomenon has been that wash-out occurs even if aqueous humor is used as the perfusion fluid[4] and even if physiological levels of pressure drop are used.[14] In light of this, how is outflow resistance in vivo maintained at a constant level? We hypothesized that, if the protein concentration in the aqueous humor entering the trabecular meshwork was higher than that in the anterior chamber, because of protein diffusion from the root of the iris, this protein could generate significant flow resistance in the trabecular meshwork as it was retarded by the extracellular matrix. Previous perfusion studies have used either protein-free media or aqueous humor collected from the anterior chamber. Thus, as the protein depot in the root of the iris was depleted during perfusion (either by the high flow rate in an in vivo experiment or by the lack of

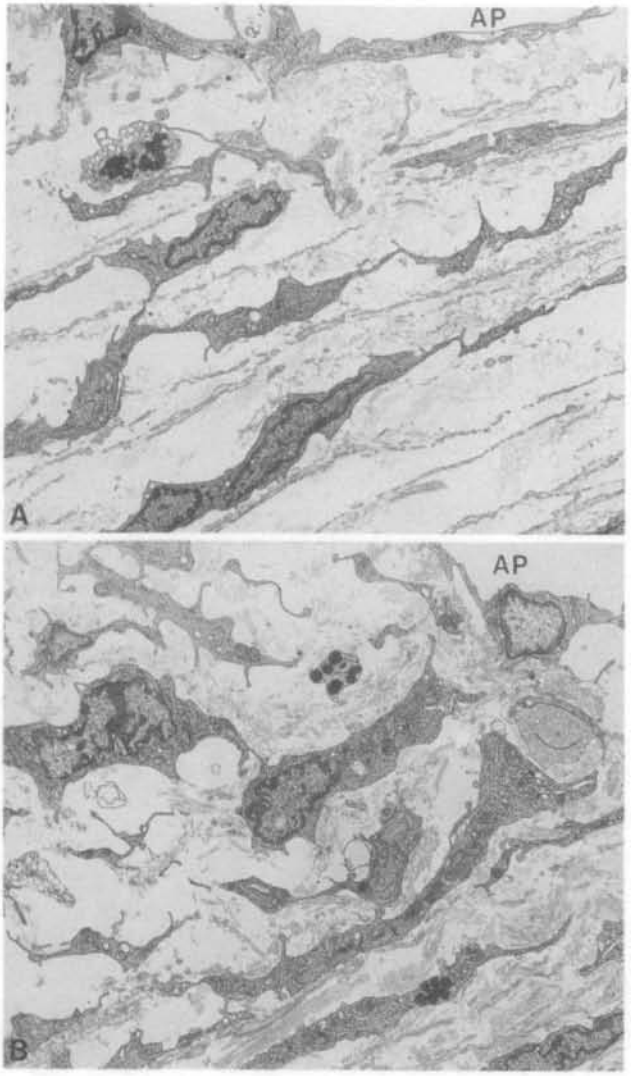

FIGURE 4. Ultrastructure of trabecular meshwork of a bovine eye (**A**) perfused for 5 hours with buffer or (**B**) perfused for 5 hours with 12% bovine serum in PBS. (AP: aqueous plexus.) Magnification ×3795.

blood flow replenishing the protein depot in an enucleated eye), the protein concentration entering the trabecular meshwork decreased as a function of time (or, more precisely, as a function of perfusion volume[14]) and the flow resistance of the system decreased progressively.

Based on this hypothesis, we conducted experiments in which enucleated bovine eyes were perfused with varying concentrations of bovine serum to investigate the effects of increased protein levels on aqueous outflow resistance. Our results (Fig. 1) showed that the eyes perfused with lower concentrations of serum invariably exhibited higher wash-out rates. The wash-out rate was significantly lower in the eyes perfused with 15% serum in buffer as compared with those perfused with buffer alone, and the wash-out rate was not significantly different from zero (Table 1).

To determine whether this reduced wash-out resulted from a nonphysiological accumulation of serum proteins, we examined the distribution of albumin in the outflow pathway as an indicator of the extent of accumulation of serum proteins in this region. Immunohistochemical evaluations of unperfused bovine eyes showed a substantial quantity of albumin in the outflow pathway that appeared to originate at the root of the iris (Fig. 2). Although eyes perfused with only buffer for 5 hours showed almost complete elimination of this albumin from the aqueous outflow system (Fig. 3A), eyes perfused with concentrations of 10% to 15% serum in PBS showed an albumin distribution (Fig. 3B) very similar to that of unperfused bovine eyes. These results are consistent with our hypothesis that wash-out is a consequence of diluting the concentration of plasma-derived protein within the trabecular meshwork resulting from depletion of protein stores in neighboring tissues.

So far, we have not determined which component of serum is responsible for the reduced wash-out rate in these enucleated bovine eyes; however, albumin has been shown previously to have an important effect on

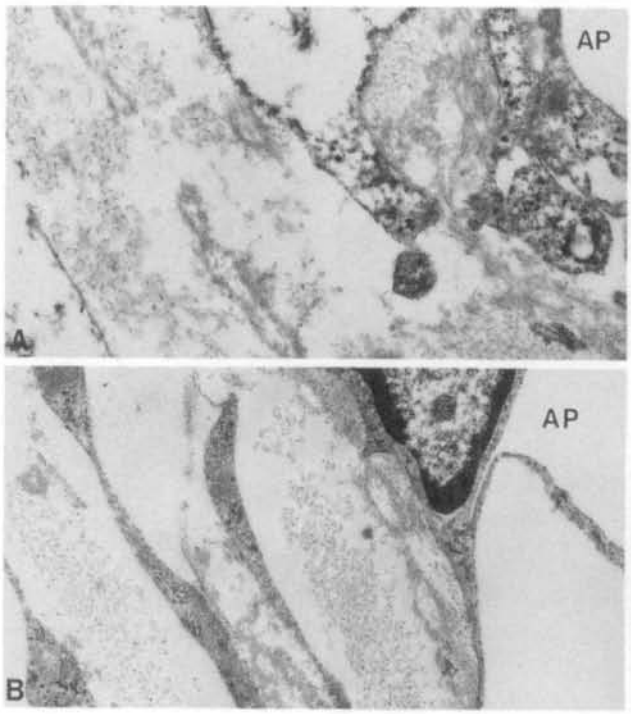

FIGURE 5. Cuprolinic blue staining of calf trabecular meshwork. (**A**) Unperfused eye. (**B**) Calf eye perfused for 5 hours with Dulbecco's PBS with 5.5 mM glucose. The black stick-like figures decorating the extracellular matrix are the sulfated proteoglycans; no difference is seen between the perfused and unperfused meshworks. (AP: aqueous plexus.) Magnification ×15,000.

the hydraulic resistance of vascular endothelia.[20] When the vascular system is perfused with buffers with an albumin concentration less than 1 mg/ml (approximately 2% of the protein content of plasma), the flow resistance of the endothelial junctions is reduced dramatically; however, if the protein content of the perfusion fluid is kept above this critical level, the flow resistance of vascular endothelia is relatively insensitive to the albumin level.[20] This latter finding contrasts with our results in the aqueous outflow pathway, in which we observed a progressive decrease in the wash-out rate as the serum concentration in the perfusion fluid was increased to approximately 15% of the protein content of plasma; however, it is important to observe that serum has many components, and careful study is necessary to determine which of the components might be involved in the generation of aqueous outflow resistance.

These results suggest a new interpretation of the effects of glycosaminoglycan-degrading enzymes on outflow facility. These enzymes may exert their effect on outflow facility in part by removing the protein-trapping capacity of the extracellular matrix and, thus, secondarily eliminating this protein layer. This would explain the dual observations that wash-out and glycosaminoglycan-degrading enzymes appear to affect the same site of flow resistance in the outflow pathway,[4,21] and yet wash-out does not remove glycosaminoglycans from this region. Our results with cuprolinic blue staining (Fig. 5) when we compared unperfused bovine eyes with eyes perfused for 5 hours with buffer showed that sulfated proteoglycans are not lost during the perfusion, confirming previous biochemical evidence.[8] These results may seem contrary to those of earlier studies suggesting that glycosaminoglycans are responsible for the bulk of aqueous outflow resistance[4,8]; however, evidence in most other connective tissues suggests that proteins (primarily proteoglycan core proteins), rather than glycosaminoglycans, are responsible for the bulk of connective tissue flow resistance.[2,22,23]

In this context, it is important to realize that different glycosaminoglycans may have potentially different effects on outflow resistance. Three classes of glycosaminoglycans can be identified in the aqueous outflow pathway: collagen-associated glycosaminoglycans (small chondroitin and dermatan sulfate proteoglycan), open-space glycosaminoglycans (large dermatan sulfate proteoglycan and hyaluronic acid), and basement membrane glycosaminoglycans (heparan sulfate proteoglycan)[24]; only the latter two would be expected to entangle aqueous proteins as they pass through the outflow pathway. We recently observed[10] an age-related loss of collagen-associated glycosaminoglycans in the human trabecular meshwork. Loss of collagen-associated glycosaminoglycans might be expected to give very different long-term results than loss of the other classes of glycosaminoglycans. Although loss of the open-space or basement membrane glycosaminoglycans might be expected to decrease outflow resistance (resulting from a decreased protein-trapping capability), a loss of collagen-associated glycosaminoglycans might increase the surface hydrophobicity of the extracellular matrix, thereby leading to a nonspecific accumulation of lipophilic proteins, increasing outflow resistance.[10]

Our results not only provide a physical basis for the wash-out effect, but also show that aqueous outflow resistance can be generated by proteins in the aqueous humor as they pass through the trabecular meshwork. To further investigate this phenomenon, we assumed that the dominant proteins in serum (albumin and globulins) are responsible for this flow resistance and that the hydrodynamic properties of albumin can be used to estimate the hydraulic effects of the serum proteins. We then used a recently developed model[18] to describe the flow resistance of albumin passing through an immobilized matrix of macromolecules. We assume the protein content of the aqueous humor in the bovine trabecular meshwork is approximately 15% that of bovine serum (7.5 mg/ml) (based on the approximate concentration needed to prevent wash-out), and the extracellular matrix and mobile plasma-derived protein in the aqueous humor are each responsible for approximately half of the measured outflow resistance in the in vivo bovine eye (after wash-out, the flow resistance of the system is roughly halved; see Fig. 1). Given these two constraints, the model predicts that the glycosaminoglycan concentration in the open spaces of the trabecular meshwork would need to be approximately 0.5 mg/ml; this is consistent with values assumed by other investigators.[2]

We used Darcy's law§ to compare the flow resistance of this hypothetical matrix with that measured experimentally. A 15% solution of albumin passing through a glycosaminoglycan matrix with a concentration of 0.5 mg/ml would have a specific hydraulic conductivity of 1×10^{-11} cm[18,25]; we considered this value representative of the serum proteins used in our experiments. Then, using Darcy's law, along with an outflow facility ($Q/\Delta P$) of 1.5 μl/min/mm Hg for the bovine eye, a viscosity of 0.74 centipoise (saline at 34°C), and a cross-sectional area facing flow of 0.125 cm^2 (the cross-sectional area for flow of the trabecular mesh-

§ Darcy's law describes the pressure decrease (ΔP) resulting from a flow (Q) per cross-sectional area (A) through a porous medium of length L: $\Delta P/Q = \mu L/KA$, where K is the "specific hydraulic conductivity" or "permeability coefficient" in Darcy's law (the units are square centimeters); each connective tissue has a characteristic value of K. Levick[23] has shown that this law can be used to describe the flow resistance of a wide variety of connective tissues.

work of a bovine eye, assuming that only half of the meshwork area is available for flow[26]), we determined the length of the matrix generating flow resistance to be approximately 100 μm, or very roughly the length of the bovine trabecular meshwork.[26] Thus, combining our experimental results with a model describing the flow resistance of serum proteins passing through an extracellular matrix[18] suggests that a thick extracellular matrix of low concentration would be necessary for proteins in the aqueous humor to generate a significant fraction of aqueous outflow resistance.

An important caveat to this calculation is that we have not included the "funneling" phenomenon recently described,[27] whereby the pores of the inner wall endothelium interact hydrodynamically with the tissue/matrix layer underlying the pores to significantly increase outflow resistance. This interaction is increased in complexity by the mobile nature of the plasma-derived proteins generating outflow resistance. The general effect of this interaction would be to increase the resistance of an extracellular matrix of given thickness and thereby reduce the length of this matrix necessary to generate the observed aqueous outflow resistance. In any case, our calculations support the concept that plasma proteins can generate a significant fraction of aqueous outflow resistance.

The human aqueous outflow system does not exhibit wash-out, in contrast to all other species investigated.[9] In the context of the above-mentioned model, there are several possibilities that may explain this difference: (1) The extracellular matrix in the trabecular meshwork of the human eye may be less dense and therefore less capable of trapping aqueous humor protein; this possibility is suggested by studies showing variable and inconclusive effects of hyaluronidase on outflow facility in the human eye as opposed to results in other species[6,21,28] and by results of microsphere transport studies[26] showing that microspheres pass through the human outflow system much more easily than that of the bovine. (2) The protein depot in the root of the human iris and ciliary body either has a greater capacity to accumulate protein or is depleted more slowly. (3) Only minor amounts of protein enter the human trabecular meshwork through the root of the iris, and protein wash-out of this region is of less consequence to outflow resistance. Finally, we note that the human aqueous outflow system is unique in one further aspect, compared with other species, in its vulnerability to primary open-angle glaucoma.[29]

Key Words

aqueous outflow resistance, extracellular matrix, serum proteins, hydraulic conductivity, albumin

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