The flow of aqueous humor is an important aspect of normal ocular health in all animals. The function of the eye relies on the normal turnover of aqueous humor to provide nutrients to and remove waste products from avascular structures within the eye, including the corneal endothelium and lens. Aqueous humor flow is also a vital component in establishing normal intraocular pressure, thereby providing the framework for appropriate visual function by maintaining structural alignment for the cornea, lens, and retina. Alterations in normal aqueous humor flow can be caused by serious ocular diseases, such as glaucoma and uveitis, and flow alterations may have damaging effects on the eye and threaten vision. Although the changes in aqueous humor flow may not be the direct cause of these damaging sequelae, a proper understanding of flow characteristics during these events may help elucidate the specific causes and effects of the diseases or enable objective evaluation of potential therapeutic interventions.

Fluorophotometry can be used for accurate noninvasive assessment of aqueous humor flow. The first objective fluorophotometer was introduced by Maurice in 1963 and was followed 3 years later by the introduction of a specific mathematical model by Jones and Maurice. This mathematical model estimates the rate of aqueous flow on the basis of a reduction in fluorescence of the aqueous humor over time following topical administration of fluorescein sodium. The model is predicated on the assumption that a steady state is reached as the fluorescein passes from the cornea into the aqueous humor and eventually exits via the aqueous humor outflow tracts. Decay curves of this steady decrease are generated from periodic fluorophotometric measurements obtained hours after initial fluorescein application to the cornea. The slopes of these decay curves are the basis for aqueous humor flow measurement, once the slopes applied to the aforementioned equations. This model has been used, with slight modification, in several species, including humans, dogs, and cats, and is currently the model most predominantly used for flow investigations.

Fluorophotometric aqueous humor flow data obtained from cats have been reported, although those previous investigations used the information largely as an adjunct to the evaluation of other aspects of aqueous humor dynamics. The protocols for those evaluations were varied and often required ophthalmic administration of large numbers of drops of fluorescein and a long interval between drop application and fluorophotometric evaluation. In those investigations, the ap-
plication of the fluorescein, fluorescein concentration, and flow measurement protocol differed considerably. Consequently, reported aqueous humor flow rates for cats range widely, from 3.6 to 22.7 μL/min.14-17 Because the locus of flow determination has been on identifying a base value with which posttreatment values can be compared, this variability has largely been ignored, which has made it difficult to compare values for cats with those for other species. Recently, a more streamlined fluorescein application protocol was established for use in dogs,18 and subsequent investigations11,12 have confirmed the viability of that technique. In cats, application of a more streamlined protocol would reduce the time necessary to perform fluorophotometry as well as eliminate the variables associated with application of an excessive number of drops of fluorescein and the need for a long interval between fluorescein application and flow measurement. Any such protocol would have to be evaluated to determine whether it successfully meets the required assumptions that ensure the accuracy and validity of the flow data. The purpose of the study reported here was to evaluate the aqueous humor flow rate in clinically normal cats by use of a noninvasive method that has been proven successful and repeatable in dogs, with the intent to compare values with those reported for other species as well as with findings of previous investigations in cats.

Materials and Methods

Animals—Twenty cats (12 females and 8 males) that weighed 2.5 to 5.2 kg were included in the study. The mean age of the cats was 11 months. The cats were purchased from 2 commercial suppliers.4 Each cat underwent a full physical examination including complete ophthalmic examination. Cats were assessed via slit-lamp biomicroscopy; indirect ophthalmoscopy, corneal fluorescein staining,5 and rebound tonometry.6 All cats were determined to be in good health, and no ocular abnormalities were detected prior to inclusion in the study. The animals were housed individually or in pairs throughout the study. This study adhered to the guidelines of the Institutional Animal Care and Use Committee at Kansas State University.

Fluorophotometry protocol—The cats were randomly assigned to 1 of 4 groups (5 cats/group). Group assignment dictated the day on which fluorophotometry would be performed. One group of 5 cats was used initially to assess the feasibility of the proposed fluorophotometry protocol. A 3-drop protocol described by Ward et al10 was used to introduce fluorescein into and ensure its homogeneous distribution within the cornea and anterior chamber. For each cat, 1 drop of 10% fluorescein sodium was applied by use of a sterile dropper bottle into each eye every 5 minutes until 3 drops/eye had been administered topically. Five minutes after instillation of the third drop in each eye, the eyes, face, and forelimbs were thoroughly rinsed to ensure no fluorescein stain remained that could be reintroduced into the eyes during grooming. If any fluorescein drops were visible within the cages, they were cleaned and bedding was removed. One hour and 55 minutes after the rinse, each cat was sedated by use of ketamine hydrochloride (5 mg/kg, IM) and medetomidine hydrochloride (0.03 mg/kg, IM) to facilitate positioning.

Two hours after rinsing of the eyes, face, and forelimbs, fluorophotometric readings of both eyes of each cat were obtained by use of a computerized scanning fluorophotometer with an anterior chamber adapter.8 The head and eyes were aligned by use of manual restraint with the aid of a small platform and a plastic eyepiece adaptor designed to approximate appropriate eye position for the holder. Fluorescein concentrations in the cornea and the aqueous humor of the midcentral portion of the anterior chamber were measured by use of a scanning ocular fluorophotometer fitted with an anterior chamber adapter. Repeated measurements were obtained at 4, 5, 6, 7, 8, 9, and 10 hours after fluorescein application. Sedative administration was repeated at reduced doses (generally 0.01 to 0.015 mg of medetomidine/kg) as necessary to achieve the desired state of compliance for positioning. During the intervals between measurements, cat’s eyes were kept closed with tape to prevent corneal desiccation.

Each of the remaining 3 groups (5 cats/group) was evaluated by use of the same 3-drop protocol on a different day. The same sedative administration and fluorophotometric technique were used for each cat, with the exception that fluorophotometric measurements of both eyes were obtained at 5, 6, 7, and 8 hours after fluorescein application.

Aqueous flow rate calculation—For each cat, fluorophotometric scans were evaluated at each time point to determine the corneal and aqueous humor fluorescein concentrations. The landmarks used for this assessment were the apex of the peak of the corneal readings and the midcentral plateau of the aqueous humor readings. These data points were then natural logarithmically transformed and plotted, and regression analysis was performed to derive the slopes of the 2 lines (lines based on the cornea and aqueous humor data). The equations used to determine the aqueous flow rate were derived by Jones and Maurice4 and later modified by Yablonski et al3 and van Best et al18:

\[
\text{Flow} = K \cdot V_a
\]

where \( A \) is mean of the slopes of the decreasing cornea and aqueous humor fluorescein concentrations, \( V_c \) is corneal volume, \( V_a \) is anterior chamber volume, \( C_a \) is corneal fluorescein concentration, \( C_c \) is anterior chamber fluorescein concentration, and \( k_{\text{aqueous}} \) is a correction factor necessary to compensate for underestimation of corneal fluorescence inherent to fluorophotometric measurements. This underestimation results because the focal diamond created by the fluorophotometer for measurement of fluorescein concentration is wider than the thickness of the cornea in cats. The correction factor is calculated as follows:

\[
k_{\text{aqueous}} = 1/(1 - Q \times X d)
\]

where \( Q \) is 0.9622, \( B \) is \(-1.848\), and \( d \) is the thickness of the cornea in millimeters. A value of 0.565 mm
has been reported for the corneal thickness in cats < 1 year of age and was used for d in the present study, resulting in \( k_{\text{water}} \) of 1.51. The corneal and anterior chamber fluorescein concentrations were obtained from the midpoint of the fluorescein decay curves. Published means for the anterior chamber volume (820 µL) and corneal volume (165 µL) in cats were used. The denominator value of 1.2 represents a second correction factor established by Jones and Maurice, which was generated to account for an inherent difference in fluorescence between the cornea and the aqueous humor that is suggested to be present in all species.

**Statistical analysis**—Regression analysis of the natural logarithmically transformed corneal and aqueous humor fluorescein concentrations obtained from 20 cats was used to create linear decay curves. Correlation coefficients were calculated to assess the fit of the 4 time points to the approximated straight line. The slopes of these curves were then compared to ensure that they were decreasing in a reasonably parallel fashion. Aqueous humor flow rates were calculated for the right and left eyes, and a mean rate for both eyes was subsequently calculated. A paired t test was used to compare the results between the right and left eyes. Eyes were excluded from flow rate calculation when correlation or slope ratios values represented extreme outliers (defined as > 3 times the interquartile range). For all comparisons, a value of \( P < 0.01 \) was considered significant.

### Results

Evaluation of corneal and aqueous humor fluorescence revealed that the ophthalmically applied fluorescein sodium was homogenously distributed within the cornea and aqueous humor at the 4-hour postapplication time point and thereafter in all cats. Fluorescein concentrations slowly decreased after the 4-hour time point.

The mean ± SD correlation coefficients for the logarithmically transformed corneal and aqueous humor fluorescein concentration data used for aqueous humor flow calculation were calculated (Table 1). These values indicated that the calculated aqueous humor flow values (Table 2) were accurate; the corresponding anterior chamber turnover rates were also determined. No significant (\( P = 0.201 \)) difference was detected between aqueous humor flow rates for the right and left eyes.

### Discussion

By use of the 3-drop protocol for fluorescein application described by Ward et al and established fluorophotometric methods developed by Jones and Maurice and modified by Yablonski et al and van Best et al, the mean baseline aqueous humor flow rate in clinically normal cats was calculated to be 5.5 µL/min. To our knowledge, the present study is the first in which this more streamlined protocol for fluorescein administration was used in cats and the only investigation to date to focus solely on the determination of normal aqueous humor flow in this species. For this reason, it was important to fully evaluate the technique to ensure the tenability of the reported results.

When fluorophotometry is used to evaluate aqueous humor flow, it is important to ensure that certain assumptions are met for the mathematical model to be valid. These assumptions are as follows: the topically applied fluorescein is homogenously distributed throughout the cornea and aqueous humor at the times at which measurements are obtained; application of fluorescein to the cornea is performed at the start of the procedure, with no subsequent applications during the procedure; and the corneal and aqueous humor fluorescein concentrations decrease at the same rate over the time points at which measurements are obtained. In dogs, Ward et al demonstrated that these assumptions could be met by use of the 3-drop protocol. Use of that same protocol in the present study proved to be equally effective in cats. Fluorophotometric readings were initially obtained for 5 cats, and homogenous, steady-state fluorescein concentrations in the cornea and aqueous humor were detected at 4 hours after fluorescein application. This time point was similar to that identified for dogs. A steady decrease in fluorescein concentration after that time point allowed flow measurements to be obtained from 5 to 8 hours after fluorescein application, enabling flow calculations to be performed in the same day.

In the present study, the correlation coefficients of the corneal and aqueous humor slopes indicated high correlation between data points and calculated slopes; thus, the slopes obtained were a reliable representation of the data points collected and a reasonable approximation of the true rate of decrease (Table 1). Similar to results of previous studies in other species, the correlation values obtained after natural logarithmic transformation also indicated that this decrease was semi-

<table>
<thead>
<tr>
<th>Table 1—Correlation coefficients and slope ratios calculated for the change in fluorescein concentration in the cornea and aqueous humor of both eyes of 20 clinically normal cats.*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Correlation coefficient</strong></td>
</tr>
<tr>
<td><strong>Eye</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Left</td>
</tr>
<tr>
<td>(0.19–0.97)</td>
</tr>
<tr>
<td>Right</td>
</tr>
<tr>
<td>(0.15–0.98)</td>
</tr>
<tr>
<td>Both</td>
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<tr>
<td>(0.15–0.98)</td>
</tr>
<tr>
<td>Values are reported as mean ± SD (range). *For each cat, 1 drop of 10% fluorescein sodium was instilled into both eyes every 5 minutes until 3 drops had been administered. Fluorophotometry was performed at 5, 6, and 7, and 8 hour after fluorescein application.</td>
</tr>
</tbody>
</table>

<p>| Table 2—Aqueous humor flow rates and anterior chamber turnover rates determined fluorophotometrically in 20 clinically normal cats.* |
| --- | --- |
| <strong>Aqueous humor flow rate</strong> | <strong>Anterior chamber turnover rate</strong> |
| <strong>(µL/min)</strong> | <strong>(% of the anterior chamber volume/min)</strong> |</p>
<table>
<thead>
<tr>
<th><strong>Eye</strong></th>
<th><strong>Flow rate</strong></th>
<th><strong>Turnover rate</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>5.05 ± 2.06 (1.17–9.89)</td>
<td>0.6 ± 0.3 (0.3–1.3)</td>
</tr>
<tr>
<td>Right</td>
<td>5.62 ± 2.40 (2.56–10.67)</td>
<td>0.7 ± 0.3 (0.1–1.3)</td>
</tr>
<tr>
<td>Both</td>
<td>5.51 ± 2.21 (1.17–10.67)</td>
<td>0.7 ± 0.3 (0.1–1.3)</td>
</tr>
<tr>
<td>Values are reported as mean ± SD (range). No significant (( P = 0.201 )) difference was detected between aqueous humor flow rates for the right and left eyes. See Table 1 for remainder of key.</td>
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</tbody>
</table>
logarithmic in nature. Analysis of slope ratios revealed a reasonably parallel rate of decay between the cornea and aqueous humor fluorescein concentrations. This allowed for the assumption that they were decreasing at the same rate, fulfilling the necessary requirements to proceed with flow calculation by use of the described equations.4,5,18

The mean correlation values and slope ratios determined for cats in the present study were not as high as those previously reported for dogs. This could be due to the inherent difficulty of working with cats or simple statistical variability that can occur from study to study. Variation among cat eyes was detected, with some of the eyes having low correlation values or slopes that were less than parallel. Such variation, which can occur as a result of placement, head or ocular movement, or reintroduction of fluorescein into the eyes, is common in fluorophotometric investigations. Evaluation of the data obtained in the present study revealed that often 1 reading stood out as possibly spurious, thereby creating a lower overall correlation. In general, the corneal measurements were often the most affected by these possibly spurious measurements. This was attributed to the thin width of the cornea, which made accurate measurement of the corneal concentration more difficult than measurement of the concentration in the large anterior chamber and especially susceptible to motion artifacts from either head or ocular movements. During the present study, sedation was used to limit these movements, but it was possible that minor ocular movements were not noticed during data collection and resulted in inaccurate readings.

The reported aqueous humor flow rates for cats range widely from 3.6 to 22.7 µL/min.14–17 A review of the veterinary medical literature revealed that the initial, more invasive investigations of flow generally resulted in higher values (estimated flow in cats, 14 to 20 µL/min).20–22 These older measurement techniques were often derivations of outflow facility or were performed under constant ocular perfusion; therefore, the derived data are less likely to accurately depict true aqueous flow. Fluorophotometry was developed as a direct measure of aqueous humor flow that can be performed in a live animal without marked alteration of the natural flow of fluid within the eye and is considered the most accurate method of aqueous humor flow rate determination. In the present study, the calculated mean aqueous humor flow rate in clinically normal cats was 5.51 µL/min, which compares favorably to values obtained during previous fluorophotometric investigations15–17 (calculated flow rate range, 5.0 to 6.2 µL/min). Examination of the components of flow calculation among the previous studies revealed that similar values were used for corneal volume (150 to 170 µL) and aqueous humor volume (700 to 820 µL), despite different approaches to volume calculation. Anterior chamber turnover rates determined in those previous investigations and in the present study were also very similar, ranging from the present study value of 0.7% of the anterior chamber volume/min (also expressed as 0.007/min) to 0.9%/min. Interestingly, closer examination of another fluorophotometric study by Hayashi et al13 revealed a similar anterior chamber turnover rate of 0.73%/min, despite a reported flow rate of 3.6 µL/min. The anterior chamber volume used for that calculation was 479 µL, which was likely the source of the gross underestimation of aqueous humor flow in that study and was addressed by the authors.14

The aqueous humor flow rate for cats (5.51 µL/min) in the present study was similar to that determined for dogs (5.22 µL/min) by use of a similar technique.10 This similarity was unexpected. Because of the large size of the anterior chamber in cats (700 to 800 µL), one would expect the aqueous humor flow rate in cats to be much higher than that of dogs, in which the anterior chamber volume is approximately 400 µL.1

An explanation for the incongruous similarity in aqueous humor flow rates may be provided by evaluation of the anterior chamber turnover rate in the 2 species. Cats have a lower turnover rate of aqueous humor in anterior chamber (0.7%/min) than do dogs (1.4%/min) and thus the appearance of a lower rate of flow per volume. These anterior chamber turnover rates are quite variable among other species, including humans (1.25%/min to 1.5%/min),4,5,26–28 owl monkeys (1.0%/min),29 cynomolgus monkeys (mean among reported values, 1.8%/min),30–37 and rabbits (1.9%/min to 2.1%/min).38,39 In a previous report,10 it was postulated that these turnover rates of aqueous humor in the anterior chamber were similar because of the similar metabolic needs of internal ocular structures among animals, an assessment that was based on information from dogs, humans, and owl monkeys. With the addition of the data obtained from cats, the mean aqueous humor turnover rate among all the aforementioned species ranges from approximately 1% to 2% of the anterior chamber volume/min, which appears to support that postulation, especially considering the inherent variability in aqueous humor flow measurement via fluorophotometry.

However, within that range, the mean turnover rate of aqueous humor in the anterior chamber in cats is half of that in dogs and nearly one-third of that in rabbits and cynomolgus monkeys. Although variability exists, the equations used for calculation of aqueous flow are fairly sensitive to small changes in the rate of aqueous turnover. Among the species for which anterior chamber turnover rate data are available, there appears to be a pattern. Higher anterior chamber turnover rates are present in animals with small anterior chamber sizes (rabbits and cynomolgus monkeys), and lower anterior chamber turnover rates are present in animals with large anterior chamber sizes (cats); other species with medium-sized anterior chambers have midrange anterior chamber turnover rates. Building on the idea that a basic metabolic requirement is necessary for mammalian eyes, this general tendency could represent modifications in species with different anterior chamber sizes to avoid excessively high aqueous flow rates (eg, animals with larger anterior chambers) or to maintain a minimum aqueous flow rate necessary for proper ocular function (eg, animals with smaller anterior chamber sizes). Future investigations into the relationship between aqueous humor flow, anterior chamber size, globe size, and the metabolic demands of the internal ocular structures of species with different anterior chamber sizes are necessary to firmly establish
whether this pattern is relevant or simply a result of variability within aqueous flow measurements. If relevant, such studies might also illuminate differences in ocular function among species that could influence understanding of ocular disease and strategies toward treatment.

References