The Source of Protein in the Aqueous Humor of the Normal Monkey Eye

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In vivo aqueous fluorophotometry, morphology, and computational modeling were combined to examine the source of protein and the pathway by which protein enters the aqueous humor of monkeys. A computational model was developed to determine the likelihood of a diffusional route for delivering plasma proteins from ciliary body capillaries via the iris to anterior chamber aqueous humor, bypassing the posterior chamber. Model predictions were compared to aqueous fluorophotometric data obtained from monkeys following a single intravenous injection of fluoresceinated horseradish peroxidase (F-HRP, 250 µg/kg body mass). Model predictions of the magnitude and time course of anterior chamber F-HRP concentration agree with the fluorophotometric measurements. For example, of anterior chamber F-HRP concentration as a percentage of initial plasma F-HRP concentration at 90 min and 180 min post-injection was predicted to be 0.02% and 0.05%, respectively, and was measured to be 0.01–0.03% and 0.03–0.06%, respectively. In addition, model predictions in the case of a constant plasma protein level also are consistent with experimental data. The steady-state anterior chamber total protein concentration as a percentage of plasma protein concentration was predicted to be 0.2% and was assayed to be 0.05–0.2%. As in our previous study of the normal rabbit eye, morphologic and tracer localization evidence combined with the good agreement between model predictions and experimental data lead to the conclusion that a significant amount of the plasma protein normally present in monkey aqueous humor originates in ciliary body capillaries and diffuses anteriorly through the iris and into the anterior chamber. Invest Ophthalmol Vis Sci 33:581–595, 1992

Although the concentration of aqueous humor protein is less than 1% of that in plasma, evidence is accumulating that at least some of these proteins may be involved in ocular pathophysiology. In particular, aqueous humor protein concentration increases in the presence of anterior uveitis,1–4 perhaps providing an objective measure of the disease severity and response to therapy. Aqueous humor proteins also have been identified as suspected participants in the obstruction of the aqueous humor outflow network from the eye in open-angle glaucoma.5 In addition, techniques that noninvasively measure aqueous humor protein concentration are being developed to determine aqueous humor flow.6–8 The physiology of aqueous humor protein content under normal conditions in primates, however, is not yet understood. Aqueous humor protein dynamics in the normal eye must be understood before the relationships between pathophysiology and aqueous humor protein content can be elucidated. The basic goal of this investigation was to examine and determine in monkeys, under normal conditions, where aqueous humor proteins originate and how they reach the aqueous humor.

The blood-aqueous barrier prevents the free exchange of proteins between plasma and aqueous humor. The anatomical components of this barrier are zonulae occulentes, or tight junctions, that connect adjacent nonpigmented ciliary epithelial cells9–12 and adjacent iridal vascular endothelial cells.11,13,14 However, no epithelium or endothelium covers the anterior iris surface. Raviola11 suggested that plasma proteins leak from ciliary body capillaries and move through iris stroma to the anterior iris surface, where they enter the anterior chamber aqueous humor. Freddo15 confirmed a portion of this pathway in rhesus monkeys, from ciliary body stroma to posterior iris stroma, and also documented the existence of tight junctions in the posterior iris epithelium. More recently, we have shown in rabbits that a significant
fraction of protein normally present in aqueous humor originates in the ciliary and iridial processes and diffuses anteriorly through iris stroma to the anterior chamber.\textsuperscript{16}

The hypothesis examined in the current investigation is that aqueous humor proteins in primates originate from ciliary body capillaries, are prevented from entering the posterior chamber by the tight junctions of the ciliary body epithelium, and diffuse through the iris root stroma into the aqueous humor present in the anterior chamber. In vivo aqueous fluorophotometry and tracer localization studies are combined on the same monkey eyes using a single tracer protein to acquire the experimental data. A computational model that simulates the anteriorly directed protein diffusion from the ciliary body stroma to the anterior chamber aqueous humor is developed for the monkey eye with parameter values estimated independently from experimental data. The fluorophotometric and tracer localization data are combined in a complementary manner with computational simulations of the experiments to determine the validity of the hypothesis.

Materials and Methods

Animal Experiments

Eight adult owl monkeys (\textit{Aotus trivirgatus}, 0.69-1.0 kg) were used in this investigation. All animals were treated in accordance with the ARVO Resolution on the Use of Animals in Research.

\textbf{Fluorescent tracer protein:} The tracer protein used in the experiments was fluorescein-labeled horseradish peroxidase (F-HRP, molecular weight \(\approx 42,000\) daltons), which was custom-conjugated for Freddo et al.\textsuperscript{16} F-HRP permitted the fluorophotometric and tracer localization studies to be performed on the same eyes.

\textbf{Aqueous fluorophotometry:} The animals were anesthetized with ketamine HCl (10 mg/kg body mass, intramuscularly), and the inguinal regions were subcutaneously injected with lidocaine HCl sodium (15 mg/1.5 ml). Both femoral veins were catheterized. The animals received sodium heparin (100 U/kg, intravenously). Supplemental anesthetic, pentobarbital sodium (5 mg/kg body mass, intravenously), was administered as needed.

After catheterization, each animal was laid onto a platform with its head positioned in front of the fluorophotometer.\textsuperscript{17,18} Anterior-to-posterior fluorophotometric baseline scans of the anterior segment were made along the pupillary axis to record anterior chamber fluorescence prior to tracer injection. F-HRP (250 mg/kg body mass) dissolved in 2 ml pyrogen-free saline was injected intravenously over a 2 min period via one of the catheterized femoral veins. Axial fluorophotometric scans of the anterior chamber were then made repeatedly to measure F-HRP content in the anterior chamber. One to 4.5 hr after F-HRP injection (post-injection), the animals were killed with an overdose of pentobarbital sodium (100 mg/kg body mass, intravenously). The eyes were enucleated and immediately immersed in fixative—phosphate-buffered 2\% paraformaldehyde and 2.5\% glutaraldehyde—for the morphologic and tracer localization studies.

During the fluorophotometric experiments at 5, 20, 40, and 60 or 90 min post-injection, blood (1 ml) was withdrawn into heparinized syringes. A blood sample prior to F-HRP injection also was obtained from three of the monkeys. The femoral vein that did not receive the F-HRP injection was used for blood sampling. These samples were centrifuged at 2000 \(\times\) g for 10 min at 4°C. The plasma was removed, diluted 1:50 with phosphate-buffered saline, and scanned with the fluorophotometer for its fluorescence. In addition, the plasma obtained prior to F-HRP injection was assayed for its total soluble protein content (Bio-Rad Protein Assay; Bio-Rad, Richmond, CA) with bovine serum albumin (BSA, Fraction V; Sigma Chemical Co., St. Louis, MO) as the standard.

At the conclusion of aqueous fluorophotometry, 150-200 \(\mu\)l of aqueous humor was withdrawn via a 25-30 G needle from the anterior chambers of six of the monkeys. Aqueous humor samples were centrifuged at 600 \(\times\) g for 1 min at 4°C. The total soluble protein concentration in the aqueous humor was measured in the same manner as that of the plasma samples.

Analysis of the fluorophotometric data included accounting for background-protein effects on fluorescence when necessary.\textsuperscript{18} The anterior chamber fluorophotometric scans were smoothed, and the average fluorescence was calculated from eight equidistant points. The baseline anterior chamber fluorescence of each eye (measured prior to F-HRP administration) was subtracted from subsequent fluorophotometric data acquired from the same eye. Anterior chamber F-HRP concentration then was calculated using the appropriate calibration curve obtained from serially diluted F-HRP solutions. The anterior chamber aqueous humor sampled from the monkeys was measured as having a total protein concentration in the range of 0.030-0.10 mg/ml, with an average of 0.049 mg/ml, a level documented to have negligible effects on tracer fluorescence.

Plasma F-HRP concentrations, taking into account the plasma dilution with phosphate-buffered saline, were obtained in a similar manner. Because background-protein effects were significant, the plasma F-
HRP concentration was determined with the appropriate calibration curve for the background-protein content. The total undiluted plasma background-protein content was measured at 40–60 mg/ml and was assumed to be 60 mg/ml in animals that did not have blood sampled prior to F-HRP injection.

**Morphology and tracer localization:** Fixed, enucleated eyes were washed with phosphate buffer at 4°C. The uvea was separated from the corneo-scleral tunic, and 150-μm thick radial sections were serially made with a Smith-Farquhar tissue chopper (DuPont Sorvall, Wilmington, DE). These chopper sections were treated for horseradish peroxidase demonstration, postfixed in 1.0% osmium tetroxide and 1.5% potassium ferrocyanide, dehydrated in a graded series of ethanol, and embedded in an Epon-Araldite mixture (EMS, Fort Washington, PA). Thick sections were cut with a glass knife, stained with toluidine blue, and examined with a Leitz Orthoplan photomicroscope (Leitz, Wetzlar, Germany). Thin sections were cut with a diamond knife on an LKB ultramicrotome (LKB Instruments, Rockville, MD), mounted on uncoated copper grids, and examined—unstained or stained with uranyl acetate and lead citrate—with a Philips-300 transmission electron microscope (Philips, Eindhoven, The Netherlands).

**Computational Model**

**Anatomical domain:** A computational model was developed to determine the potential of an anteriorly directed diffusion route via the iris by which plasma proteins may reach anterior chamber aqueous humor. The model domain includes the ciliary processes, the portion of the ciliary body stroma along and between ciliary process stroma, and the entire iris. A schematic of the relevant portion of monkey anterior segment anatomy is shown in Figure 1. The ciliary processes are modeled as radially oriented rectilinear fin-like extensions that protrude into the posterior chamber about the pupillary axis. A continuous, circumferential band of ciliary body stroma interfaces with the discrete processes.

The protein source is modeled as a network of uniformly distributed capillaries in the posterior portion of the ciliary body and process stroma, indicated by the cross-hatched region in Figure 1. Protein leaking from these capillaries into interstitium is assumed to diffuse anteriorly through stroma toward the root of the iris. Upon reaching the anterior iris surface, protein enters the anterior chamber aqueous humor. Protein is presumed to leave the anterior chamber with aqueous humor outflow, principally through the angle region.

**Analysis:** The rate at which plasma protein leaks from the fenestrated ciliary capillaries is assumed to be proportional to the difference between the protein concentration in the plasma and that in the ciliary interstitium. After leaving the capillaries, this protein is assumed to be transported through the ciliary and iris stroma solely by molecular diffusion down a concentration gradient (Fick’s first law of diffusion). The protein transport is modeled as two-dimensional; x and r are the anterior and radial directions, respectively, defined in Figure 1.

The local interstitial concentration of protein in a volume element of stroma is affected by (1) the local protein influx from the capillaries within the element and (2) protein exchange by diffusion across the surfaces of the elements. This is expressed by an equation that represents the mass balance of protein in a rectilinear element of ciliary process stroma:

\[ \frac{\partial C}{\partial t} = K_{rad} (C_p - C) + D_x \frac{\partial}{\partial x} \left( \frac{\partial C}{\partial x} \right) + D_r \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial C}{\partial r} \right) \]  

and by a mass balance of protein in a cylindrical stromal element of the continuous circumferential band of ciliary body or iris:

\[ \frac{\partial C}{\partial t} = K_{rad} (C_p - C) + D_x \frac{\partial}{\partial x} \left( \frac{\partial C}{\partial x} \right) + D_r \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial C}{\partial r} \right) \]  

where \( C \) is the time- and space-dependent protein concentration in the interstitial fluid of ciliary or iris stroma, \( C_p \) is the time-dependent protein concentration in the plasma.
tion in plasma, \( K_{\text{rate}} \) is the transcapillary exchange rate constant, and \( D \) is the molecular diffusivity of protein within the interstitial space of the stroma. Term (a) represents the rate of change of protein mass contained within a stromal element of volume. Term (b) is the source term, corresponding to the rate at which protein is introduced into the element as a result of transcapillary exchange. No protein is assumed to leak from the iris vasculature. Consequently, term (b) is set to zero in iris stroma. In addition, no significant protein leakage occurs from the limited number of capillaries in the region connecting the iris to the posterior portion of the ciliary body and processes. Therefore, term (b) is set to zero within the entire unshaded region in Figure 1. Terms (c) and (d) correspond to the total net flux of protein into the element resulting from molecular diffusion. Because the objective is to investigate the potential of the anterior pathway via iris stroma to anterior chamber aqueous humor, no protein is assumed to leak across the ciliary or iris epithelium along the surfaces exposed to the posterior chamber. Also, no protein flux is assumed to occur between the ciliary body stroma and the adjacent dense and complex ciliary muscle structure, which, because of its relatively small interstitial volume fraction, possesses a lower permeability to protein than the loose connective tissue of the ciliary body and process stroma depicted in Figure 1.

The mass balance of protein in the anterior chamber is represented by the following equation:

\[
\frac{V_a}{\Delta t} \frac{dC_a}{d\ell} = -D(A_\ell \phi) \frac{\partial C_a}{\partial x} - fC_a
\]

where \( a \) denotes the anterior chamber, \( C_a \) is the anterior chamber protein concentration, \( I \) denotes iris stromal values, \( C_I \) is the interstitial protein concentration in the iris, \( A_\ell \) is the total cross-sectional area of iris stroma across which diffusion occurs, \( \phi \) is the interstitial volume fraction, and \( f \) is the aqueous humor outflow rate. The rate of change of protein mass in the anterior chamber, term (a), equals the difference between the rate of protein influx from the anterior iris surface, term (b), and the rate of protein outflow via the anterior chamber angle, term (c). As already discussed, the posterior chamber aqueous humor entering the anterior chamber through the pupil is assumed to contain no protein. Also, the anterior chamber is assumed to be well mixed.

Equations (1)–(3) are solved numerically with finite differences. \(^{20} \) Centered differences are used at interior nodes of process and iris stromal elements, and one-sided differences are used at surface nodes of stromal elements. The initial protein concentration in the process stroma, iris stroma, and anterior chamber is set to zero. The boundary conditions are the following. (1) At the posterior surface of the processes, the protein flux is zero (this applies to all surfaces directly exposed to posterior chamber aqueous humor). (2) At the anterior surface of the iris, the interstitial protein concentration is equal to the anterior chamber protein concentration.

The plasma protein concentration can be appropriately modeled for the particular conditions and protein examined. In this investigation, the plasma protein concentration is modeled in two different ways. To simulate the aqueous fluorophotometry experiments with F-HRP—the case of a single intravenous tracer injection exponentially eliminated from plasma—the plasma concentration can be described by the following relation:

\[
C_p = C_{p0} e^{-\frac{t}{\tau}}
\]

where \( C_p \) is the plasma protein concentration, \( C_{p0} \) is the initial plasma concentration, and \( \tau \) is the time decay constant of protein clearance from the plasma. To simulate the case of a normal steady level of plasma protein concentration, \( C_p \) is set equal to a constant value.

Parameters: The geometry and dimensions of the monkey model domain (Fig. 1) were determined by light microscopy of tissue specimens from the eyes used in the fluorophotometric experiments. The measured dimensions are given in Figure 1 and Table 1. The pupil radius during the fluorophotometric experiments was an average of 1.5 mm. The number of major ciliary processes in owl monkey eyes was counted to be about 100. Approximately 30 minor ciliary processes, with dimensions 1/4 to 1/5 of those of the major processes, are neglected in the model.

Parameters that characterize the plasma decay of F-HRP concentration following a single intravenous injection of F-HRP were calculated from the blood samples withdrawn at several times post-injection. \( C_{p0} \) and \( \tau \) were measured to be an average of 5.4 mg/ml and 108 min, respectively.

**Table 1. Baseline monkey model parameters**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( h_l )</td>
<td>( x )-Direction height of iris stroma</td>
<td>0.1 mm</td>
</tr>
<tr>
<td>( d_{\text{sh}} )</td>
<td>Thickness of individual ciliary process stroma</td>
<td>0.1 mm</td>
</tr>
<tr>
<td>( n_{\text{cp}} )</td>
<td>Number of ciliary processes</td>
<td>100</td>
</tr>
<tr>
<td>( \phi )</td>
<td>Effective porosity of ciliary and iris stroma*</td>
<td>0.4</td>
</tr>
<tr>
<td>( V_a )</td>
<td>Anterior chamber volume</td>
<td>290 ( \mu l )</td>
</tr>
<tr>
<td>( f )</td>
<td>Aqueous humor outflow rate</td>
<td>1.5 ( \mu l/min )</td>
</tr>
<tr>
<td>( K_{\text{rate}} )</td>
<td>Transcapillary exchange rate constant*</td>
<td>( 6.9 \times 10^{-3} \text{ sec}^{-1} )</td>
</tr>
<tr>
<td>( D )</td>
<td>Molecular diffusivity*</td>
<td>3.0 E-7 cm²/sec</td>
</tr>
</tbody>
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* Value for albumin is assumed.
The remaining model parameter values (Table 1) were determined in a manner similar to that used by the authors for corresponding parameters of rabbits. The porosity, \( \phi \), is defined as the ratio of void volume available to a specific protein to the total stromal volume, excluding the epithelial volume. In the absence of specific data from monkeys, the porosity of ciliary process stroma in monkeys is taken to be the same as that calculated for the rabbit, 0.4. Ciliary body and iris stroma porosity is assumed to equal the ciliary process porosity. The transcapillary exchange rate constant, \( K_{\text{rate}} \), in the ciliary processes was assumed to be the same as that calculated for rabbit processes, \( K_{\text{rate}} = K/(V_p\phi) = 6.9 \times 10^{-4} \text{l/s} \), where \( K \) is the transcapillary exchange volume flow constant calculated for rabbits and \( V_p \) is the total stromal volume of rabbit ciliary and iridial processes. The rate of protein leakage from capillaries in the ciliary body stroma is assumed to be the same as that from capillaries in the ciliary processes. However, the density of ciliary body capillaries was observed microscopically to be about half the capillary density of the processes. \( K_{\text{rate}} \) in the ciliary body, therefore, is taken to be \( 3.45 \times 10^{-4} \text{l/s} \).

### Results

#### Aqueous Fluorophotometry Experiments

The mean anterior chamber F-HRP concentration measured in the monkey eyes is shown in Figure 2 as a function of time following intravenous injection of the tracer protein. F-HRP slowly appeared in the anterior chamber and reached a concentration of 0.0028–0.0030 mg/ml at 240 min post-injection. The maximum anterior chamber F-HRP concentration apparently occurred at a post-injection time ≥240 min. The aqueous humor, sampled from the monkeys at the conclusion of fluorophotometry, contained an average total protein concentration of 0.049 mg/ml.

The average initial plasma concentration and characteristic plasma decay constant of F-HRP were measured to be 5.4 mg/ml and 108 min, respectively. The average total plasma protein content was assayed to be 54 mg/ml.

### Morphology and Tracer Localization Studies

The distribution of F-HRP in these eyes was examined. A light micrograph of an anterior segment chopper section, which cuts through a single ciliary process from an owl monkey, is shown in Figure 3 (top). F-HRP is present throughout the ciliary process stroma and at the iris root in the anterior chamber angle region. In addition, the ciliary muscle is stained significantly less for HRP presence than are the ciliary process and iris root stroma.

The anatomical components of the blood-aqueous barrier of the monkeys remained intact. No evidence of F-HRP leakage from iris blood vessels was found (Fig. 3, bottom left). Although F-HRP penetrated intercellular clefts between pigmented ciliary epithelial
Fig. 3. Monkey morphology and F-HP (dark reaction product) localization. (Top) Light micrograph of a 200-μm chopper section reacted for the demonstration of horseradish peroxidase. Dark F-HP reaction product fills the entire stroma of the ciliary body and processes. F-HP can be followed from the ciliary body stroma along a channel (arrow) to the iris root and through the iris stroma to the anterior iris surface (X62). (Bottom left) Transmission electron micrograph of an iris stromal vessel demonstrates granular F-HP reaction product confined to the vessel lumen (X8100). (Bottom right) Transmission electron micrograph demonstrates dark (black) F-HP reaction product permeating the intercellular clefts between adjacent pigmented ciliary epithelial cells (PCE) and between the apices of the pigmented and nonpigmented ciliary epithelial (NPCE) layers. Further movement toward the posterior chamber between adjacent nonpigmented epithelial cells is blocked by an apico-lateral junctional complex that includes a tight junction (arrow) (X60,000).

Computational Model

Simulation of fluorophotometric experiments: The parameters and dimensions given in Table 1 and Figure 1 were used in the computational model to simulate F-HP transport to anterior chamber aqueous humor following the single intravenous F-HP injection given in the fluorophotometric experiments. The F-HP concentration in the anterior chamber, iris interstitium, and ciliary body and process interstitium were predicted as a function of time and position. The anterior chamber F-HP concentration (Fig. 4a) is predicted to become significant about 30 min post-injection. The concentration initially increases slowly, then grows at an increasing rate until 70 min post-injection. The anterior chamber F-HP concentration is predicted to reach a maximum value

cells and between apices of pigmented and nonpigmented cells, tight junctions apparently prevented it from moving deep into the intercellular clefts between nonpigmented cells and entering the posterior chamber (Fig. 3, bottom right). No vesicular transport of F-HP across the blood-aqueous barrier endothelium or epithelium was observed.
at 230 min post-injection that is 0.055% of the initial tracer protein concentration in the plasma. The anterior chamber F-HRP concentration decreases toward zero at post-injection times greater than 230 min.

The interstitial F-HRP concentration within tissue stroma as a function of x from the posterior surface of the ciliary process stroma to the anterior iris surface is shown at two different radial positions in Figures 4b and c. The concentration in the iris stroma is much less than in the process stroma. The interstitial F-HRP concentration in Figures 4d and e is presented as a function of radial position within the ciliary and iris stromas. The tracer concentration in the iris is significantly greater near the anterior chamber angle than throughout the remaining portion of the iris. The mean interstitial F-HRP concentration is shown in Figure 4f as a function of time post-injection. The mean interstitial tracer concentration within the ciliary body and process stroma is similar, rising rapidly to a maximum value at about 60 min that is approximately 50% of the initial plasma tracer concentration and then declining rapidly. The mean interstitial iris concentration rises and declines slowly about a maximum value that is 0.11% of the initial plasma tracer concentration.

**Simulation of normal steady-state conditions:** The computational model also was used to predict the protein concentration normally present in the anterior chamber and the interstitium of the ciliary body and iris for a constant level of plasma protein concentration. Because albumin is the characteristic aqueous humor protein upon which model parameters were based for simulation of the F-HRP bolus experiments, the same parameters and dimensions (Table 1, Fig. 1) were used to simulate steady plasma protein concentration.

The results of these steady-state computations are shown in Figure 5. The anterior chamber protein concentration is predicted to increase for several hours. A steady-state anterior chamber protein concentration of about 0.22% of the plasma protein concentration eventually is reached after more than 10 hours (Fig. 5a). The interstitial protein concentration in the iris is approximately 4% of plasma protein concentration in the iris root region and significantly less throughout the remainder of the iris stroma (Fig. 5e). The mean interstitial protein concentration in the iris reaches a steady-state value that is 0.37% of the plasma protein concentration, compared to about 92% and 94% of the plasma protein concentration for the ciliary body and processes, respectively (Fig. 5f).

**Discussion**

In vivo fluorophotometric, morphologic, and computational modeling methods were combined in the current investigation to examine the existence and significance of a diffusional pathway via the iris for transporting plasma protein from ciliary body capillaries to anterior chamber aqueous humor.

The predictions of the computational model (Fig. 4) can be compared to the aqueous fluorophotometric data obtained in monkeys following a single intravenous injection of F-HRP (Fig. 2). The model predicts that the anterior chamber concentration should begin to rise significantly about 30 min post-injection. In the experiments, except for one eye, the anterior chamber F-HRP concentration rise was detected 20–60 min post-injection.

The predicted and measured anterior chamber tracer protein concentrations agree reasonably well over the entire range of post-injection times examined. For example, the anterior chamber F-HRP concentration as a percentage of initial plasma F-HRP concentration at 90 min and 180 min post-injection was predicted to be 0.02% and 0.05%, respectively; it was measured to be 0.01–0.03% and 0.03–0.06%, respectively. Consequently, the rate of increase in measured anterior chamber tracer concentration agrees with the model prediction.

The maximum anterior chamber F-HRP concentration as a percentage of initial plasma F-HRP concentration was predicted to be 0.06%, and the ratio measured at the corresponding post-injection time (230 min) was 0.05–0.06%.

The time post-injection of maximum anterior chamber F-HRP concentration was predicted to be 230 min and was measured to be >240 min.

Following the aqueous fluorophotometry measurements, tracer localization studies that were performed on these same eyes confirmed the presence of the tracer protein throughout the ciliary body stroma and iris root (Fig. 3, top). F-HRP was not found to leak from iris vessels (Fig. 3, bottom left) or across the tight junctions between nonpigmented ciliary epithelial cells (Fig. 3, bottom right).

The normal physiologic case of a constant protein concentration also was examined, and the model predictions (Fig. 5) were compared to experimental data. The steady-state anterior chamber total protein concentration as a percentage of plasma protein concentration was predicted to be 0.2% and was assayed to be 0.05–0.2%. The model predicts that the interstitial protein concentration in the iris, about 0.4% of plasma protein concentration, should be much lower than in the ciliary body and process stroma, about 90% of plasma protein concentration. This is consistent with previous observations by Allansmith et al and Radius and Anderson.

Only a limited number of studies have examined the interstitial protein distribution in the anterior seg-
A: Posterior Ciliary Process Surface
B: Ciliary Process-Ciliary Body Interface
C: Ciliary Body-Iris Interface
D: Anterior Iris Surface

A: Medial Iris Surface (Pupillary Margin)
B: Lateral Iris Surface (Anterior Chamber Angle)

A: Posterior Ciliary Process Surface
B: Ciliary Process-Ciliary Body Interface
C: Anterior Iris Surface

A: Medial Ciliary Process Surface
B: Ciliary Process-Ciliary Body Interface
C: Lateral Ciliary Body Surface

A: Posterior Ciliary Process Surface
B: Ciliary Process-Iris Interface
C: Anterior Iris Surface

A: Medial Ciliary Process Surface
B: Ciliary Process-Ciliary Body Interface
C: Lateral Ciliary Body Surface

A: Posterior Ciliary Process Surface
B: Ciliary Process-Iris Interface
C: Anterior Iris Surface

A: Posterior Ciliary Process Surface
B: Ciliary Process-Iris Interface
C: Anterior Iris Surface

A: Posterior Ciliary Process Surface
B: Ciliary Process-Iris Interface
C: Anterior Iris Surface
Fig. 4. Monkey computational model predictions from a simulation of a single intravenous F-HRP injection. Concentrations normalized by the initial plasma F-HRP concentration (C\text{Po}) are shown as a function of postinjection time. (A) Anterior chamber F-HRP concentration (C\text{Ca}) left vertical axis, normalized C\text{Ca}/C\text{Po}; right vertical axis, estimated C\text{Ca} value (mg/ml) with C\text{Po} = 5.4 mg/ml. (B) Interstitial F-HRP concentration (C\text{iCP}) in ciliary process (CP) and iris stroma as a function of position x at location r = 6.98 mm. (C) Interstitial F-HRP concentration (C\text{iCP}) in ciliary process (CP), ciliary body (CB), and iris stroma as a function of position x at location r = 7.04 mm. (D) Interstitial F-HRP concentration (C\text{iCP}) in ciliary process (CP) and ciliary body (CB) stroma as a function of position r at location x = 0.24 mm. (E) Interstitial F-HRP concentration (C\text{iCP}) in iris stroma as a function of position r at location x = 0.1 mm. (F) Mean interstitial F-HRP concentration (C\text{inter}) in the continuous circumferential band of ciliary body, ciliary processes, and iris. Iris concentration increased by a factor of 100.

ment of primate eyes. In autopsied human eyes, immuno­fluorescence was used by Allansmith et al\textsuperscript{21} to determine the albumin and immunoglobulin distribution. The stromal tips of ciliary processes were bright with fluorescence, and iris stroma was minimally fluorescent. These authors suggested this protein distribution may be a result of protein transport from the iris to the anterior chamber by aqueous humor flow through the tissues. In owl monkeys, Radius and Anderson\textsuperscript{22} documented the albumin distribution in normal eyes following intravenous injection of fluorescent Evans’ blue dye, which irreversibly binds to serum albumin. The brightest fluorescence was observed in tissues that surround the anterior chamber angle, including the iris root and trabecular meshwork. Intense fluorescence also was found in ciliary processes, but no fluorescence was detected in the iris except within vessels. These studies indicate that a strong gradient in protein concentration exists from the ciliary body capillaries to the iris root and therefore are consistent with an anteriorly directed diffusion protein route in primates.

Extravascular albumin concentration recently was measured in monkeys and rabbits by Toris et al\textsuperscript{23} with three different methods. Two of the methods correspond to examination of the time-dependent ratio of tracer albumin concentration in uveal tissues to that in plasma 120 min after a single intravenous injection of fluorescein isothiocyanate-labeled albumin. In monkeys, the extravascular tracer concentration as a percentage of plasma tracer concentration in the iris measured by the two methods—1.8% ± 4.0 and 3.6% ± 0.5 (mean ± standard error)—is greater than that predicted by the computational model described in this report (approximately 0.1%). However, their measurements in the pars plicata (13.5% ± 7.8 and 12.3% ± 1.3) are less than the model prediction (approximately 40%). In rabbits, extravascular tracer concentration as a percentage of plasma tracer concentration measured by one of the two methods (iris, 10.8% ± 3.7; pars plicata, 68.6% ± 8.0) are consistent with the rabbit computational model predictions previously reported (iris, about 2%; ciliary and iridal processes about 25%),\textsuperscript{1} while the results of the second method (iris, 20.9% ± 3.6; pars plicata, 26.0% ± 2.5) are not. A third method used by Toris et al gives the ratio of endogenous, or normal steady-state, albumin concentrations in the iris and pars plicata to that in plasma. In monkeys, their measurement of extravascular steady-state albumin concentration in the pars plicata to the plasma albumin concentration (10.9% ± 1.1) is less than that predicted by the computational model (approximately 90%), while their measured extravascular albumin concentration in the iris as a percentage of the plasma albumin concentration (2.4% ± 0.6) is greater than the model prediction (approximately 0.4%). In rabbits, their reported steady-state pars plicata albumin concentration as a percentage of plasma albumin concentration (39.2% ± 17.5) is less than the predictions of our previously described rabbit computational model\textsuperscript{1} and the extravascular steady-state measurements made by Bill (about 75%).\textsuperscript{24} Although the results of Toris et al differ from those of the present authors as well as Bill, the significant standard errors reported by Toris et al for all three of their methods limit any implications. In addition, the steady-state ratios of tissue to plasma tracer concentrations reported by Toris et al had values less than those measured by the same authors with their two transient methods.

It is worthwhile to compare the results of the current investigation in monkeys with those of our previous studies in rabbits\textsuperscript{16} after administration of the same dose of F-HRP (Fig. 6a).\textsuperscript{23} First, the anterior chamber F-HRP concentration in the monkeys is much less than in the rabbits, about an average of 30 times less at 60 min post-injection. Second, the time course of F-HRP in the anterior chamber of monkeys is longer than that in the anterior chamber of rabbits. The time post-injection to maximum anterior chamber F-HRP concentration is at least 4 times longer in the monkeys than in the rabbits. Third, the initial plasma F-HRP concentration and the plasma F-HRP time decay constant were about the same in monkeys and rabbits. Fourth, the total soluble protein content in anterior chamber aqueous humor was 10-fold less in the monkeys than in the rabbits. Fifth, F-HRP was present throughout ciliary body stroma and reached the iris root in monkeys and rabbits. Finally, no F-HRP leakage from the iris vasculature or across the ciliary and iridal epithelia was observed in rabbits or monkeys. Simulations by the computa-
tional monkey and rabbit models predict all of these species differences in F-HRP transport to anterior chamber aqueous humor. A comparison of the anterior chamber F-HRP concentrations in monkeys and rabbits predicted by the models is shown in Figure 6b.

The comparison of monkey and rabbit fluorophotometric data suggests that protein diffusion from the ciliary body to the anterior chamber is much more impeded in monkeys than in rabbits. This is because of readily identifiable anatomical differences between the two species. Monkeys have a longer and narrower pathway for anteriorly directed protein diffusion from the ciliary body than rabbits. Unlike primates, rabbits have iridial processes in addition to ciliary processes. Furthermore, the iridial and ciliary processes of rabbits are directly attached to posterior iris stroma. The protein route in monkeys has an average pathway length from ciliary body capillaries to aqueous humor (0.8 mm) that is twice as long as in rabbits (0.4 mm). Also, the total cross-sectional area of the pathway from ciliary body and ciliary process stroma to iris stroma in monkeys is 3 mm², while in rabbits the total cross-sectional area from ciliary and iridial process stroma to iris stroma is 8 mm².

The time for diffusional transport is proportional to \( t/D \), where \( t \) is the characteristic length along which the tracer diffuses. The length \( l \) from the ciliary body to aqueous humor via the iris in monkeys is about twice as large as that in rabbits, and \( D \) is assumed to be the same in both species. Consequently, the time course of anteriorly directed diffusional transport to aqueous humor should be approximately 4 times greater in monkeys than in rabbits. This was fluorophotometrically observed following intravenous F-HRP injection. The time until an appreciable amount of F-HRP, or protein, can be expected to enter the anterior chamber is approximately \( t/\sqrt{D} \), about 40 min in monkeys and 10 min in rabbits. This agrees with the fluorophotometric measurements.

The significance of the time delay due to tracer diffusion from the ciliary body to the anterior iris surface on the overall transport of plasma proteins to aqueous humor can be examined computationally. In the computational model developed for the current in-

![Graph](https://via.placeholder.com/150)

**Fig. 5.** Monkey computational model predictions from a simulation of constant plasma protein concentration. Concentrations normalized by plasma protein concentration \( (C_p) \) are shown as a function of time following a step function from zero plasma protein concentration to \( C_p \). (A) Anterior chamber protein concentration \( (C_a) \), (B) Interstitial protein concentration \( (C) \) in ciliary process \( (CP) \) and iris stroma as a function of position \( x \) at \( r = 6.98 \text{ mm} \). (C) Interstitial protein concentration \( (C) \) in ciliary process \( (CP) \), ciliary body \( (CB) \), and iris stroma as a function of position \( x \) at \( r = 7.04 \text{ mm} \). (D) Interstitial protein concentration \( (C) \) in ciliary process \( (CP) \) and ciliary body \( (CB) \) stroma as a function of position \( r \) at \( x = 0.24 \text{ mm} \). (E) Interstitial protein concentration \( (C) \) in iris stroma as a function of position \( r \) at \( x = 1.0 \text{ mm} \). (F) Mean interstitial protein concentration \( (C_{\text{mean}}) \) in the continuous circumferential band of ciliary body, ciliary processes, and iris. Iris concentration increased by a factor of 100.

![Graph](https://via.placeholder.com/150)

**Fig. 6.** Comparison of monkey and rabbit anterior chamber F-HRP concentration as a function of time following a single intravenous F-HRP injection with the same dose (250 mg/kg body mass). (A) Aqueous fluorophotometry data. (B) Computational model predictions; initial plasma F-HRP concentration assumed to be the average of the monkey and rabbit values measured during the fluorophotometric experiments, \( C_{p0} = 5.65 \text{ mg/ml} \).
vestigation, the protein tracer must diffuse through stroma before reaching the anterior iris surface where it enters aqueous humor. If the time delay corresponding to the diffusion is ignored and protein is assumed to pass directly from the vasculature into the anterior chamber aqueous humor, the mass balance of tracer in the anterior chamber is governed by the following equation:

\[ \frac{dC_a}{dt} = f(\alpha C_p - C_a) \]  

(5)

where \( \alpha \) can be thought of as a sieving coefficient with a value determined by the steady-state anterior chamber protein concentration. The anterior chamber concentration predicted by equation (5) with \( C_p \) given by equation (4) is shown in Figure 7. Two differences in the time course of anterior chamber concentration are apparent between these results and the predictions of the computational model that includes the diffusion through stroma (Fig. 4a). First, initial tracer influx into the anterior chamber occurs immediately post-injection if the diffusion delay is ignored but occurs 20 min post-injection if the diffusion delay is included. Second, the time post-injection to peak anterior chamber concentration is 140 min if the diffusion delay is ignored but is 230 min if the diffusion delay is included.

![Graph showing the significance of diffusion delay](image)

Fig. 7. Significance of the diffusion delay to the anterior chamber. Solid line: monkey computational model simulation of a single intravenous F-HRP injection with the time delay due to diffusion through stroma neglected, anterior chamber F-HRP concentration \( C_a \) normalized by the product of initial plasma F-HRP concentration \( C_{p0} \) and a sieving coefficient \( \alpha \). Dashed line: monkey computational model simulation of a single intravenous F-HRP injection with the time delay due to diffusion through stroma included, anterior chamber F-HRP concentration \( C_{a0} \) normalized by the initial plasma F-HRP concentration \( C_{p0} \).

Our investigation does not support the hypothesis that an adequate amount of protein enters the posterior chamber to account for normal aqueous humor protein levels. A protein route to posterior chamber aqueous humor via the ciliary epithelium would require a ciliary epithelial sieving coefficient in rabbits approximately four to nine times larger than that in monkeys to account for differences in aqueous humor protein content. No morphological evidence of such a large difference in the epithelial permeability properties between these two species was found. Furthermore, no F-HRP was microscopically found to cross the ciliary epithelium and reach the posterior chamber in either the rabbit or monkey eyes used in the fluorophotometric experiments. Clearly, tissue sections are discrete, precluding a claim that absolutely no tracer protein entered the posterior chamber aqueous humor. In addition, transport from the vitreous body is another possible route by which proteins may reach posterior chamber aqueous humor. Overall, no conclusive statements regarding potential protein routes to posterior chamber aqueous humor can be made. The significance of any pathway by which proteins first enter the aqueous humor in the posterior chamber and flow into the anterior chamber can be determined by comparing the protein concentrations in the anterior and posterior chamber aqueous humor. However, little data of these relative protein concentrations exist. Two studies\(^5,^6\) measured similar protein concentrations in the anterior and posterior chambers. A third investigation\(^2^7\) found the protein concentration in the posterior chamber to average 35% less than that in the anterior chamber of the same eye. In these studies of aqueous humor protein content, the aqueous humor samples were obtained by paracentesis. Given the small posterior chamber volume (about 30 \( \mu \)l), paracentesis samples are likely to be contaminated with plasma because of mechanical damage to the ciliary epithelium, thus altering the protein content of the posterior chamber sample.

Another possible route by which protein may reach aqueous humor originates in permeable limbal capillaries\(^2^8\) and passes through limbal corneo-sclera stroma to the anterior chamber. F-HRP is evident in the limbal and corneal stromal of anterior segment tissue sections (Fig. 3, top). The presence there of tracer protein may be a result of limbal vessel leakage, migration from the ciliary body, or transport with aqueous humor outflow through the anterior chamber angle. While quantifying the protein pathway originating in the limbus is not yet possible, an aqueous humor protein source in the limbal capillaries could, in principle, be significant.

The data regarding alternative protein pathways to aqueous humor and the current investigation of ante-
riorly directed protein diffusion via the iris to the anterior chamber clearly have some experimental and modeling limitations. First, the tracer protein, F-HRP, is assumed to behave as a representative aqueous humor protein. This is reasonable because HRP has molecular properties similar to those of albumin, which is the characteristic protein of both plasma and aqueous humor. Both are globular proteins with similar molecular weights (HRP, MW \( \approx 42,000 \) daltons; albumin, MW \( \approx 69,000 \) daltons), hydrodynamic radii (HRP, \( r_s \approx 25-30 \) Å; albumin, \( r_s \approx 33-35 \) Å), and frictional ratios (HRP, \( f_r \approx 1.36 \); albumin, \( f_r \approx 1.29 \)).

Second, F-HRP may have systemic effects after protracted periods. One monkey unexpectedly died during a fluorophotometric experiment 4.5 hr after intravenous injection of the tracer. Although the data obtained from this animal were consistent with the data obtained from all of the other monkeys, the data were not included in this report.

Third, the computational model of protein diffusion from the ciliary body to the iris and into the anterior chamber assumes the anterior chamber aqueous humor is well mixed. Natural convection currents, normal head and eye movements, and accommodation are assumed to effectively enhance anterior chamber mixing following protein diffusion across the anterior iris surface into the anterior chamber. The variation in anterior chamber tracer protein concentration measured along the pupillary axis generally was on the order of the fluorophotometric measurement errors.\(^{18}\)

Fourth, the computational model is two-dimensional. The numerous processes, however, are discrete and fin-like. The stroma of a single ciliary process is physically separated from the stroma of a neighboring ciliary process by a distance 4 times the stromal thickness of an individual ciliary process. A three-dimensional monkey model, with the additional dimension in the circumferential direction about the pupillary axis, was developed to more realistically model the protein diffusion from the individually separated processes through the continuous iris to the anterior chamber. With the baseline parameters (Table 1) and geometric dimensions (Fig. 1), the three-dimensional monkey model predictions of anterior chamber protein concentration for transient and steady-state simulations differ by about 4% from the predictions of the two-dimensional monkey model. Effects on the time course of anterior chamber protein content are negligible. Therefore, the simpler and less costly computational model is described and used in this investigation.

Fifth, bulk flow within the process and iris interstitium is neglected in the model. If a bulk flow does exist, however, it would most likely flow from the ciliary body processes in an anterior direction. Bill\(^{32,33}\) demonstrated that labeled albumin perfused into the anterior chambers of rabbits did not penetrate the ciliary processes when the perfusion was conducted in vivo, but the albumin did penetrate the processes when the perfusion was performed in freshly sacrificed rabbits. Bill therefore hypothesized that any tissue fluid flow is normally from the ciliary processes. An anteriorly directed flow from the ciliary body processes would augment diffusive protein transport from the processes to the anterior chamber. Consequently, the neglect of convective protein transport in the computational models is likely to underestimate the contribution of an anteriorly directed protein route to aqueous humor. An estimate of the significance of this bulk flow can be obtained by calculating a value for the Peclet number (\( Pe \)), a ratio of convective protein transport (proportional to the pressure gradient) to diffusive protein transport (proportional to the concentration gradient). \( Pe = u l_r^2/D_l \), where \( u \) is the anteriorly directed interstitial velocity in the \( x \) direction, \( l_r \) is the process radial length across which protein is transported, and \( l_s \) is the anterior-to-posterior length along which protein is transported. For the maximum expected bulk flow of 0.3 \( \mu \)l/min\(^{32,34}\) from the process stroma to the iris stroma, the \( Pe \) in monkeys is of order 1, implying that convective and diffusive protein transport are comparably important. Any bulk flow from the processes, however, may be directed not only toward the anterior iris surface but also toward neighboring tissues such as the suprachoroidal spaces. Furthermore, because anterior convection can only increase anterior chamber protein content, its contribution must be small considering the good agreement found when diffusion alone is considered.

The computational model of an anteriorly directed protein route via the iris to aqueous humor depends in part on the values of stromal porosity, protein diffusivity, transcapillary exchange, aqueous humor flow rate, and anatomical dimensions. The dimensions were measured directly from tissue specimens, and the model parameter values were independently determined from previously reported measurements for rabbits.\(^{16}\) If a parameter value was reported as a range, the value chosen for the model was the conservative estimate—ie, the value that leads the model to underestimate protein transport to the anterior chamber. Several conservative estimations were made. (1) About 30 minor ciliary processes, which are approximately one-third the size of the major ciliary processes included in the computational model, were excluded. (2) The smallest porosity value in the range estimated from reported measurements was used in
Table 2. Monkey model parameter sensitivity

<table>
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<tr>
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<th>Baseline (Table 1)</th>
<th>(\phi)</th>
<th>(D (\text{cm}^2/\text{sec}))</th>
<th>(K_{\text{rat}} (\text{sec}^{-1}))</th>
<th>(h_1 (\text{mm}))</th>
<th>(f (\text{ml/min}))</th>
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</thead>
<tbody>
<tr>
<td>Transient* simulations</td>
<td>(\frac{C_t}{C_{\text{opt,max}}}) (%)</td>
<td>0.0552</td>
<td>0.2027</td>
<td>0.0823</td>
<td>0.027</td>
<td>0.106</td>
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<td>(t_{\text{max}}) (min)</td>
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<td>224</td>
<td>229</td>
<td>264</td>
<td>206</td>
<td>264</td>
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<tr>
<td>Steady-state simulations</td>
<td>(\frac{C_f}{C_p}) (%)</td>
<td>0.21</td>
<td>0.110</td>
<td>0.328</td>
<td>0.114</td>
<td>0.415</td>
</tr>
</tbody>
</table>

* Model predictions following a single intravenous F-HRP injection. \(C_t/C_{\text{opt,max}}\), maximum anterior chamber tracer concentration; \(t_{\text{max}}\), postinjection time of maximum anterior chamber tracer concentration.

† Model predictions of a constant plasma albumin level. \(C_f/C_p\), anterior chamber albumin concentration at a time of \(t = 1000\) min.

the models. The porosity with respect to F-HRP and albumin was taken to be 0.4 from the range 0.5 ± 0.1.

(3) The molecular diffusivity used in the model, which is based on the standard temperature of 20°C, may be 1.5 times less than the diffusivity at the physiological temperature of 37°C.

The model parameters have a degree of uncertainty in their values. In addition, parameter values vary with physiology and pathology. Therefore, the sensitivity of model predictions to these parameters was examined with a series of model computations performed over a range of parameter values likely encountered in pathophysiology as well as in normal physiology. The results of these calculations are presented in Table 2. Considering a reasonable degree of error in parameter estimation and deviations in normal physiology, the model is relatively insensitive to individual parameter values.

Overall, considering the limitations discussed, this investigation demonstrates that a major portion of protein normally in the aqueous humor of monkeys originates in ciliary body capillaries and diffuses through stroma to the anterior iris surface, where it enters the anterior chamber. This conclusion was reached by using a computational model in a complementary manner with experimental data to confirm the hypothesis. Our study suggests that an additional component of the blood-aqueous barrier is ciliary body and iris stromas themselves, which restrict macromolecular movement from plasma to anterior chamber aqueous humor. Also, the time delay associated with protein diffusion from ciliary body capillaries through stroma to anterior chamber aqueous humor can be significant and should be considered in studies of aqueous humor dynamics that involve proteins or relatively large molecules.

Clinically relevant applications of the computational model include the following.

First, the values of model parameters and anatomical dimensions can be altered (Table 2) to mimic the actions of pharmacologic agents and their effects in aqueous humor protein concentration.

Second, the model can be modified appropriately to represent specific physiologic or disease conditions. In particular, in cases of anterior uveitis, a protein route across ciliary epithelium to the posterior chamber and a protein source in the iris can be added to aid in the interpretation and evaluation of uveitic aqueous humor protein data.

Third, immunological factors, or proteins, that are components of the mechanism of anterior chamber-associated immune deviation appear to be produced in the ciliary body and iris.35-36 As a result, the influence of their transport on the characteristics of the immunological privilege of the anterior chamber may be examined in part by the computational model.

Finally, the aqueous humor outflow network is contiguous with the portion of the anterior iris surface across which the majority of aqueous humor proteins diffuse into the anterior chamber. Therefore, the outflow network normally may be exposed to a protein concentration greater than the protein concentration in the central region of the anterior chamber. This may influence any role aqueous humor proteins may have in open-angle glaucoma.

In view of the computational model’s success in predicting the available experimental data, it can now be used with confidence to predict aqueous humor protein concentrations under various conditions, to enhance evaluation of aqueous humor protein data, and to strengthen or develop new hypotheses related to aqueous humor protein concentration.

Key words: monkey, aqueous fluorophotometry, blood-aqueous barrier, protein transport, computational model

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References