Fluorophotometric determination of aqueous humor flow rate in clinically normal dogs

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Objective—To determine aqueous humor flow rate in clinically normal dogs, using fluorophotometry.

Animals—20 clinically normal Beagles.

Procedure—A study was performed on 5 dogs to establish an optimal protocol for fluorophotometric determination of aqueous humor flow rate. This protocol then was used to measure aqueous humor flow rate in 15 dogs. Corneas were loaded with fluorescein by topical application, and corneal and aqueous humor fluorescein concentrations were measured 5, 6.5, and 8 hours after application. Concentration-versus-time plots were generated, and slopes and ratios of the fluorescein concentration in the cornea and aqueous humor from these graphs were used to calculate flow rates. Calculations were performed by use of automated software provided with the fluorophotometer and by manual computation, and the 2 calculation methods were compared.

Results—The protocol established for the 5 dogs resulted in semilogarithmic and parallel decay of corneal and aqueous humor concentrations. Manually calculated mean ± SD aqueous humor flow rates for left, right, and both eyes were 5.58 ± 2.42, 4.86 ± 2.49, and 5.22 ± 1.87 µl/min, respectively, whereas corresponding flow rates calculated by use of the automated software were 4.54 ± 3.08, 4.54 ± 3.10, and 4.54 ± 2.57 µl/min, respectively. Values for the left eye were significantly different between the 2 computation methods.

Conclusions and Clinical Relevance—Aqueous humor flow rates can be determined in dogs, using fluorophotometry. This technique can be used to assess pathologic states and medical and surgical treatments that alter aqueous humor dynamics. (*Am J Vet Res* 2001;62:853–858)

Aqueous humor flows through the anterior segment of the eyes to provide metabolic support to the transparent avascular ocular structures. In normal eyes, the rates of production and drainage are balanced to provide a stable **intraocular pressure (IOP)**. In certain pathologic states, especially glaucoma and anterior uveitis, the aqueous humor flow rate is altered. Surgeries and drugs used in the treatment of glaucoma also alter the flow rate. Information of the flow rate in clinically normal dogs would be helpful in assessing ocular diseases that affect aqueous humor flow and in assessing and developing treatments for dogs with glaucoma.

Methods for measuring the rate of aqueous humor flow can be categorized as pressure-derivative methods or isopiestic methods.[1] Pressure-derivative analyses such as tonographic and suction cup methods measure the change in ocular pressure or volume following controlled alterations in pressure, volume, or both. Isopiestic analyses such as photogrammetry, radiometry, and fluorophotometry measure aqueous flow without altering pressure or volume and are the most reliable methods for determining aqueous humor flow rate.[1] Mean flow rate in clinically normal humans as assessed by use of fluorophotometry is 2.6 to 2.7 µl/min,[2,3] but the flow rate has not been directly measured by use of isopiestic methods in clinically normal dogs.

Anterior segment fluorophotometry has been used widely in humans, nonhuman primates, and laboratory rodents to directly measure total aqueous humor flow rate because of its accuracy and noninvasiveness.[4-7] In this technique, the cornea is loaded with fluorescein by iontophoresis[2,4] or topical application of drops.[1,3,5] Fluorescein gradually leaves the cornea and enters the anterior chamber, where it exits with the outflow of aqueous humor. Within several hours, a steady state is reached in which the rates of egress from the cornea and anterior chamber are equal. During this time, serial measurements of fluorescein concentrations in the cornea and aqueous humor are obtained with an optical sensor placed in front of the subject's eye, and decay curves are generated. The slope of these lines is proportional to the rate of aqueous humor flow, and the flow rate can be calculated by reference to equations derived from the differential equation relating the rate of egress of total ocular fluorescein to the rate of egress of fluorescein from the anterior chamber.[4,5]

Fluorescein concentrations can be measured noninvasively, using an optical detector with appropriate excitation and emission filters. The computerized scanning ocular fluorophotometer is the instrument of choice for these measurements because of the accuracy of its fluorescein measurements, superior spatial resolution, and ease of operation. It is widely used in research and human clinical settings. In addition to offering extremely accurate analysis of ocular fluorescein concentrations, this fluorophotometer has an integral automated software system that will automatically calculate aqueous humor flow rate. This software system is routinely used in humans, but its accuracy in dogs has not been established. The purposes of the study reported here were to establish a satisfactory protocol for the fluorophotometric evaluation of aqueous humor flow rate in clinically

normal dogs and to assess the usefulness of the fluorophotometer's automated software for determination of aqueous humor flow rate in dogs.

Materials and Methods

Animals—Twenty clinically normal Beagles were used in the study. Results of ocular examinations, including indirect ophthalmoscopy, slit lamp biomicroscopy, gonioscopy, and applanation tonometry,[a] were normal in all dogs. All animal procedures were conducted in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by an institutional animal care and use committee.

Fluorophotometry protocol—Five Beagles were used in the initial phase of the study to develop an optimal fluorophotometry protocol for use in dogs. One drop of 10% fluorescein sodium was applied to both corneas of each dog at 5-minute intervals for a total of 15 minutes (a total of 4 drops; 4-drop protocol). Five minutes after the last drop was applied, the ocular surfaces and lids were thoroughly rinsed to ensure that fluorescein did not remain in the tear film or periocular area. Paws of the forelimbs and areas of gross fluorescein staining elsewhere on the body were rinsed to ensure that fluorescein could not be introduced into the tear film by a dog rubbing its eyes. Thirty minutes after the last drop of fluorescein was applied, fluorescein concentrations were measured in the cornea and mid-central aqueous humor, using a computerized scanning ocular fluorophotometer with an anterior chamber adapter.[b] Fluorescein measurements were repeated at hourly intervals for 10 hours. For each scan, dogs were sedated with a combination of tiletamine hydrochloride-zolazepam (6.5 to 11 mg/kg of body weight) and butorphanol tartrate (0.4 mg/kg) administered IM. After a 1-week washout period, the study was repeated, using 3 drops of fluorescein at 5-minute intervals for a total of 10 minutes (a total of 3 drops; 3-drop protocol).

Fifteen additional Beagles were used for the remainder of the study. The 3-drop protocol was followed, except that scans were made only 5, 6.5, and 8 hours after application of the final drop of fluorescein.

Determination of aqueous humor flow rate—Analyses of the fluorescein data were accomplished by use of the automated analysis system software provided with the fluorophotometer and by manual computation. Corneal concentrations used in the fluorophotometer's calculation of flow rate were selected by the operator from visual inspection of the fluorophotometric scans, but aqueous humor concentrations were chosen automatically by the fluorophotometer. Corneal and aqueous humor concentrations used in manual computation were identified directly from the scans, using visual inspection (**Fig 1**). For both methods the computations of Jones and Maurice[4] as modified by Yablonski et al[5] were used.

Derivation of calculation of aqueous humor flow rate—To calculate the aqueous humor flow rate, we assumed that the cornea was homogenously loaded with fluorescein at the beginning of the measurements and that after corneal loading additional fluorescein did not enter the cornea from the tear film. We also assumed that once a steady state was reached, all fluorescein leaving the cornea entered the aqueous humor, and all fluorescein leaving the aqueous humor did so by outflow of aqueous humor.

On the basis of these assumptions, a model was developed (**Fig 2**). From this model, the **concentration of fluorescein in the cornea** (C_c), the **concentration of fluorescein in the aqueous humor** (C_a), and the rates of change in these

concentrations were used to calculate aqueous humor flow rates. At steady state, rate of fluorescein egress from the cornea is defined by the following equation:

$$dC_c/dt = k_{c.ca}(C_a - C_c)$$

where $k_{c.ca}$ is the transfer coefficient between the cornea and aqueous humor referred to the corneal volume. Rate of fluo-
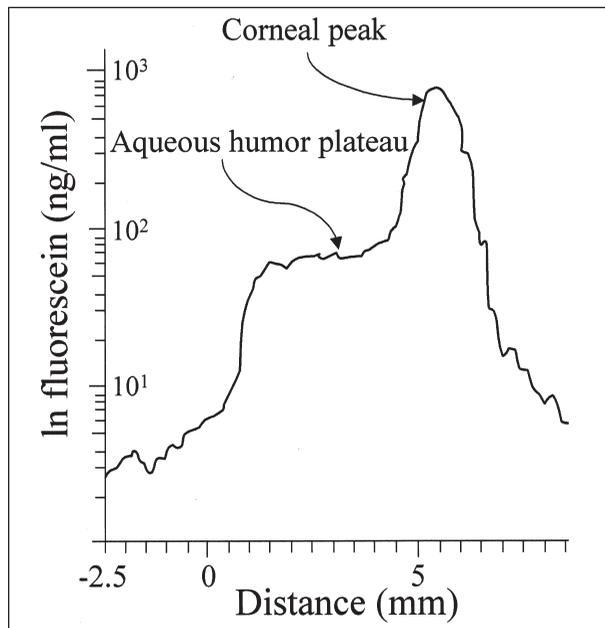

Figure 1—Results of a fluorophotometric scan of the right eye of a representative dog obtained 5 hours after administration of the last of 3 drops of fluorescein. Notice the tall thin corneal peak and the lower aqueous humor plateau.
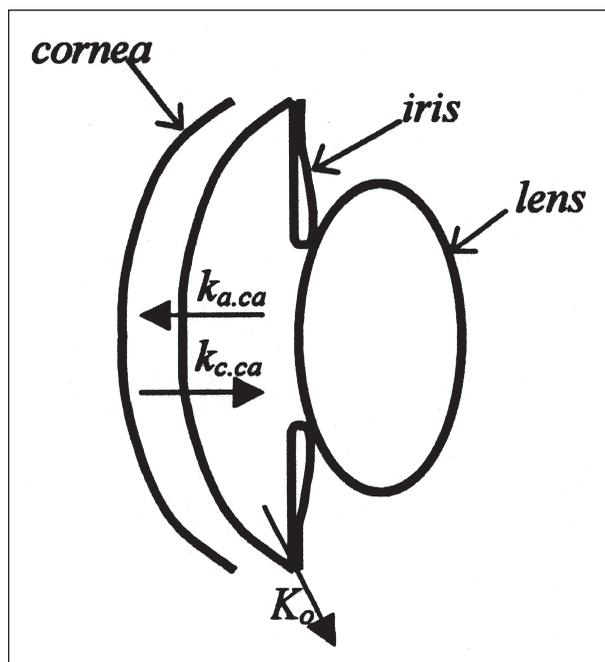

Figure 2—Schematic representation of the model used to derive aqueous humor flow rate calculations in dogs. $k_{a.ca}$ = Transfer coefficient between cornea and aqueous humor referred to the anterior chamber volume. $k_{c.ca}$ = Transfer coefficient between cornea and aqueous humor referred to the corneal volume. K_o = Anterior chamber loss coefficient.

rescein egress from the anterior chamber is defined by the following equation:

$$dC_a/dt = K_oC_a + k_{a.ca}(C_c - C_a)$$

where K_o is the anterior chamber loss coefficient, and $k_{a.ca}$ is the transfer coefficient between cornea and aqueous humor referred to the anterior chamber volume. At steady state the amount of fluorescein entering the cornea from the aqueous humor is negligible, so the anterior chamber egress equation reduces to $dC_a/dt = K_oC_a$.

The rate of egress of fluorescein from an eye is defined as dm_t/dt, where m_t is the total mass of fluorescein in the eye. Because we assumed that fluorescein leaves an eye only by egress with aqueous humor outflow, it follows that $dm_t/dt = -m_aK_o = -V_aC_aK_o$, where V_a is the anterior chamber volume. Dividing by m_t, the equation becomes:

$$(dm_t/dt)/m_t = -V_aC_aK_o/m_t$$

Because $(dm_t/dt)/m_t = d\ln m_t/dt$, then $d\ln m_t/dt = -V_aC_aK_o/m_t$.

At steady state, the rates of change of m_t, C_c, and C_a are all equal, and their slopes are represented by the differential expressions $d\ln m_t/dt$, $d\ln C_c/dt$, and $d\ln C_a/dt$, respectively (**Fig 3**). The **terminal slope of each of these curves** (**A**) can be determined as follows:

$$A = -V_aC_aK_o/m_t$$

Because $m_t = m_a + m_c$, then $m_t = V_aC_a + V_cC_c$. Thus, the equation can be expressed as follows:

$$A = -V_aC_aK_o/(V_aC_a + V_cC_c)$$

This can be further manipulated to yield the equation:

$$K_o = -A \times ([V_aC_a + V_cC_c]/V_aC_a), \text{ which can be expressed as}$$
$$K_o = -A \times (1 + [V_cC_c/V_aC_a])$$

Two correction factors were necessary. The first (spatial resolution correction) was needed because the diamond-shaped intersection of the fluorophotometer's excitation and emission beams is slightly larger than the corneal thickness. Therefore, corneal fluorescence is underestimated. In their study, van Best et al[3] demonstrated that the correction factor is as follows:

$$k_{spat.res.} = 1/(1 - Q \cdot e^{Bd})$$

where Q is 0.9622, B is -1.848, and d is the thickness of the cornea in millimeters. Mean thickness of the central cornea has been reported for dogs between 1 and 7 years old (0.560 mm) and for dogs weighing between 7 and 18 kg (0.552 mm)[8]; our dogs fit both criteria, and the value of 0.555 mm was a compromise between those 2 values. For a corneal thickness of 0.555 mm, $k_{spat.res.}$ is 1.53. The **second correction factor** (**r_{ca}**) accounts for the apparent difference between C_c and C_a, even when those 2 compartments are in equilibrium. Jones and Maurice[4] determined in humans that the apparent fluorescences differed at equilibrium by a factor of 1.2, and the fluorophotometer manufacturer suggests that this value be used for all species. Including these correction factors, the equation becomes:

$$K_o = -A \times (1 + [1.53 \; V_cC_c/1.2V_aC_a])$$

In this equation, A is the average slope of the corneal and aqueous humor decay curves; ideally, these slopes should be nearly equal. The V_c was determined to be 100 µl by measuring the mean volume of saline (0.9% NaCl) solution displaced by corneas excised from 3 size-matched dogs. Estimates of the population average obtained from the literature may be used for V_a, or values may be established for each patient. We used a previously published population average of 400 µl.[9] The ratio of C_c to C_a is obtained from the midpoint of the corneal and aqueous humor decay curves.

Thus, the aqueous humor flow rate was determined as follows:

$$\text{Flow} = K_oV_a$$

Statistical analysis—Correlation coefficients of the corneal and aqueous humor fluorescein decay curves were calculated to ensure that 3 time points reasonably approximated a straight line. Corneal and aqueous humor fluorescein decay curves were compared to ensure that their slopes were decreasing in parallel. Aqueous humor flow rates were calculated, using the automated fluorophotometer software and manual calculation, for the left eye, right eye, and the mean of both eyes. Values for left and right eyes were compared for each computation method, using a paired t-test. Results from the 2 computation methods were compared, using the Student t-test. Values of $P < 0.05$ were considered significant.

Results

Administering fluorescein in accordance with the 4-drop protocol, fluorescein was measurable in the cornea and aqueous humor by the earliest fluorophotometric scan in the initial 5 dogs. However, corneal concentrations were erratic and inconsistent at 0.5, 1, 2, 3, and 4 hours. Gross observation of the corneas for these early time points revealed that fluorescein was not homogeneously distributed throughout the cornea, resulting in inconsistent readings. In some dogs, corneal fluorescein concentrations approached the maximal detection limit in the eye.[10] Therefore, we chose not to use this protocol.

Administering fluorescein in accordance with the 3-drop protocol provided more uniform corneal staining by 3 to 4 hours after application with corneal concentrations well within the fluorophotometer's accuracy range. From 4 through 10 hours, a semilogarithmic decrease in corneal and aqueous humor concentrations
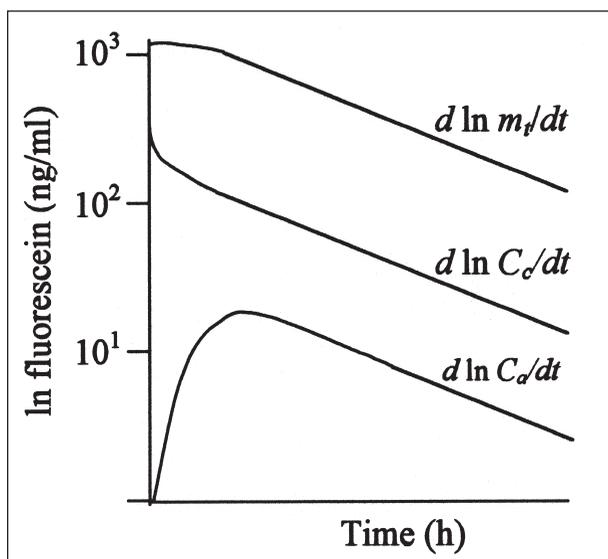

Figure 3—Graph used to calculate aqueous humor flow rate in dogs. The terminal slopes of the lines represent the rate of change in total ocular fluorescein mass ($d\ln m_t/dt$), rate of change in fluorescein concentration of cornea ($d\ln C_c/dt$), and rate of change in fluorescein concentration of aqueous humor ($d\ln C_a/dt$).
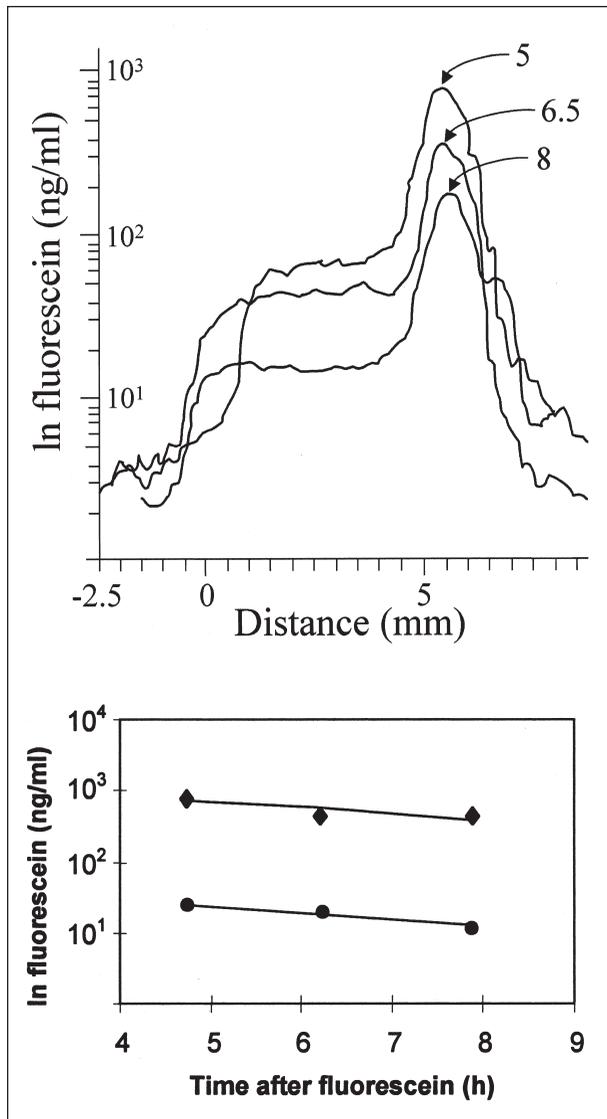
Figure 4—Results of fluorophotometric scans of the right eye of the dog in Figure 1 obtained 5, 6.5, and 8 hours after application of fluorescein (top) and logarithmically transformed fluorescein concentrations of the cornea (diamond) and aqueous humor (circle) determined from those scans (bottom). Slopes of the 2 lines should be equal, and the slope and concentration ratios are used to calculate aqueous humor flow rate.

was detected, with a strong correlation between the natural logarithm of the fluorescein concentrations and time. Slopes of the corneal and aqueous humor decay curves were approximately equal between these times as well. On the basis of these observations, the 3-drop protocol with scan times of 5, 6.5, and 8 hours

Table 2—Aqueous humor flow rates determined by fluorophotometry that were calculated by use of automated fluorophotometer software and manual computation

	Aqueous humor flow rate (μl/min)	
Eye	Fluorophotometer software	Manual
Left	4.54 ± 3.08 (−1.65–9.91)*	5.58 ± 2.42 (2.61–9.34)*
Right	4.54 ± 3.10 (0.29–11.33)	4.86 ± 2.49 (1.47–10.69)
Both	4.54 ± 2.57 (−0.36–10.62)	5.22 ± 1.87 (2.17–9.87)

Values reported are mean ± SD (range).
*Values differ significantly ($P = 0.02$).

after application was chosen for determination of aqueous humor flow rate.

Graphs were made of results obtained at 5, 6.5, and 8 hours (**Fig 4**). Subsequently, the logarithmically transformed fluorescein concentrations were plotted versus time, revealing the semilogarithmic decay and similarity of corneal and aqueous humor slopes. Correlation coefficients between corneal and aqueous humor lines of best fit were determined (**Table 1**). Ratios between slopes of corneal and aqueous humor decay curves also were determined.

Aqueous humor flow rates were determined by use of the automated fluorophotometer software and manual computation methods (**Table 2**). We did not detect a significant difference between values for the left and right eyes for either method. For the left eye, the manually calculated aqueous humor flow rate was significantly higher than the flow rate calculated by use of the fluorophotometer software. There was not a significant difference between the 2 computation methods for the right eye or mean of both eyes.

Discussion

It is imperative when using fluorophotometric calculations that the cornea be homogeneously stained with fluorescein and that concentrations be measured during the period in which they are decreasing semilogarithmically from the cornea and aqueous humor.[4] Because the method reported here has not been used in dogs, we had to establish a protocol for fluorescein administration and measurement times that would satisfy these requirements. We started with a commonly used protocol for humans in which the cornea is stained with 1 drop of 10% fluorescein every 5 minutes for a total of 4 drops, and fluorescein concentrations in the cornea and aqueous humor are measured 4, 5, and 6 hours after the final application of fluorescein.[3] Administration in accordance with this protocol did not result in homogeneous corneal staining for the early evaluation points. Administration of fluorescein in accordance with the 3-drop protocol resulted in

Table 1—Correlation coefficients and ratio of the slopes for fluorescein concentration in the cornea and aqueous humor derived from regression lines of logarithmically transformed fluorescein concentrations versus time

	Correlation coefficients		
Eye	Cornea	Aqueous humor	Slope ratios
Left	0.81 ± 0.28 (0.002–1.00)	0.95 ± 0.07 (0.83–1.00)	1.05 ± 0.54 (0.04–2.01)
Right	0.85 ± 0.23 (0.27–1.00)	0.93 ± 0.12 (0.52–1.00)	1.14 ± 0.55 (−0.06–2.06)
Both	0.83 ± 0.17 (0.46–0.99)	0.94 ± 0.07 (0.76–1.00)	1.09 ± 0.36 (0.26–1.53)

Values reported as mean ± SD (range).

homogenous corneal staining by 3 to 4 hours for all dogs. Graphs of the natural logarithm of fluorescein concentrations in cornea and aqueous humor versus time were linear between 4 and 10 hours after fluorescein administration. They may have remained linear beyond 10 hours, but points beyond 10 hours were not evaluated. Slopes of the corneal and aqueous humor lines were approximately equal. Analysis of these results indicated that administration in accordance with the 3-drop protocol is valid for dogs if measurements are obtained between 4 and 10 hours after fluorescein administration. Although only 2 measurement points during this period are needed to establish decay curves necessary for determination of slopes and concentration ratios, use of additional points provides greater accuracy. Most investigators use at least 3 time points. We chose to evaluate the values for the time points 5, 6.5, and 8 hours after fluorescein administration because they were fairly evenly distributed throughout the range of 4 to 10 hours.

Correlation coefficients for the decay curves of natural logarithm of fluorescein concentration versus time were generally quite high (Table 1), indicating that corneal and aqueous humor concentrations of fluorescein were decreasing semilogarithmically over the period studied and that 3 measurements were adequate to describe the decay in fluorescein concentrations. The ratio of the slopes for the concentrations in the cornea and aqueous humor were near unity, indicating that they were decreasing in parallel. A few dogs had low correlation coefficients or disparate slopes. The most likely explanation for these findings is reintroduction of fluorescein into the tear film after measurements had begun or inaccurate measurement of corneal fluorescein concentrations as a result of movement of the dog (slight movements do not affect aqueous humor measurements because of the large volume of the anterior chamber in relation to the spatial resolution of the fluorophotometer). Reintroduction of fluorescein into the tear film was unlikely because of the thorough rinsing following instillation of the last fluorescein drop, but movement of dogs during scans can be encountered. We have not been able to obtain adequate patient cooperation to perform fluorophotometry without chemical restraint and have evaluated a number of sedation regimens for use in dogs to minimize motion during fluorophotometric readings.[11-14] The combination of tiletamine-zolazepam and butorphanol appears optimal. However, a small number of dogs continued to have sporadic head movements. Potential solutions to this problem include zealous manual restraint or use of general anesthesia. We have found that aggressive manual or mechanical restraint only aggravates the problem. We have avoided volatile and injectable general anesthetics, because they have effects on the cardiovascular system and acid-base balance that could alter aqueous humor dynamics.[15] Because the number of subjects that had troublesome head motion was small, it did not affect the overall calculations for our moderate sample size.

As an alternative to finding solutions to the motion problem, poor correlation coefficients or incongruous slopes could be assumed to represent motion artifacts and eliminated from the data set. For example, if 0.7 is arbitrarily chosen as a minimally acceptable correlation coefficient, and only slope ratios between 0.5 and 1.5 are considered acceptable, 3 dogs would have been eliminated from our study. However, mean ± SD flow rate for the remaining 12 dogs (5.53 ± 1.93 µl/min) is not appreciably different from the values calculated for all 15 (5.22 ± 1.87 µl/min). Another approach is to discard data from individuals with correlation coefficients or slope disparities that are statistically defined as extreme outliers. If correlation coefficients or slope ratios > 3 times the interquartile range are defined as extreme outliers,[16] and we eliminated data from such dogs, 1 dog would have been eliminated from the study. Mean ± SD flow rate for the remaining 14 dogs is 5.20 ± 2.00 µl/min. Fluorophotometry studies in humans can be affected by poor fixation of the subject, and such data points simply are discarded.[2]

We calculated mean aqueous humor flow rates of approximately 4.5 µl/min by use of the automated fluorophotometer software and 5.0 to 5.5 µl/min by use of manual calculations. Although these figures do not differ drastically, there was a significant difference for the left eye. We believe that the method of data acquisition used by the automated software system is inherently inaccurate when applied to dogs and, therefore, could result in miscalculation of aqueous humor flow rates. The software initiates its calculations of flow rate by prompting an investigator to place a cursor on the peak corneal concentration of each scan. This is typically a high narrow peak, and its position within the scan is always obvious. The instrument then automatically chooses the aqueous humor concentration located midway between the previously selected corneal peak and the rostral aspect of the lens, which is identifiable in humans as a result of intense lenticular autofluorescence in that species. Unfortunately, lenses of dogs do not autofluoresce intensely at the emission peak of fluorescein sodium, making the aqueous humor fluorescein concentration used in the calculations somewhat arbitrary. The propensity for erroneous data acquisition when using the automated software is particularly evident in the negative flow values calculated for some dogs (Table 2). Barring reintroduction of fluorescein into the tear film after initiation of measurements, de novo synthesis of fluorescein by the anterior segment, or retrograde flow of aqueous humor from the systemic circulation into the anterior chamber, such results are impossible. Mathematic manipulation of the data in manual computation of aqueous humor flow rate was identical to that used by the fluorophotometer's software, but we were able to more accurately assess fluorescein concentrations of the aqueous humor by direct inspection of the aqueous humor and corneal sections of the scans. Therefore, manually calculated flow rates are more reliable.

Aqueous humor flow rate in dogs has been indirectly estimated from the facility of aqueous humor outflow, which is 0.2 to 0.3 µl/min • mm Hg in clinically normal dogs.[17,18] Facility of outflow is related to aqueous humor flow rate by the equation[19] $f = (C[IOP - EVP]) + f_u$, where f is aqueous humor flow rate, C is facility of outflow, EVP is episcleral venous pressure,

and f_u is the rate of uveoscleral aqueous humor outflow. Assuming a normal IOP of approximately 20 mm Hg[20] and a normal episcleral venous pressure of approximately 11 mm Hg,[21] an estimate of trabecular aqueous humor flow rate in dogs is 2.3 µl/min. A value of 2.5 µl/min has been reported in the veterinary literature.[22] Analysis of the data from the study reported here indicated a flow rate approximately twice that estimate. In humans, fluorophotometrically measured aqueous humor flow rates also are substantially greater than tonographic estimates.[2] One reason for this discrepancy is that tonography does not measure uveoscleral outflow, whereas fluorophotometry measures total outflow. Barrie et al[23] found that uveoscleral outflow accounts for approximately 15% of total outflow in clinically normal dogs. Adding this to the tonographic estimate would yield mean total flow of 2.9 µl/min, which still differs slightly from our results. The additional discrepancy could be accounted for by technical artifacts associated with tonography, which is influenced by ocular rigidity, corneal curvature, and changes in intraocular blood volume and episcleral venous pressure induced by the tonometer.[1,19] Fluorophotometric measurements are independent of these variables, and fluorophotometry is widely accepted as the method of choice for determining aqueous humor flow rates.[1] Whereas several aforementioned assumptions were made in the derivation of the equations used in fluorophotometry, these assumptions have consistently proven valid.[1,4-6] When fluorophotometry was applied to perfused enucleated eyes of rhesus monkeys, calculated flow rates correlated closely with the known perfusion rate, providing convincing evidence of the accuracy of the fluorophotometric technique.[24]

Although the left and right eyes of any specific dog had similar flow rates, there was considerable variability in flow rates among dogs. The lowest measured flow rate (by manual computation) was 1.47 µl/min, and the highest was 10.69 µl/min. A degree of variability also has been reported in clinically normal humans, with reported ranges of 0.2 to 32.0 µl/min,[3] 1.5 to 4.6 µl/min,[2] and 1.61 to 5.27 µl/min.[25] Interestingly, K_o in our dogs (0.014/min, as calculated from the equation $K_o = f/V_a$) was nearly identical to that reported in clinically normal humans (0.013 to 0.015/min)[4,5] and owl monkeys (0.010/min).[6] This variable describes the fractional egress of aqueous humor from the anterior chamber per unit of time. The similarity in values suggests that the metabolic demands placed on the aqueous humor by the anterior segment are essentially the same among these species and that the higher flow rate in dogs is necessitated by the larger anterior chamber volume, which is approximately 400 µl in dogs, 174 µl in humans,[5] and 300 µl in owl monkeys.[6]

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