

Fluorophotometric determination of aqueous humor flow rates in red-tailed hawks (*Buteo jamaicensis*)

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Objective—To determine aqueous humor flow rate (AHFR) in an avian species by use of anterior segment fluorophotometry.

Animals—9 healthy red-tailed hawks (*Buteo jamaicensis*; 4 males and 5 females) that ranged from 8 months to 8 years of age.

Procedures—A protocol was developed for fluorophotometric determination of AHFR. Topical administration of 10% fluorescein was used to load the corneas, and corneal and aqueous humor fluorescein concentrations were measured approximately 5, 6.5, and 8 hours later. Concentration-versus-time plots were generated, and slopes and cornea-to-aqueous humor concentration ratios from these plots were used to manually calculate flow rates.

Results—Mean \pm SD AHFRs for the right eye, left eye, and both eyes were 3.17 ± 1.36 $\mu\text{L}/\text{min}$ (range, 1.67 to 6.21 $\mu\text{L}/\text{min}$), 2.86 ± 0.88 $\mu\text{L}/\text{min}$ (range, 2.04 to 4.30 $\mu\text{L}/\text{min}$), and 2.90 ± 0.90 $\mu\text{L}/\text{min}$ (range, 1.67 to 4.42 $\mu\text{L}/\text{min}$), respectively. The AHFRs were similar for right and left eyes. These flow rates represented a mean aqueous humor transfer coefficient of 0.0082/min, which is similar to that of mammalian species.

Conclusions and Clinical Relevance—The AHFR in red-tailed hawks was similar to that of most mammalian species, and the fractional egress was almost identical to that of other species. This information will allow a greater understanding of aqueous humor flow in avian eyes, which is crucial when evaluating diseases that affect avian eyes as well as medications that alter aqueous humor flow. (*Am J Vet Res* 2012;73:551–555)

The structure and function of avian eyes, which are unique and highly specialized, are dependent on the flow of AH to provide for physiologic metabolism of the cornea and lens and maintain the exceptional visual acuity common to avian species. Raptor eyes contain a large volume of AH, and in clinically normal eyes, the rate of AH production by the ciliary processes of the ciliary body and removal of AH via the aqueous sinus, which is analogous to the canal of Schlemm in humans¹ and intrascleral venous plexus in domestic animals² and is located circumferentially around each eye, are balanced to maintain physiologic IOP.^{3,4} Similar to the situation in primates and other mammalian species, the aqueous sinus of birds connects directly with extracellular spaces and forms a major pathway for AH outflow. Thus, AH flows directly from the lumen of the aqueous sinus into the intrascleral plexus of veins and eventually into the episcleral and subconjunctival system of veins.^{3,5}

Diseases of the eyes in birds have been described in the literature. In free-ranging birds of prey, lesions secondary to trauma to the globe have consistently been the most commonly reported ophthalmologic

ABBREVIATIONS

AH	Aqueous humor
AHFR	Aqueous humor flow rate
IOP	Intraocular pressure
K_a^o	Aqueous humor transfer coefficient
V_a	Anterior chamber volume

findings.^{6,7} Glaucoma, which is common in humans and domestic mammals, is quite rare in birds. There is a single case report of presumed primary glaucoma in an owl⁸ as well as secondary glaucoma in Slate turkeys⁹ and experimentally induced glaucoma in chickens.^{10–13} Although there are only few reports^{7,14–18} of glaucoma in birds, most of these are of secondary glaucoma resulting from severe traumatic injury that leads to uveitis, posterior or peripheral anterior synechia, lens luxation, or hyphema and that may be unilateral or bilateral depending on the cause. The reason glaucoma is uncommon in birds is not known. It was suggested in 1 report¹⁴ that primary glaucoma has not been widely detected in birds because of the width of the iridocorneal angle. Results of another report³ support this assertion. Furthermore, it was also suggested that the aqueous sinus is protected and unlikely to collapse because of its location in a scleral furrow at the limbus, that there is a large artery in the septum that divides the sinus, and that the actions of the ciliary muscle and strands that

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cross the ciliary cleft tighten the trabecular meshwork in the sinus and widen the iridocorneal angle.³

The rarity of naturally occurring glaucoma in birds suggests that avian eyes have mechanisms to cope with severe increases in IOP or optic nerve characteristics that confer the ability to withstand such increases without the development of disabling visual sequelae. Unfortunately, little is known about physiologic dynamics of AH in birds. Therefore, the primary purpose of the study reported here was to investigate the rate of production of AH, which is 1 variable of the physiologic dynamics.

Materials and Methods

Animals—Nine healthy red-tailed hawks (*Buteo jamaicensis*; 4 males and 5 females) that ranged from 8 months to 8 years of age were used in the study. The hawks were permanently captive birds housed at local raptor rehabilitation and educational facilities or were birds evaluated by members of the University of Tennessee Veterinary Medical Center Avian and Zoological Medicine Service. Prior to inclusion in the project, all birds were assessed as clinically normal on the basis of results of physical examination, ophthalmic examination (including slit-lamp biomicroscopy, indirect ophthalmoscopy, and applanation tonometry), and hematologic analysis (CBC and serum biochemical analysis). All procedures were approved by the University of Tennessee Animal Care and Use Committee, and appropriate scientific collections permits were obtained from the Tennessee Wildlife Resources Agency.

Determination of corneal volume, V_a , and corneal thickness—Anterior chamber volumes were measured via aspiration of AH from 5 young adult red-tailed hawks (most likely males as determined on the basis of size, iris color, and plumage color) that were clinically normal on the basis of results of ophthalmologic examination but were euthanized for reasons unrelated to the present study. Mean V_a values were used in all calculations. Corneas were excised from the same 5 hawks used for V_a determination. Mean corneal volume was determined by displacement of a metered volume of saline (0.9% NaCl) solution by the excised corneas, and the resulting value was used in all calculations. Mean central corneal thickness of 4 adult red-tailed hawks was measured in vivo by use of high-resolution ultrasonography.^a

Fluorophotometry protocol—Each hawk received 1 drop of 10% fluorescein^b topically at 5-minute intervals 3 times, similar to the procedure described for dogs.¹⁹ This resulted in consistent, homogenous staining of the cornea within 3 to 4 hours after fluorescein administration. Five minutes after application of the third drop of fluorescein, the corneal surfaces, conjunctival recesses, areas under the nictitating membranes, and periocular feathers were thoroughly rinsed with a sterile eye-irrigation solution to remove excess fluorescein from the tear film and prevent reintroduction of fluorescein into the eye. At 5, 6.5, and 8 hours after administration of the third drop of fluorescein, corneal and AH fluorescein concentrations were measured with a computerized scanning ocular fluorophotometer fit-

ted with an anterior chamber adapter.^c The hawks were manually restrained during the scanning procedure.

Determination of AHFR—Corneal and AH concentrations were obtained from the concentration-versus-time decay curves, and the resulting data were analyzed by use of a method reported in 1 study,²⁰ modified as described elsewhere.²¹ Briefly, fluorescein was allowed to equilibrate within the corneal stroma to ensure the rates of decrease from the corneal stroma and AH were equal. The K_o of fluorescein from an eye can be calculated by use of the following equation²¹:

$$K_o = -A(1 + \{[k_{sr} V_c C_c] / [1.2 V_a C_a]\})$$

where A is the mean slope of the corneal and AH decay curves, k_{sr} is the spatial resolution correction factor, V_c is the volume of the cornea, C_c is the concentration of fluorescein in the cornea, and C_a is the concentration of fluorescein in the midcentral anterior chamber. The ratio of C_c to C_a is obtained from the midpoint of the corneal and AH decay curves. A spatial resolution correction factor is necessary to account for the fact that the intersection of the fluorophotometer emission and excitation beams (ie, the focal diamond) is slightly larger than the corneal thickness; this correction factor is calculated as a function of corneal thickness by use of the following equation²²:

$$k_{sr} = 1 / (1 - [0.9622 \cdot e^{-1.848 \cdot d}])$$

where e is the natural logarithm and d is the corneal thickness. The AHFR is the product of K_o and V_a . Because the derivation of K_o is predicated on corneal and AH fluorescein concentrations decreasing semilogarithmically and in parallel, scans resulting in natural logarithm fluorescein concentration-versus-time correlation coefficients < 0.75 or in which the ratio of the slopes of the cornea and AH decay curves were < 0.5 or > 1.5 were discarded.¹⁹

Statistical analysis—Correlation coefficients of the corneal and AH fluorescein decay curves were calculated by use of the Pearson product moment correlation to ensure that values for 3 time points approximated a straight line. Slopes of corneal and AH fluorescein decay curves were compared to ensure that they were decreasing in parallel. All variables were calculated for the left eye, right eye, and the mean of both eyes; for discarded scans, the variables for the retained eye alone were reported as the mean of both eyes. Left and right eyes were compared for each variable by use of a paired t test. Values of $P < 0.05$ were considered significant.

Results

Mean corneal volume, V_a , and corneal thickness were 163 μ L, 355 μ L, and 480 μ m, respectively. All birds were extremely cooperative for fluorophotometric scanning, which resulted in repeatable results. Corneal and AH fluorescein concentrations decreased semilogarithmically between 5 and 8 hours after fluorescein administration (Figure 1). There was excellent correlation among time points ($r = 0.93$ for corneal fluorescein concentrations and $r =$

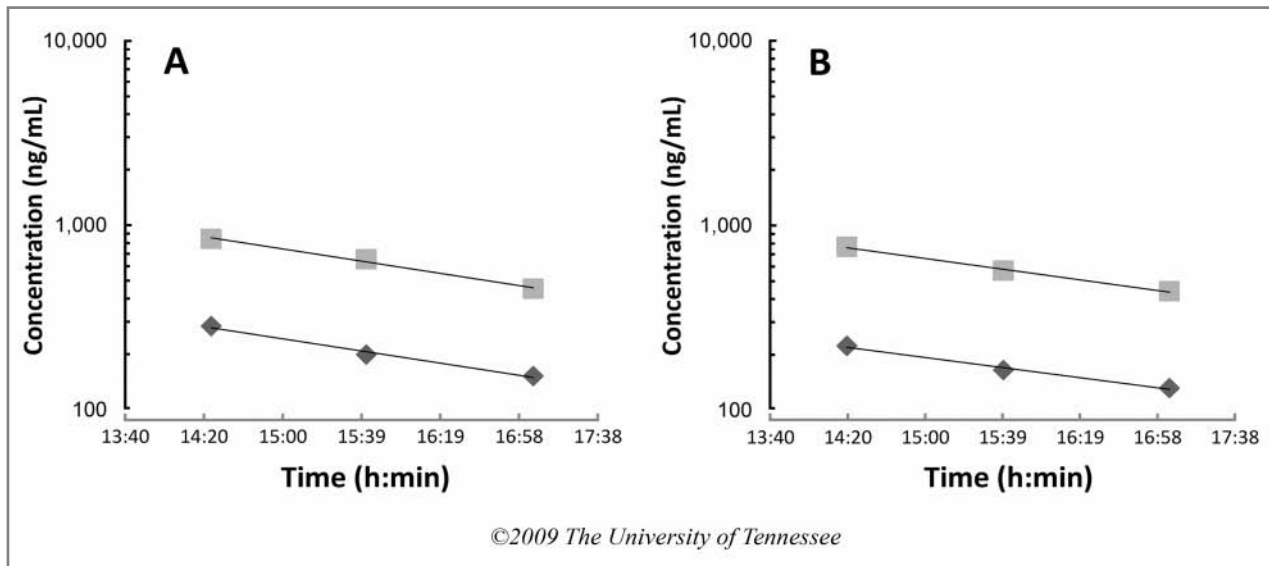


Figure 1—Logarithmically transformed fluorescein concentrations of the cornea (squares) and AH (diamonds) determined from fluorophotometric scan of the left (A) and right (B) eyes of 9 red-tailed hawks (*Buteo jamaicensis*). The slopes of the 2 lines decrease in parallel. The AHFR is determined from slopes of the lines and concentration ratios. (Illustration by D. K. Haines © 2009 The University of Tennessee).

Table 1—Comparison of AHFR, V_a , and K_o among species.

Species	AHFR ($\mu\text{L}/\text{min}$)	V_a (μL)	K_o (/min)*	Reference
Dog	5.22	400	0.0131	19*
Human	2.70	180	0.0150	23
Human	2.60	157	0.0166	24
Rabbit	1.84	250	0.0074	25*
Cat	7.52†	696	0.0108	26
Red-tailed hawk	2.90	355	0.0082	—

*Value calculated as $\text{AHFR}/(V_a)$. †Value calculated as described in the reference.
 — = Not applicable; represents results determined in the present study.

0.98 for AH fluorescein concentrations). The corneal and AH decay curves had a high degree of parallelism, with a mean ratio for the slopes of the corneal decay curves to the slopes of the AH decay curves of 1.16. Despite this, 4 scans resulted in slope ratios, correlation coefficients, or both that were outside acceptable ranges and were discarded from further analysis.

On the basis of the slopes of the natural logarithm fluorescein concentration-versus-time decay curves, mean \pm SD K_o was calculated as $0.0089 \pm 0.0038/\text{min}$, $0.0081 \pm 0.0025/\text{min}$, and $0.0082 \pm 0.0025/\text{min}$ for right eyes ($n = 8$), left eyes (6), and both eyes (9), respectively. Mean AHFR calculated from K_o was 3.17 ± 1.36 for right eyes and 2.86 ± 0.88 for left eyes; mean AHFRs did not differ significantly ($P = 0.55$) between right and left eyes. The mean AHFR value for both eyes was $2.90 \pm 0.90 \mu\text{L}/\text{min}$; this value was within the range of values reported for most mammals.^{19,23–26} (Table 1). During the scanning period, fluorescein concentrations ranged from 83 to 5,384 mg/mL.

Discussion

Analysis of results of the study reported here indicated that anterior chamber fluorophotometry can be

applied to a raptorial species. Successful use of fluorophotometry is contingent on several factors. Fluorescein concentrations in the cornea and AH must approximate a straight line for the scanning period when plotted semilogarithmically, fluorescein concentrations must be within the linear range of the fluorophotometer, and during the scanning period, the plots of the corneal fluorescein concentration-versus-time curve and AH fluorescein concentration-versus-time curve must decrease in parallel. If any of these conditions is not met, the assumptions on which the mathematical derivation of AHFR is predicated will be violated, resulting in erroneous AHFR calculations.²⁰

For the present study, we used a protocol developed for dogs in which 3 drops of 10% sodium fluorescein were applied topically at 5-minute intervals and in which scans were obtained between 5 and 8 hours after fluorescein administration.¹⁹ The hawks were, with few exceptions, remarkably cooperative for the scans and did not require sedation. The requirements for accurate fluorophotometry were met as indicated by the correlation coefficients and cornea-to-AH slope ratios nearing unity in most cases. Fluorescein concentrations resulting from use of this protocol were within the linear range of the fluorophotometer²⁷ (approx 10 to 10,000 ng/mL) during the scanning period 5 to 8 hours after fluorescein administration.

Four scans had slope ratios outside of the prescribed acceptable range; these data points were not included in the final AHFR calculations. It is presumed that motion artifact (head movement or movement of the nictitating membrane) may have contributed to these results. In contrast to the dogs in some previous studies conducted by our research group, the red-tailed hawks in the present study maintained a calm, attentive posture in front of the fluorophotometer, even without chemical restraint. The hawks did not struggle or appear stressed, and only a few scans had to be repeated because of obvious movement of the head or nictitating

membrane. It is not known whether the use of chemical restraint would have affected these results or whether manual restraint and the position of the head and body may have altered systemic blood pressure, thus affecting IOP and AH dynamics. This may need to be considered when performing scans on other avian species in the future. Fluorophotometric measurement of AHFR is not affected by ocular rigidity, corneal curvature, or iatrogenic changes in intraocular blood volume and episcleral venous pressure, which is in contrast to effects for results of other methods (ie, tonography) of determining flow rate.^{27,28} Further investigations are needed to evaluate the effect of systemic blood pressure on AH dynamics in avian eyes.

In 1 study,¹⁴ the IOP in healthy avian eyes was 11 to 16 mm Hg and 20 to 25 mm Hg in birds of prey and psittacines, respectively, as measured via applanation tonometry. In another study^d of 31 bird of prey species, the IOP ranged from 9 mm Hg in small raptor species to 40 mm Hg in larger species, as measured by use of a rebound tonometer. The differences in the values for these studies may be partially explained by differences in the methods used to measure IOP because rebound tonometry consistently registers significantly higher pressures than does applanation tonometry in some raptor species.²⁹ However, it is important to mention that IOPs measured by use of applanation tonometry can differ dramatically between raptor species and that applanation tonometry alone does not distinguish between conditions that affect the rate of inflow or outflow of AH.³⁰

Several methods have been used to measure the AHFR, including tonographic techniques (which measure changes in ocular pressure or volume following induced alterations in pressure or volume) and isopiestic techniques (photogrammetry, radiometry, and fluorophotometry).^{19,27,31} Fluorophotometry is a reliable, noninvasive method for measurement of AHFR without altering pressure or volume within an eye.^{19,27} Briefly, the technique involves flooding the cornea with fluorescein, which eventually diffuses into the corneal stroma and anterior chamber. The fluorescein then exits the anterior chamber along with the AH. Several hours after fluorescein administration, a steady state is reached in which the rates of aqueous flux from the cornea into the anterior chamber and from the anterior chamber out of the eye are equal. Corneal and AH fluorescein concentrations are measured with a fluorophotometer over a period of several hours. The resulting fluorescein concentration-versus-time decay curves can be analyzed mathematically to yield the AHFR.

Anterior segment fluorophotometry has been used in humans,^{22–24,32} nonhuman primates,^{33,34} laboratory rodents,³⁵ rabbits,^{25,36} cats,²⁶ and dogs¹⁹ to directly measure total AHFR because of its accuracy, repeatability, and noninvasiveness. In 1 study²³ in humans, the mean AHFR was 2.7 $\mu\text{L}/\text{min}$. In clinically normal dogs,¹⁹ cats,²⁶ rabbits,²⁵ and nonhuman primates (owl monkey [*Aotus trivirgatus boliviensis*]),³³ mean AHFR is 5.22, 7.52, 1.84, and 2.75 $\mu\text{L}/\text{min}$, respectively. In 1 study⁴ in chickens, the calculated AHFR was 9.9 to 16.6 $\mu\text{L}/\text{min}$, depending on the age of the chicken. However, investigators in that study⁴ used a technique involving

IV administration of fluorescein, which is complicated by plasma binding of fluorescein, systemic conversion of fluorescein to a fluorescent metabolite, and entry of fluorescein into the eyes via numerous routes.²⁷ Therefore, systemic administration of fluorescein has been replaced by topical application in current fluorophotometric techniques. To our knowledge, no studies have been performed in which investigators accurately determined the AHFR in avian eyes by use of fluorophotometry techniques that use topical application of fluorescein.

Similar to the lens of dogs, the lens of red-tailed hawks does not autofluoresce. This is in contrast to the lens in humans, which has a strong autofluorescent peak known as Gray's peak.³⁷ The automated AHFR software included with the fluorophotometer used this autofluorescent peak (in combination with an operator-defined corneal peak) to locate the midcentral anterior chamber for acquisition of the AH fluorescein concentration. Therefore, attempting to calculate AHFR with the software will lead to capricious values for AH fluorescein concentrations and increase the likelihood of erroneous results. To circumvent this problem, fluorescein concentrations were obtained directly from the graphs generated by the fluorophotometer, and AHFRs were then calculated manually by use of these values.

The mean AHFR value for both eyes of the hawks in the present study was 2.90 $\mu\text{L}/\text{min}$, which was within the range of values reported for most mammals^{19,23–26} (Table 1). It should be mentioned that when AHFRs among various species are standardized on the basis of V_a , the resulting K_o is remarkably similar (AH turnover, approx 0.01/min). This suggests that a similar fractional egress per unit of time is necessary among species to maintain the metabolism of the anterior segment structures.

It has been suggested⁴ that the AHFR is greater in birds, compared with the AHFR in mammalian species. The AHFR in domestic chickens is 10 to 15 $\mu\text{L}/\text{min}$.⁴ This value is much greater than the values determined in the present study for red-tailed hawks. The difference could be attributable to an actual difference in the values between chickens and hawks, but it could also be associated with differences in methods because investigators in that other study⁴ used fluorescein administered IV. Most current fluorophotometric evaluations are conducted via topical introduction of fluorescein to avoid the impact that systemic metabolism of fluorescein can have on AHFR calculations.²⁷ In addition, investigators in that aforementioned study⁴ used an excitation filter set at 365 nm, despite the fact that the maximum excitation for sodium fluorescein is 480 nm.³⁸ Therefore, the fluorescein concentrations those other investigators⁴ used for the AHFR calculations may have been erroneous. Interestingly, a technique of repetitive sampling of AH after administration of para-aminohippuric acid was used in another study³⁹ to calculate an AH turnover rate of 1.7% to 2%/min in clinically normal leghorn hens, which closely approximated the results of the study reported here.

The AHFR of red-tailed hawks in the present study was similar to that of humans. Although anatomy of the iridocorneal angle may differ, the physiologic process of

AH inflow and outflow in avian species is qualitatively similar to that of humans, nonhuman primates, and other mammalian species.^{3,5,40} Further investigations are required to assess physiologic comparisons as well as to determine the response of commonly used anti-glaucoma medications in avian species.

- a. I3 high-resolution ultrasound, Innovative Imaging Inc, Sacramento, Calif.
- b. AK-Fluor 10% fluorescein (100 mg/mL), AKORN, Buffalo Grove, Ill.
- c. FM-2 FluorotronMaster, OcuMetrics, Mountain View, Calif.
- d. Bayón A, Vecino E, Albert A, et al. Evaluation of intraocular pressure obtained by two tonometers, and their correlations with corneal thickness obtained by pachymetry in raptors (abstr). *Vet Ophthalmol* 2006;9:432.

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