

Tear volume, turnover rate, and flow rate in ophthalmologically normal horses

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Objective—To determine tear volume, turnover rate, and flow rate in ophthalmologically normal horses by use of fluorophotometry.

Animals—12 mares free of ophthalmic disease.

Procedures—2 μL of 10% sodium fluorescein was instilled onto 1 eye of each horse, and tear samples were collected via microcapillary tubes from the inferonasal conjunctival cul-de-sac at 0, 2, 4, 6, 10, 15, and 20 minutes after instillation. Collected tear samples were then measured for fluorescein concentrations with a computerized scanning ocular fluorophotometer. A decay curve plot of concentration changes over time was used to determine tear flow rate and volume through 2 different mathematical treatments of the data (the including method and the excluding method).

Results—Fluorescein concentration in tears decreased in a first-order manner. The including method yielded a mean tear volume of 360.09 μL , a turnover rate of 12.22%/min, and a flow rate of 47.77 $\mu\text{L}/\text{min}$. The excluding method yielded values of 233.74 μL , 13.21%/min, and 33.62 $\mu\text{L}/\text{min}$, respectively. Mean \pm SD correlation coefficients for the natural logarithm of the fluorescein concentration versus time were 0.93 ± 0.12 for the including method and 0.98 ± 0.03 for the excluding method.

Conclusions and Clinical Relevance—The excluding method yielded more accurate results. A tear flow rate of 33.62 $\mu\text{L}/\text{min}$ and a tear volume of 233.74 μL imply a complete recycling of the tear volume in approximately 7 minutes and suggest that increased dosing regimens or constant infusion methods for topical administration of ophthalmic drugs may be indicated when treating horses for corneal disease in which high ocular surface concentrations are needed. (*Am J Vet Res* 2010;71:671–676)

Choice of dosing volume and frequency for topical administration of ophthalmic drugs used in the treatment of horses with ocular diseases has historically been largely empirical. A major impediment to the development of more quantitatively legitimate dosing regimens is that the volume and turnover rate of tears in healthy horses are unknown. Such information is important because the tear fluid has a dilutional effect on exogenously applied drugs. Therefore, determination of tear volume and flow rate in equine eyes will help establish more rigorous scientific standards for topical ophthalmic treatment regimens through better estimation of drug kinetics in tear film.

Most of the tear film is produced by the main lacrimal gland and the gland of the nictating membrane and ejected onto the ocular surface through multiple small ductules. Tears are distributed across the corneal

ABBREVIATIONS	
In[FI]	Natural logarithm of the fluorescein concentration
MIC ₉₀	Minimal concentration required to inhibit the growth of 90% of organisms
STT	Schirmer tear test

surface by the sweeping actions of the eyelids and nictating membrane, and they are removed from the ocular surface by movement into the nasolacrimal system, absorption into ocular tissues, and evaporation.^{1,2}

The most commonly used method of assessing the lacrimal system in domestic animals is the STT, which involves use of a small strip of filter paper imbued with dye and placed in the inferior conjunctival cul-de-sac for 60 seconds. However, the STT does not measure the tear flow rate per se. It does yield a relative measure of tear film volume through comparison of the distance by which tears wick up the paper strip in a given animal to published reference values, but it does not measure an absolute volume.³ Studies^{4,5} in which the STT was used to assess relative tear volumes in horses have been performed to establish reference limits and to assess the influence of environmental factors and signalment on those values.

Whereas various techniques, including lacrimal scintigraphy,⁶ lacrimal streak dilution,⁷ and mathematical modeling,^{8,9} have been used to quantify absolute tear

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turnover and flow rates in humans, fluorophotometry has emerged as the gold standard.¹⁰⁻¹³ Mishima et al¹⁰ used fluorophotometry to measure rates of fluorescein decay in human eyes,¹⁰ and the mathematical treatment of the data has been corroborated by several investigators and remains the basis for most fluorophotometric tear flow studies. The purpose of the study reported here was to use fluorophotometry to measure the tear volume, turnover rate, and flow rate in ophthalmologically normal horses.

Materials and Methods

Horses—Twelve mares typically used for teaching purposes at the University of Tennessee College of Veterinary Medicine were used for the study. The mean age was 11.1 years (range, 5 to 15 years), and the mean body weight was 478 kg (range, 426 to 525 kg). Four of the 12 horses were also receiving a nutraceutical^a (various omega-3 fatty acids, minerals, and vitamins) to evaluate its effects on the gastric mucosa. All horses were judged to have healthy eyes by means of slit-lamp biomicroscopy and indirect ophthalmoscopy prior to commencement of the study. The STT value in all horses was within reference limits, exceeding 15 mm of wetting in 60 seconds in all tests.⁵ All experiments were carried out in compliance with the US Animal Welfare Act and with the approval of the University of Tennessee Institutional Animal Care and Use Committee.

Experimental protocol—Although tear production in horses does not vary diurnally,⁵ all experiments were performed at the same time of day (between 9:00 AM and 11:00 AM) for consistency. The horses received no general sedatives or local nerve blocks that might otherwise confound tear production and measurement. Horses were acclimated to the procedures for 7 days prior to data collection by application of saline (0.9% NaCl) solution eye-drops and tear collection from the inferonasal conjunctival cul-de-sac 2 to 3 times/d in a manner identical to that described for the actual data acquisition.

A 2- μ L glass microcapillary tube^b with a rubber bulb stopper was used to instill 2 μ L of 10% sodium fluorescein^c onto the upper bulbar conjunctiva of the left eye without touching the eye. Immediately thereafter (0 minutes), a tear sample was collected from the inferior conjunctival fornix by everting the inferior eyelid and gently placing a 2- μ L microcapillary tube against the conjunctiva for 2 to 3 seconds. The volume of the collected sample was calculated by measuring the length of fluid contained within the microcapillary tube and extrapolating the volume from the volume-to-length ratio of the microcapillary tube. The contents of the microcapillary tube were then expelled into a 1.5-mL Eppendorf tube^d containing 1.0 mL of buffered saline solution. The collection procedure was repeated at 2, 4, 6, 10, 15, and 20 minutes. Fluorescein concentrations were measured with a computerized scanning ocular fluorophotometer^e fitted with a cuvette holder. Samples were placed in the glass cuvette, and 1.0 mL of buffered saline solution was added to provide an adequate volume for reading.

Calculations of tear parameters—Actual tear film concentrations were calculated from the diluted samples by use of the following formula (formula 1):

$$C(t) = \frac{C_f(t) \cdot V_d}{V_c}$$

in which $C(t)$ is the actual tear film concentration at each sample collection time, $C_f(t)$ is the concentration of the diluted samples as measured by use of the fluorophotometer, V_d is the dilution volume (ie, 2.0 mL), and V_c is the collected volume of each sample.

The tear volume was calculated from the resulting fluorescein concentrations by use of 2 methods: the including and excluding methods. In the including method (also known as the dilution method¹⁰), the actual tear film concentration at 0 minutes ($C(t=0)$) is included in the analysis and the volume is calculated by mathematically determining the change in the original fluorescein concentration immediately following instillation and thorough mixing with the tear film. If C_i is the original concentration of the fluorescein solution instilled into the tear film (10% or 10^8 ng/mL), V_i is the original volume of instilled fluorescein solution (2.0 μ L), and $C(0)$ is the postinstillation concentration in the tear film at 0 minutes (presuming that thorough mixing and homogenization occur essentially instantaneously upon instillation of fluorescein into the tear film), then the tear film volume (V) is given by a dilution ratio as follows (formula 2):

$$V = V_i \cdot \left[\frac{C_i}{C(0)} - 1 \right]$$

where $C(0)$ is obtained from the measured concentration at 0 minutes.

In the excluding method (also known as the zero time method¹⁰), $C(t=0)$ is excluded from the analysis and the mathematical treatment is the same as that described for the dilution method, except that $C(0)$ is extrapolated from the regression equation of the $\ln[FI]$ versus time plot from 2 to 20 minutes.

To confirm the tear volume calculations, tear volume was estimated on the basis of geometric considerations. Tears were assumed to be homogeneously distributed, and tear film thickness was assumed to be equal on the corneal surface and under the eyelids.¹⁰ The volume contributed by the marginal tear strips was ignored. A schematic model was used in which the globe of the eye was taken to be a sphere with a radius (r) of 20 mm (in approximate accordance with measurements reported elsewhere¹⁴) and in which the conjunctival cul-de-sac extended to the equator of the sphere such that the surface of the globe covered by tears would approximate a hemisphere. The volume of the hemisphere without tears was calculated as follows:

$$\frac{1}{2} \left(\frac{4}{3} \pi r^3 \right)$$

and the volume of the hemisphere with tears was calculated as follows:

$$\frac{1}{2} \left(\frac{4}{3} \pi (r+d)^3 \right)$$

in which d is the thickness of the tear film. The tear film volume was subsequently calculated by subtracting the

volume of the hemisphere without tears from the volume with tears. The thickness of the tear film in horses is not known, but estimates in humans vary from approximately 3 to 40 μm .^{15,16} Both of these extremes were used to calculate a range of plausible volumes in horses, which were then compared with the volume determined by use of the excluding method.

The tear turnover rate was defined by the percentage decrease in fluorescein concentration from an initial time (0 minutes) and was calculated as follows (formula 3):

$$T(t) = 100 \cdot \frac{C(t) - C(t + 1)}{C(t)}$$

in which $T(t)$ is the turnover rate, $C(t)$ is the concentration at 0 minutes, and $C(t + 1)$ is the concentration 1 minute later. Assuming a semilogarithmic decay pattern, the concentration at any time (t) is represented by use of the following formula (formula 4):

$$C(t) = C(0) \cdot e^{-kt}$$

where k is the decay constant, which is the slope of the regression line of the semilogarithmic plot of the concentration versus time. The regression lines were established with the method of least squares by use of commercially available software.^f By substituting $C(t)$ into T , the turnover rate could then be simplified as follows (formula 5):

$$T = 100 \cdot (1 - e^{-k})$$

Tear flow rate (T_f) was determined by multiplying the decay constant k by the tear volume V as follows (formula 6)¹³:

$$T_f = k \cdot V$$

Comparison between right and left eyes and consistency of parameters over time—In addition to the preceding calculations, tear parameters were measured in the right eye as well as the left in 3 of the horses by use of the excluding method to compare right and left eye values. In an additional 3 horses, the left eye parameters were remeasured 8 to 9 days after the original measurements were made to assess the consistency of parameters over time.

Statistical analysis—Tear volumes, turnover rates, and flow rates calculated by use of the including and excluding methods were compared between horses receiving the nutraceutical and those not receiving the nutraceutical by use of a Student t test. Tear volumes, turnover rates, and flow rates calculated via the including method were compared with those obtained via the excluding method in all 12 horses by use of a paired t test. Values are reported as mean \pm SD. In horses in which both right and left eye measurements were obtained, right and left eyes were compared with a paired t test. In horses remeasured 8 to 9 days after the original measurement, original and repeated values were compared with paired t tests. In all statistical analyses, a

value of $P < 0.05$ was considered significant. Correlation coefficients and coefficients of determination were calculated for $\ln[\text{Fl}]$ -versus-time plots for both methods of calculation.

Results

Tear volume, turnover rate, and flow rate did not differ significantly ($P > 0.05$; power > 0.80) between horses receiving and those not receiving the nutraceutical. Therefore, data from all horses were combined for the remainder of the analysis.

Including method—With the including method, in which the concentration of tears at 0 minutes was used in calculations (formula 2), a mean tear volume of 360.09 μL was obtained (Table 1). The decay constant, as determined from the slope of the regression line of $\ln[\text{Fl}]$ versus time, was 0.1319. The mean tear turnover rate was 12.22%/min, and the mean tear flow rate was 47.77 $\mu\text{L}/\text{min}$. The mean \pm SD correlation coefficient for $\ln[\text{Fl}]$ versus time was 0.93 ± 0.12 , and the mean coefficient of determination was 0.88 ± 0.18 . Seven of the 12 horses had concentrations immediately following fluorescein instillation (0 minutes) that were lower than the subsequent measurement at 2 minutes.

Excluding method—The regression lines were recalculated for data from the 2-minute to 20-minute measurements, without use of the 0-minute values (Figure 1; Table 1). Tear film concentration at 0 minutes was then equal to the y-intercept of the calculated regression line and did not depend on the assumption of instantaneous homogenization of fluorescein in the tear film immediately after instillation. The decay constant was 0.1437, and mean tear volume was 233.74 μL . The mean turnover rate was 13.21%/min, and the mean tear flow rate was 33.62 $\mu\text{L}/\text{min}$. The mean \pm SD correlation coefficient for $\ln[\text{Fl}]$ versus time was 0.98 ± 0.03 , and the mean coefficient of determination was 0.95 ± 0.06 .

Tear volume from geometric considerations—When the radius of the globe of the eye was assumed to be 20 mm and values of 3 and 40 μm were used as the thickness of the tear film, the calculated range of tear

Table 1—Mean \pm SD values for tear parameters in left eyes of 12 ophthalmologically normal horses calculated by use of including and excluding methods.*

Parameter	Including method	Excluding method
Volume (μL)	360.09 \pm 111.67	233.74 \pm 90.96
Turnover rate (%/min)	12.22 \pm 5.20	13.21 \pm 5.22
Flow rate ($\mu\text{L}/\text{min}$)	47.77 \pm 24.34	33.62 \pm 19.46
Decay constant for instilled fluorescein	0.1319 \pm 0.0593	0.1437 \pm 0.0603
Natural logarithm of initial tear film concentration	13.56 \pm 0.27	13.72 \pm 0.37

*In the including method, the actual tear film concentration at 0 minutes was included in the analysis and the volume is calculated by mathematically determining the change in the original fluorescein concentration immediately following instillation and thorough mixing with the tear film. In the excluding method, the initial tear film concentration was excluded from the analysis and was then extrapolated from the regression equation of $\ln[\text{Fl}]$ versus time from 2 to 20 minutes. Values for all parameters differ significantly ($P < 0.05$) between methods.

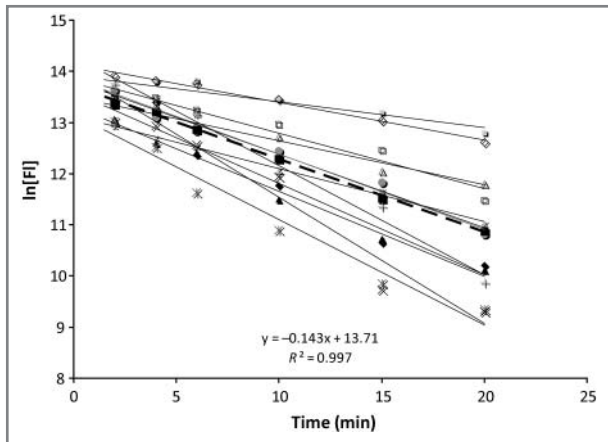


Figure 1—Semilogarithmic plot of time versus concentration of fluorescein in the tear film from 2 to 20 minutes after instillation of 2 μL of 10% sodium fluorescein onto the upper bulbar conjunctiva of the left eye in 12 ophthalmologically normal horses (individual solid lines). Mean values for all horses (black squares) and the regression line of the mean (dashed line) and associated equation are indicated. Data were calculated by use of the excluding method, in which the initial tear film concentration was excluded and then extrapolated from the regression equation. Individual horse data are included to show the goodness of fit of the data for each horse. Notice that there is minimal variation in slopes among horses.

Table 2—Mean \pm SD values for tear parameters in right and left eyes of 3 ophthalmologically normal horses, as calculated by use of the excluding method.*

Parameter	Right eye	Left eye
Volume (μL)	362.88 \pm 55.05	276.38 \pm 68.01
Turnover rate (%/min)	13.36 \pm 4.62	11.36 \pm 9.38
Flow rate ($\mu\text{L}/\text{min}$)	53.49 \pm 24.38	30.89 \pm 22.12

Differences between right and left eyes were not significant (ie, $P > 0.05$) for any parameter.
See Table 1 for key.

Table 3—Mean \pm SD values for tear parameters measured in the left eye of 3 ophthalmologically normal horses twice, 8 to 9 days apart, as calculated by use of the excluding method.*

Parameter	First measurement	Second measurement
Volume (μL)	226.35 \pm 145.50	116.35 \pm 6.94
Turnover rate (%/min)	16.86 \pm 6.19	15.80 \pm 5.69
Flow rate ($\mu\text{L}/\text{min}$)	35.55 \pm 8.19	20.51 \pm 9.50

First and second measurements did not differ significantly (ie, $P > 0.05$) for any parameter.
See Table 1 for key.

film volumes in ophthalmologically normal horses was 7.54 to 100.73 μL . Similarly, when a tear film volume of 233.74 μL was used to solve for the volume of the globe hemisphere with tears and the resulting value was used to solve for tear film thickness, the data predicted a tear film thickness in ophthalmologically normal horses of approximately 92.6 μm .

Right eye versus left eye and consistency across time—In the 3 horses in which right and left eyes were measured, there were no significant differences in tear volume, turnover rate, or flow rate (Table 2). Similarly, values of parameters did not change across time in the 3 horses in which measurements were made twice (Table

3). However, the small sample sizes for these analyses resulted in low statistical power, so it is possible that true differences could exist but that larger samples would be needed to detect them.

Discussion

In the present study, tear volume, turnover rate, and flow rate were determined in ophthalmologically normal horses. Fluorescein administration and tear collection appeared to be well tolerated by all horses, with no obvious reflex lacrimation (Figure 1). This finding is consistent with that of another study⁵ in which no significant difference was detected between results of STTs I and II in horses, implying a relative lack of reflex lacrimation in response to placement of a paper tear strip into the inferior conjunctival cul-de-sac. In each horse in the present study, fluorescein concentrations decreased according to first-order kinetics between 2 and 20 minutes after instillation of fluorescein. An axiom in ocular pharmacokinetics is that the highest concentration of a topically applied compound in the tears must occur immediately after instillation, with concentrations decreasing thereafter because of dilution by newly formed tears.¹⁷ However, our data indicated higher concentrations at 2 minutes after instillation rather than immediately after instillation (0 minutes; ie, $C(2) > C(0)$) in 7 of 12 horses. Given that it is physiologically impossible for fluorescein concentration to increase following initial instillation into the tear film, the lower concentrations measured at 0 minutes were most likely unrepresentative of the actual concentration attributable to the fluorescein not having had adequate time to distribute homogeneously in the tear film. In such instances, the primary assumption leading to volume calculation as determined by use of the including method would be violated, and the artifactually lower concentrations at 0 minutes would alter the regression model by reducing the decay constant. The result would be overestimation of tear volume and flow rate and underestimation of tear turnover rate. Thus, the excluding method should provide more accurate estimations of tear volume, turnover rate, and flow rate in horses.

Comparing the including method analysis with the excluding method analysis, there was typically an increase in the correlation strength and coefficients of determination with concomitant reduction in range and SD with the latter, corroborating that the excluding method may be the more robust approach. Similar decreases in range were detected when tear volumes and flow rates were calculated in humans with the excluding method.¹⁰ Researchers in that study also surmised that the excluding method was the more accurate method, hypothesizing that a combination of reflex lacrimation and poor homogenization in the first few minutes following fluorescein instillations created artifacts that were problematic for the dilution method. Our observations were similar.

All horses in the present study had a semilogarithmic decline of fluorescein concentration with a simple exponential decay pattern, indicative of first-order kinetics over the period evaluated (Figure 1). Coefficients of determination for these plots were > 0.9 , indicating

that a single exponential equation was appropriate. Mishima et al¹⁰ reported similar instances of decay with a single turnover rate in human subjects, but most of their subjects had an initial rapid decay, followed by a slower decay after 4 to 5 minutes. The single turnover rate was evident in subjects who claimed to be irritated with the instillation of fluorescein, producing a high turnover rate. The decreased decay rate was associated with subjects who claimed the least irritation with the technique. It was concluded that the more rapid decay in the first 4 to 5 minutes after fluorescein instillation was caused by reflex lacrimation, and that the slower decay after 5 minutes was the actual physiologic tear turnover. Similar biphasic decay in humans was detected in another study.¹³

The horses in the present study appeared to tolerate the application of fluorescein well (ie, there were no signs of blepharospasm or epiphora), and care was taken not to directly contact the conjunctiva with the tip of the capillary tube during instillation. However, contact with the inferior conjunctival fornix surface to collect tears was inevitable and this may have resulted in reflex lacrimation, which would increase the turnover rate. If irritation had been caused by fluorescein instillation or tear collection, it appeared to be fairly constant with each horse and did not result in much fluctuation of the tear turnover rate. We could not assess our horses for increased decay rates during the early period after instillation because we did not have enough sampling points prior to 5 minutes with which to establish a regression line. Increasing the number of samples collected within the first few minutes might have revealed the biphasic pattern detected in humans.^{10,13} Interestingly, the physiologic tear turnover rate (ie, the rate when those early points associated with reflex lacrimation were excluded) in humans was 13% to 18%/min (depending on subject age), which was close to our value (calculated via the excluding method) of approximately 13%/min.

Studies of tear volume and flow rate in other species are limited. The mean tear volume, turnover rate, and flow rate in humans have been reported to be 7.0 μL , 13.1% to 30.0%/min, and 1.2 $\mu\text{L}/\text{min}$, respectively.^{10,12,13} Rabbits reportedly have a tear volume of 7.5 μL and a flow rate of 7%.¹⁸ Cows in 1 study¹⁹ had a flow rate of 32.6 $\mu\text{L}/\text{min}$, which is comparable to our flow rate of 33.62 $\mu\text{L}/\text{min}$ for an animal with a comparably sized eye. The tear volume was not determined in that study; therefore, the turnover rate could not be calculated.

The tear volume predicted by the excluding method (233.74 μL) was in approximate agreement with the higher end of the estimate calculated on the basis of geometric considerations of the globe in horses (100.73 μL). Because the geometric estimation is based on both the radius of the approximated sphere of the globe and the tear film thickness, it is likely that the geometric estimation would agree even more closely with our kinetically calculated value if the tear film thickness in horses was known to be thicker than the human tear film thickness estimates used in our calculations. It is reasonable to assume that the tear film is thicker in horses than in humans in that the horse ocular surface area is much greater, and a thicker film would be needed to maintain stability stretched over that greater surface area. Our

geometric estimates predicted a tear film thickness of 92.6 μm , which is twice as thick as even the thickest estimate in humans, but corroboration of this prediction is required.

Knowing the tear volume and tear turnover rate is clinically important because these variables affect the concentration versus time profiles of topically applied drugs. Consider, for example, topical administration of tobramycin for the treatment of a corneal ulcer infected by *Pseudomonas aeruginosa*. The concentration in the tear film immediately upon instillation can be calculated with the following equation:

$$C(0) = \frac{C_i \cdot V_i}{V + V_i}$$

in which C_i is the concentration of the tobramycin eye-drops, V_i is the instillation volume of the tobramycin eyedrops, and V is the volume of tears. Assuming an instillation volume of 0.200 mL (as is commonly suggested for application via a subpalpebral catheter²⁰), assuming a tear volume of 234 μL (0.234 mL) as calculated with the excluding method, and given that the concentration of commercially available tobramycin is 0.3% (or 3 mg/mL), the concentration immediately following instillation can be calculated as follows:

$$C_0 = \frac{3.0 \cdot 0.200}{0.234 + 0.200} = 1.382 \text{ mg/mL}$$

In other words, the concentration in the tear film is diluted by more than half immediately upon instillation into the tears. In addition, using the tear turnover rate of 13.21%/min (as calculated via the excluding method for horses in the present study), the concentration $C(t)$ at any time t after initial administration that results from dilution with newly formed tears can be calculated as follows:

$$C(t) = C(t-1) - 0.1321(C(t-1))$$

Assuming the MIC_{90} for tobramycin against *P aeruginosa* is 2 $\mu\text{g}/\text{mL}$,²¹ by 50 minutes following instillation, the tobramycin would be diluted to a concentration (1.16 $\mu\text{g}/\text{mL}$) lower than the MIC_{90} ; therefore, a dosing interval of every 30 to 45 minutes might be selected to ensure maintenance of a concentration that exceeds the MIC_{90} . This is a simplification in that it does not allow for factors other than lacrimal dilution that can influence tear film concentrations (ie, tissue binding, protein binding, and tear film metabolism), but it does serve as a good first approximation. In addition, infectious keratitis in horses is a disorder of the corneal epithelium and stroma, not the tear film, so concentrations in corneal tissue would provide more valuable information but are much more difficult to obtain.

Obvious caveats to the foregoing discussion include the fact that the horses in our study were limited to females of a narrow age range, which was necessitated because those were the only horses available. Some authors claim that tear flow decreases

with advancing age in humans,²² whereas 1 study¹¹ revealed no relationship between tear flow and age. In another study,²³ older dogs were found to have reduced tear production, compared with younger dogs²³; however, another study⁵ in horses revealed no such correlation.⁵ In humans, tear flow rates appear to be little affected by sex across most age ranges,²⁴ and sex differences in tear flow rates in horses have not been found.⁵

More importantly, results of the study reported here provided kinetic data for tear parameters in ophthalmologically normal horses, but the horses with ocular disease that might benefit from dosing regimens derived from these data will have altered kinetics. It is intuitive that the lacrimation associated with corneal disease would result in higher tear volumes, turnover rates, and flow rates, and those parameters will vary widely from case to case. In fact, tear film turnover rates can increase up to 300% in humans after mild touching of the cilia of the lower lid with a small piece of filter paper, even after application of topical anesthetic.¹¹ Therefore, the present data should be used only as a first approximation until additional studies similar to the one reported here establish kinetic parameters in horses with corneal disease.

- a. Seabuck Complete, SeaBuck Equine LLC, Salt Lake City, Utah.
- b. Microcapillary tube, Fischer Brand, Fischer Scientific, Pittsburgh, Pa.
- c. Ak-Fluor, Akorn, Buffalo Grove, Ill.
- d. 1.5-mL Eppendorf tube, Fischer Brand, Fischer Scientific, Pittsburgh, Pa.
- e. Fluorotron Master, OcuMetrics, Mountain View, Calif.
- f. Microsoft Excel 2008 for Mac, version 12.2.3, Microsoft Corp, Redmond, Wash.

References

1. Lucarelli MJ, Dartt DA, Cook BE, et al. The lacrimal system. In: Kaufman PL, Alm A, eds. *Adler's physiology of the eye*. 10th ed. St Louis: Mosby, 2003;30–43.
2. Gum GG, Gelatt KN, Ofri R. Physiology of the eye. In: Gelatt KN, ed. *Veterinary ophthalmology*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 1999;151–181.
3. Williams DL. Analysis of tear uptake by the Schirmer tear test strip in the canine eye. *Vet Ophthalmol* 2005;8:325–330.
4. Marts BS, Bryan GM, Prieur DJ. Schirmer tear test measurement and lysozyme concentration of equine tears. *J Equine Med Surg* 1977;1:427–430.
5. Beech J, Zappala RA, Smith G, et al. Schirmer tear test results in normal horses and ponies: effect of age, season, environment, sex, time of day and placement of strips. *Vet Ophthalmol* 2003;6:251–254.
6. Sørensen T, Jensen FT. Tear flow in normal human eyes. Determination by means of radioisotope and gamma camera. *Acta Ophthalmol (Copenh)* 1979;57:564–581.
7. Norm MS. Tear secretion in normal eyes. Estimated by a new method: the lacrimal streak dilution test. *Acta Ophthalmol (Copenh)* 1965;43:567–573.
8. Zhu H, Chauhan A. A mathematical model for tear drainage through the canaliculi. *Curr Eye Res* 2005;30:621–630.
9. Ehlers N. A calculation of the average tear flow. *Acta Ophthalmol (Copenh)* 1967;45:273–274.
10. Mishima S, Gasset A, Klyce SD Jr, et al. Determination of tear volume and tear flow. *Invest Ophthalmol* 1966;5:264–276.
11. Jordan A, Baum J. Basic tear flow. Does it exist? *Ophthalmology* 1980;87:920–930.
12. Occhipinti JR, Mosier MA, LaMotte J, et al. Fluorophotometric measurement of human tear turnover rate. *Curr Eye Res* 1988;7:995–1000.
13. van Best JA, Benitez del Castillo JM, Coulangeon LM. Measurement of basal tear turnover using a standardized protocol. European concerted action on ocular fluorometry. *Graefes Arch Clin Exp Ophthalmol* 1995;233:1–7.
14. McMullen RJ Jr, Gilger BC. Keratometry, biometry and prediction of intraocular lens power in the equine eye. *Vet Ophthalmol* 2006;9:357–360.
15. King-Smith PE, Fink BA, Fogt N, et al. The thickness of the human precorneal tear film: evidence from reflection spectra. *Invest Ophthalmol Vis Sci* 2000;41:3348–3359.
16. Prydal JI, Artal P, Woon H, et al. Study of human precorneal tear film thickness and structure using laser interferometry. *Invest Ophthalmol Vis Sci* 1992;33:2006–2011.
17. Shell JW. Pharmacokinetics of topically applied ophthalmic drugs. *Surv Ophthalmol* 1982;26:207–218.
18. Chrai SS, Patton TF, Mehta A, et al. Lacrimal and instilled fluid dynamics in rabbit eyes. *J Pharm Sci* 1973;62:1112–1121.
19. Slatter DH, Edwards ME. Normal bovine tear flow rates. *Res Vet Sci* 1982;33:262–263.
20. Martin CL. Ophthalmic pharmacology. In: Martin CL, ed. *Ophthalmic disease in veterinary medicine*. London: Manson Publishing, 2005;41–70.
21. Wilhelmus KR, Gilbert ML, Osato MS. Tobramycin in ophthalmology. *Surv Ophthalmol* 1987;32:111–122.
22. Mathers WD, Lane JA, Zimmerman MB. Tear film changes associated with normal aging. *Cornea* 1996;15:229–234.
23. Hartley C, Williams DL, Adams VJ. Effect of age, gender, weight, and time of day on tear production in normal dogs. *Vet Ophthalmol* 2006;9:53–57.
24. Records RE. The tear film. In: Tasman W, Jaeger EA, eds. *Duane's foundations of clinical ophthalmology*. Vol 2. Rev ed. Philadelphia: JB Lippincott Co, 1992;1–22.