La Lettre de la SFEROV

Bulletin de liaison n°26 . janvier 2007

Editorial

Le Bureau de la SFEROV vous présente ses meilleurs vœux de santé et de réussite pour l'année 2007.

Chaque tour de calendrier nous donne la fausse impression que « cette fois est la bonne », c'est-à-dire que cette nouvelle année sera celle de tous les aboutissements.. Et puis un cycle recommence, c'est sans doute naturel.

Après avoir terminé 2006 en voyant la défunte 5ème année d'études ressortir du chapeau de la DGER, nous avons bien compté sur nos doigts pour comprendre que pour les malheureux étudiants, 6 égale 7. Et que penser de nos enseignants qui venaient juste de réussir à comprimer cinq années de savoir en quatre et qui vont maintenant avoir quatre années de maigre budget pour en financer cinq. Les voies du ministère sont décidément impénétrables. Pour pimenter un peu le tout, la formation complémentaire et continue devient obligatoire ; souci légitime de nos édiles qui savent bien combien la formation continue est primordiale face aux pitreries de la pensée unique. L'exercice de style sera pourtant difficile pour les rédacteurs chargés de la communication.

Pour notre spécialité, la gageure est aussi d'importance : la réglementation française est en place, les DESV fonctionnent et la VAE est active... mais il faut satisfaire tout le monde et rendre compatibles les systèmes européens avec la réglementation française. L'organisation du dépistage des MHOC n'échappe pas non plus à cette curieuse stratégie ; nous aurons l'occasion d'en reparler.

L'exercice du pouvoir est diablement compliqué, nous le savons tous. Informer le pouvoir, est un peu de notre responsabilité. Pour cela, une dose de corporatisme doit être exploitée, mais avec le discernement nécessaire pour ne pas heurter le nombrilisme chronique cher au fonctionnement libéral.

De la science, rien que de la science, s'étaient juré les fondateurs de la SFEROV. Las, il fallut se rendre à l'évidence, il fallait aussi faire un peu de politique avec juste une formation sur le tas et donc beaucoup d'insuffisances en la matière. Une bonne pincée de bon sens, voire d'humour, suffit heureusement souvent pour nous mettre sur le bon chemin. 2007 sera donc un nouveau cycle au cours duquel le bureau de la SFEROV se mobilisera encore pour faire entendre la voix de ses membres, votre voix, tout en continuant sa mission première de faire partager l'ophtalmologie vétérinaire.

P.-F. ISARD

Cotisations 2007

L'appel de cotisations est lancé par notre trésorier, P.E. Lallement ; vous trouverez en annexe le bulletin d'inscription pour l'exercice 2007 à télécharger au format PDF

Ateliers de Formation 2007

- Implantation : le 12 janvier 2007, à l'ENV Toulouse. (Moniteurs : P. Lazard - PF Isard) Atelier Complet
- Techniques de Microchirurgie et Phacoémulsification : les 5 et 6 juillet 2007 à Rueil-Malmaison. (Moniteurs : AS Augsburger – V. Meunier – B. Cantaloube. PF Isard – P. Lazard)
- Electrophysiologie visuelle fonctionnelle, en décembre 2007 à l'ENV Lyon. (Moniteurs : P. Lazard- P.E. Lallement- Ph. Durieux)

Renseignements et Inscriptions : Frank Famose. Tel : 05 61 71 24 02 Fax : 05 61 71 65 52 @ : frank.famose@wanadoo.fr

Prix SFEROV

Créé en 2004 , le Prix SFEROV, est attribué par un jury composé des membres du Conseil d'Administration pour récompenser un travail original récent (publication, mémoire, thèse..) en ophtalmologie vétérinaire. Le Prix 2006, sous la forme d'un chèque de 1000 €, est attribué à Thomas Dulaurent (T 2005) pour son travail de thèse sur l'angle irido-cornéen. Le prix lui sera officiellement remis le 12 janvier au cours de la journée de formation à l'implantation, à l'ENVT.

Actualités en génétique

Parmi les nouveautés de cette fin d'année, en génétique ophtalmologique, on retrouve deux découvertes qui concernent des gènes étudiés depuis de longues années : le gène « prcd » et le gène « merle ».
Rappelons qu'à l'heure actuelle 17 races peuvent présenter une forme d'atrophie progressive de la rétine associée au locus prcd-PRA et dont les trois principales sont le Caniche le Cocker et le Labrador. Cette

constatation illustre la notion d'effet fondateur et l'existence d'une origine commune à toutes ces races.
Par ailleurs, sur le plan physiopathologique, il est intéressant de noter que malgré la très grande variété des expressions cliniques de cette affection dans les différentes races concernées, c'est la même et unique mutation qui est en cause (mutation ponctuelle TGC♦ TAC).
Un test de mutation était disponible depuis près de deux ans, alors que la publication de la découverte n'avait pas encore été effectuée. Désormais ANTAGENE collabore avec le laboratoire américain initial et propose un service en français pour traiter les échantillons, les regrouper pour bénéficier de remise, et les acheminer aux Etats-Unis, pour les trois races citées précédemment.
Le gène Merle concerne l'ophtalmologie dans la mesure où certains individus merles peuvent présenter des malformations oculaires et notamment une microphtalmie. La découverte récente du gène responsable et de la mutation, va permettre d'avancer enfin dans l'étude de cette curiosité génétique à transmission autosomique dominante.
C'est sur le centromère du chromosome CFA 10 que l'équipe de C. André au CNRS de Rennes avait situé le locus qui contient le gène responsable. Dans ce gène appelé SILV (pour silver) la mutation apparaît comme une insertion d'un élément court répété. De nombreuses études seront nécessaires pour comprendre l'expression ophtalmologique de cette mutation, et votre participation est activement requise si vous pouvez rencontrer des individus avec malformations oculaires dans votre clientèle.

Ph. PILORGE

Un peu de Biblio : en annexe, quelques articles sélectionnés par Alain Régnier.

Contacts

Le Bureau de la SFEROV est toujours à votre disposition. Merci de privilégier les contacts e-mail ou fax.

Président : P.F. ISARD
Centre d'Ophtalmologie Vétérinaire. 4550 Les Grands Vignobles. 74320 Sevrier. T :0450190101. F : 0450190985. @ : contact@ceovet.com

Vice Président (relation avec les instances nationales) P. LAZARD.
Clinique Vétérinaire. 80 rue Pereire. 78100 Saint Germain en Laye.T : 0134513133. F : 0134515516. @ : ladelou@wanadoo.fr

Vice Présidente (congrès européen 2008) V. MEUNIER. 30 rue Faÿs. 94300. Vincennes. T:0143982107.@v.meunier@free.fr

Vice Président (relations internationales) M. VERNEUIL. Clinique Vétérinaire. Bd de la Libération. Chemin du Micocoulier. 83490 Le Muy T : 0494458787. F: 0494458611. @ : marc.verneuil@cegetel.net

Secrétaire : Ph. PILORGE. Clinique Vétérinaire.
68-70 rue Papu. 35000 Rennes. T : 0299590122. F : 029959615
@ : p.pilorge@wanadoo.fr

Secrétaire adjoint (ateliers et manifestations) F. FAMOSE Clinique Vétérinaire. 42 rue Lucien Servanty. 31700 Blagnac. T: 0561712402 F: 0561716552. @ : frank.famose@wanadoo.fr

Trésorier : P.E. LALLEMENT .Clinique Vétérinaire. 20 avenue des Tignes. 06400 Cannes. T: 0493390727. F: 0493683558.@:pelallement@aol.com.

Membres :

B. CANTALOUBE (relations partenaires commerciaux).Clinique Vétérinaire. 61 route de Sète. 34300 Agde. T+F: 0467773064. @ : b.cantaloube@wanadoo.fr

Ph. DURIEUX. Clinique Vétérinaire. 29 avenue Joffre. 77100 Meaux. T: 0164341155. F: 0164333235. phil.durieux2@wanadoo.fr

F. GOULLE. Clinique Vétérinaire. 1 rue du Dr Nancel Penard. 33600 Pessac T : 0556450475. F : 0556150079. @ : frederic.goulle@wanadoo.fr

A. REGNIER (validation scientifique) ENVT. 23 chemin des Capelles. 31076 Toulouse Cédex 3. T : 0561193832. F : 0561193203. @ : a.regnier@envt.fr

F. RIVAL. Clinique Vétérinaire.192 avenue de Romans. 26000 Valence . T : 0475433391. F : 0475431460. @ : franck.rival@wanadoo.fr

SOCIETE FRANCAISE D'ETUDES ET DE RECHERCHES
EN OPHTALMOLOGIE VETERINAIRE

Bulletin d'adhésion 2007

à renvoyer accompagné obligatoirement du règlement correspondant à :

P.E. LALLEMENT. Trésorier SFEROV

Clinique Vétérinaire. 20 avenue des Tignes. 06400 Cannes
Tel : 04 93 39 07 27
Fax : 04 93 68 35 58
@ : pelallement@aol.com

NOM, Prénom.....

Inscrit à l'Ordre des Vétérinaires sous le numéro.(si première adhésion) :.....
Adresse Professionnelle (en cas de changement ou de première adhésion) :
.....
Tél .....Fax :.....

E-mail (écrire très lisiblement SVP):

La liste des inscrits à la SFEROV pour l'année en cours est consultable sur le site web : www.sferov.org
Cela permet à chacun de pouvoir vérifier l'état de sa cotisation (aucun reçu n'étant prévu, sauf demande expresse)
Si vous ne souhaitez pas que vos coordonnées soient visibles sur le site web veuillez nous le préciser :

Coordonnées visibles : OUI ◊ NON ◊

Année d'obtention du CESOV, ou mention du diplôme équivalent (si première adhésion) :.....
Je soussigné Dr.....certifie avoir pris connaissance des statuts de la SFEROV
(consultables sur le site web : www.sferov.org)
m'engage à payer la cotisation de l'année 2007 qui s'élève à : **95 €** : cotisation comprenant l'adhésion à SFEROV 55 € + ESVO 40 € . Attention clôture des inscriptions réglementée par l'ESVO le 1er Février 2007.
Si vous souhaitez ne pas faire partie des associations européennes et internationales en ophtalmologie vétérinaire, vous ne payez que **55 €** .

Règlement à l'ordre de la SFEROV

.....
A conserver pour votre comptabilité :
Adhésion SFEROV + ESVO pour 2007 payée le : .....(91 €)
Adhésion SFEROV pour 2007 payée le : .....(55 €)
Chèque n°..... Banque .....
La TVA n'est pas récupérable sur ces règlements

Duration of corneal anesthesia following topical administration of 0.5% proparacaine hydrochloride solution in clinically normal cats

Daniel R. Binder, PhD, and Ian P. Herring, DVM, MS

Objective—To determine duration of corneal anesthesia following topical administration of 0.5% proparacaine hydrochloride solution in domestic shorthair (DSH) cats.

Animals—20 clinically normal DSH cats.

Procedures—Baseline corneal touch threshold (CCT) was established by use of a Cochet-Bonnet aesthesiometer. Treatment consisted of a single 50-µL topical application of an ophthalmic preparation of 0.5% proparacaine solution to a randomly selected eye of each cat. The corneal touch threshold was assessed 1 and 5 minutes after application to the cornea and at 5-minute intervals thereafter for 60 minutes.

Results—Corneal sensitivity, as determined by Cochet-Bonnet aesthesiometry, was significantly reduced from baseline for 25 minutes following topical administration of ophthalmic proparacaine. Maximal anesthetic effect lasted 5 minutes.

Conclusions and Clinical Relevance—As determined by Cochet-Bonnet aesthesiometry, duration of anesthetic effects on the cornea induced by a single topical application of an ophthalmic preparation of 0.5% proparacaine solution in DSH cats is considerably shorter than the reported duration of corneal anesthesia in dogs. (*Am J Vet Res* 2006;67:1780–1782)

Topical administration of ophthalmic anesthetic agents, such as 0.5% proparacaine solution, is regularly used to facilitate diagnostic and therapeutic procedures such as tonometry, corneal and conjunctival scraping, corneal suture and foreign body removal, and intracameral injection. Proparacaine is commonly used because of its predictability and minimal adverse effects following topical administration.[1]

Although widely used in veterinary medicine, duration of corneal anesthesia induced by topical administration of proparacaine has only recently been quantified in a study[2] on dogs. Results of that study revealed a significant anesthetic effect from a single drop of ophthalmic proparacaine for up to 45 minutes after topical administration, with a maximal effect lasting 15 minutes. Duration of anesthesia was prolonged with multiple-drop administration. Previous to that study, knowledge on the efficacy and duration of this drug in companion animals was solely based on clinical experience,[3] rather than objectively determined data. A similar lack of objective data currently exists on the duration of corneal anesthesia induced by topical administration of proparacaine in cats.

In a limited number of other studies,[4-6] duration of corneal anesthesia induced by topical administration of proparacaine in other species, including rabbits and humans, has been investigated. Considering the variation in corneal innervation and sensitivity found among species,[7] the duration of corneal anesthesia for this drug in cats should not be assumed on the basis of data obtained from other species. The purpose of the study reported here was to establish the duration of the anesthetic effect following topical administration of an ophthalmic preparation of 0.5% proparacaine solution in clinically normal DSH cats by use of Cochet-Bonnet aesthesiometry.

Abbreviations

DSH	Domestic shorthair
CTT	Corneal touch threshold

Materials and Methods

Animals—Twenty adult DSH cats were used in the study. Cats were included if they had no history of corneoconjunctival disease or evidence of corneal or adnexal disease, as determined by slit-lamp biomicroscopic examination. Cats with abnormal Schirmer tear test values were excluded from the study. Cats with Schirmer tear test values of > 5 mm/min were considered to have Schirmer tear test values within reference range. The study group comprised 13 spayed females and 7 neutered males. Mean ± SD age was 5.5 ± 3.1 years. The Animal Care and Use Committee of Virginia Tech approved the protocol for all procedures in the study.

Treatment—One randomly selected eye was tested in each cat (10 right eyes, 10 left eyes). A commercially available anesthetic ophthalmic solution was used, containing 0.5% proparacaine hydrochloride[a] and inactive ingredients (0.01% benzalkonium chloride preservative, glycerin, purified water, sodium chloride, and hydrochloric acid or sodium hydroxide to adjust the pH to between 5.0 and 6.0). Treatment consisted of topical administration of 50 µL of the ophthalmic anesthetic agent, and time of treatment was designated as time 0. Corneal sensitivity measurements began 1 minute after time 0. The same investigator (DRB) performed all treatments and measurements. To maintain consistent efficacy, the proparacaine solution was stored in a refrigerator during the study and anesthetic from the same bottle was used throughout the study.[8]

rReceived March 31, 2006.
Accepted May 10, 2006.
From the Department of Small Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0442.
Presented in part at the 36th Annual Meeting of the American College of Veterinary Ophthalmologists, Nashville, Tenn, October 2005.
The authors thank Ellen Binder and Heather Johnston for technical assistance and Daniel Ward and Stephen Werre for help with statistical analysis.
Address correspondence to Dr. Herring.

Measurement of corneal sensitivity—A Cochet-Bonnet aesthesiometer[b] with a 0.12–mm cross-sectional diameter nylon monofilament was used to measure sensitivity of the central portion of the cornea. To minimize environmental influences, examinations of all cats were conducted in the same facility in which temperature and humidity were monitored and recorded. To determine corneal sensitivity, the filament of the aesthesiometer was advanced slowly toward the globe and applied perpendicular to the central portion of the cornea. Pressure was increased only until a slight deflection of the filament was evident. Corneal sensitivity, defined as the CTT, was recorded as the length of the aesthesiometer filament in centimeters that induced a blink reflex on at least 3 of 5 stimulations for a specific filament length. After statistical analysis, data were converted to pressure measurements (g/mm^2) by use of the manufacturer's conversion table to allow comparisons to previous study results.

Each cat's baseline CTT was established before treatment by the use of an initial length of 5.0 cm for the aesthesiometer filament.[9] If a blink reflex was not detected, the filament length was decreased in 0.5-cm increments and testing was repeated until a blink reflex was evident on at least 3 of 5 stimulations at a specific filament length. The CTT was assessed 1 and 5 minutes after topical application of the proparacaine solution and then at 5-minute intervals thereafter until 60 minutes after application. For the measurement obtained 1 minute after treatment, the initial length of the nylon filament was matched to the individual cat's baseline CTT and then decreased in the same manner as for pretreatment measurements. For subsequent measurements, the initial length of the nylon filament was the shortest length that did not result in a positive blink reflex for at least 3 of 5 attempts during the preceding time point.[6,10] When a blink reflex was detected for at least 3 of 5 stimulations, the length of the filament was increased by 0.5 cm and testing was repeated. The CTT after treatment was recorded as the longest filament length that elicited a blink reflex on 3 of 5 stimulations. The CTT was recorded as 0 when no blink reflex to stimulation was observed.

Statistical analysis—Filament length was modeled by use of a software program[c] to perform a mixed-effects repeated-measures ANOVA. Comparisons of the means from each posttreatment time to the time 0 mean were performed by use of the Bonferroni correction to hold the familywise error rate to 0.05. A repeated-measures ANOVA was also used to evaluate for differences in response between eyes receiving treatment (ie, left eyes vs right eyes). Values of $P < 0.05$ were considered significant.

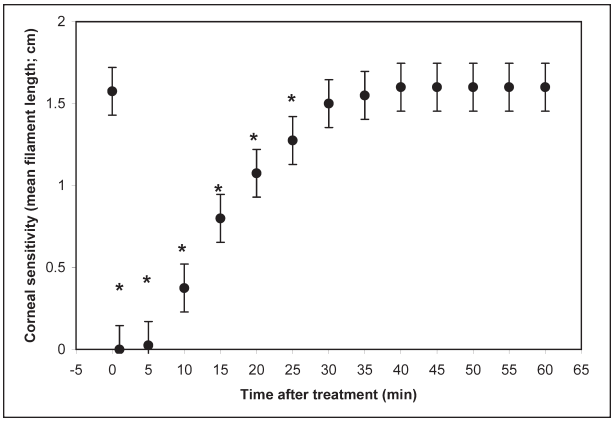

Figure 1—Mean (95% confidence limits) aesthesiometer filament length for determination of corneal sensitivity in 20 cats before and after topical administration of 1 drop of an ophthalmic preparation of 0.5% proparacaine hydrochloride solution in 1 eye. Baseline filament length is designated as time 0. A lack of elicited blink reflex in response to corneal stimulation by use of a 0.5-cm length of filament is designated as a filament length of 0. Subsequent times are minutes after administration. *Significantly ($P < 0.05$) different filament length from baseline.

Results

Mean filament length before treatment with 1 drop of proparacaine was 1.58 cm (95% confidence limits, 1.43 and 1.72 cm). No cat responded to the maximal stimulation (filament length of 0.5 cm) 1 minute after the application of the corneal anesthetic. The CTT before treatment differed significantly from the CTT after treatment up to and including the 25-minute posttreatment measurement (**Figure 1**). Maximum effect was observed through 5 minutes, as only 1 cat responded to maximal stimulation at that time point. No significant difference was found in CTT measurements between treated left and right eyes at any time.

Discussion

Results of our study indicate that topical administration of an ophthalmic preparation of 0.5% proparacaine solution induces demonstrable corneal anesthesia in cats for up to 25 minutes, as determined by use of Cochet-Bonnet aesthesiometry. Maximum effect was only observed for a 5-minute duration. Onset of topical anesthesia was rapid, as no blink reflex could be elicited at 1 minute after treatment. Similarly, a rapid corneal sensitivity recovery rate was seen in each cat.

Mean baseline central CTT in our study differs from that of a previous report,[11] in which mean ± SD central CTT in DSH cats was reported as 1.79 ± 2.33 g/mm^2 and 1.74 ± 1.65 g/mm^2 for right and left eyes, respectively. After conversion of filament length measurements to force measurements, the mean baseline CTT measurements in our study were 4.64 g/mm^2 (right eyes) and 5.16 g/mm^2 (left eyes), which are appreciably higher than the previously reported values for DSH cats.[11] It should be noted that the range of central CTT values for DSH cats in the previous report was quite wide,[11] as evidenced by the large SD, and the mean baseline CTT values in our study fall within this range. Possible reasons for the differences in corneal sensitivity values between these studies include investigator variability in application of the aesthesiometer filament to the cornea; differences in investigator interpretation of the blink response; and variation in the testing environment between the 2 studies, as temperature and humidity differences affect filament stiffness. Because of these variables, it is difficult to directly compare values between the 2 studies, but the differences do highlight the importance of controlling these variables when a Cochet-Bonnet aesthesiometer is used. Because these variables were controlled within our study and baseline corneal sensitivity was not significantly different from corneal sensitivity at the end of the posttreatment evaluation period, results of our study remain valid.

Duration of corneal anesthesia following topical administration of proparacaine in DSH cats differs somewhat from results previously reported for dogs,[2] in which maximal anesthetic effect was observed for 15 minutes and a significant decrease in corneal sensitivity was observed up to 45 minutes following application

of 1 drop of proparacaine to the eye. The difference in results of these 2 studies may be related to unique physiologic characteristics of corneal innervation or pharmacodynamics of topically applied medications in each species. The amount of proparacaine used in our study was exactly 50 µL, whereas the study conducted in dogs was reported as a single drop from the commercial container. The volume of a single drop from the commercial solution[a] container used in both studies measures approximately 25 µL, suggesting that the applied dose in cats of our study does not account for the decreased duration of anesthetic effect relative to that of dogs.

Corneal sensitivity in cats has been shown to vary between DSH cats and brachycephalic breeds, with brachycephalic breeds demonstrating a significantly higher CTT relative to DSH cats.[11] Our study involved evaluation of only DSH cats; thus, these results cannot be extrapolated to other breeds. In addition, all cats in our study were deemed healthy at the beginning of the study. It remains to be determined whether disease states that affect corneal sensitivity would have an effect on the efficacy of topical administration of ophthalmic proparacaine, as has been demonstrated for humans.[5]

The Cochet-Bonnet aesthesiometer has been widely applied in the investigation of corneal sensitivity in veterinary ophthalmology.[9,11-14] Limitations of this instrument include subjective interpretation of filament deflection and variability in filament stiffness with temperature and humidity. We attempted to minimize these problems by having a single investigator perform all tests and by performing all tests in the same physical environment. We also recorded the daily temperature and humidity in the testing room during each session to ensure that environmental conditions were nearly identical across testing days.

Following onset of topical anesthesia, the aesthesiometer filament was initially set at the shortest length that did not result in a positive blink during the prior measurement. If a blink reflex was then elicited at that length, the filament was lengthened by 0.5 cm, and testing was repeated, as previously described.[6,10] By starting with a shorter filament length (a more noxious stimulus) and then lengthening the filament, it is possible that conditioning to the stimulus could have occurred to some degree, which would confound the results. However, corneal sensitivity returned to values identical to baseline by the end of the study period, suggesting that any such effect was negligible.

Objective studies on the duration of corneal anesthesia following topical administration of proparacaine in other animals are limited. In rabbits, the onset of action reportedly is < 1 minute and duration of anesthesia is 63 minutes.[4] In humans with clinically normal eyes, maximal effect of 0.5% proparacaine solution is 11.7 minutes,[5] with a complete recovery time of 34.9[5] to 45[6] minutes. Comparison of our results with those of studies in other species, including the previously described dog study,[2] suggests that corneas of DSH cats recover more rapidly from topical administration of proparacaine than those of other species.

a. Ophthetic, Allergan Inc, Irvine, Calif.
b. Cochet-Bonnet aesthesiometer, Luneau Ophtalmologies, Chartres Cedex, France.
c. MIXED procedure of the SAS System, version 8.02, SAS Institute Inc, Cary, NC.

References

1. Ward DA. Clinical ophthalmic pharmacology and therapeutics. Part 3. In: Gelatt KN, ed. *Veterinary ophthalmology*. 3rd ed. Baltimore: Lippincott Williams & Wilkins, 1999;336–354.
2. Herring IP, Bobofchak MA, Landry MP, et al. Duration of effect and effect of multiple doses of topical ophthalmic 0.5% proparacaine hydrochloride in clinically normal dogs. *Am J Vet Res* 2005;66:77–80.
3. Forstom C. Ophthaine (proparacaine hydrochloride): a local anesthetic for ophthalmic surgery (lett). *Vet Rec* 1964;76:385.
4. Liu JC, Steinemann TL, McDonald MB, et al. Topical bupivacaine and proparacaine: a comparison of toxicity, onset of action, and duration of action. *Cornea* 1991;12:228–232.
5. Weiss JS, Goren MB. The effect of corneal hypesthesia on duration of proparacaine anesthetic eyedrops. *Am J Ophthalmol* 1991;112:326–330.
6. Polse KA, Keener RJ, Jauregui MJ. Dose-response effects of corneal anesthetics. *Am J Optom Physiol Opt* 1978;55:8–14.
7. Chan-Ling T. Sensitivity and neural organization of the cat cornea. *Invest Ophthalmol Vis Sci* 1989;30:1075–1082.
8. Stiles J, Krohne S, Rankin A, et al. The efficacy of 0.5% proparacaine stored at room temperature. *Vet Ophthalmol* 2001;4:205–207.
9. Barrett PM, Scagliotti RH, Meredith RE, et al. Absolute corneal sensitivity and corneal trigeminal nerve anatomy in normal dogs. *Prog Vet Comp Ophthalmol* 1991;1:245–254.
10. Nomura K, Singer DE, Aquavella JV. Corneal sensation after topical anesthesia. *Cornea* 2001;20:191–193.
11. Blocker T, van der Woerdt A. A comparison of corneal sensitivity between brachycephalic and domestic short-haired cats. *Vet Opthalmol* 2001;4:127–130.
12. Weigt AK, Herring IP, Marfurt CF, et al. Effects of cyclophotocoagulation with a neodymium:yttrium-aluminum-garnet laser on corneal sensitivity, intraocular pressure, aqueous tear production, and corneal nerve morphology in eyes of dogs. *Am J Vet Res* 2002;63:906–915.
13. Good KL, Maggs DJ, Hollingsworth SR, et al. Corneal sensitivity in dogs with diabetes mellitus. *Am J Vet Res* 2003;64:7–11.
14. Brooks DE, Clark CK, Lester GD. Cochet-Bonnet aesthesiometer-determined corneal sensitivity in neonatal foals and adult horses. *Vet Ophthalmol* 2000;3:133–137.

Effects of topical administration of an aldose reductase inhibitor on cataract formation in dogs fed a diet high in galactose

Peter F. Kador, PhD; Daniel Betts, DVM, MS; Milton Wyman, DVM, MS; Karen Blessing, BA; James Randazzo, BS

Objective—To determine effects of a topical formulation of an aldose reductase inhibitor (ARI) on the development of sugar cataracts in dogs fed a diet high in galactose.

Animals—Ten 6-month old Beagles.

Procedures—Dogs were fed a diet containing 30% galactose, and after 16 weeks, 6 dogs were treated topically with a proprietary ARI formulation and 4 dogs were treated with a placebo. Cataract formation was monitored by means of slit-lamp biomicroscopy and fundus photography. Dogs were euthanized after 10 weeks of treatment, and lenses were evaluated for degree of opacity, myo-inositol and galactitol concentrations, and concentration of the ARI.

Results—All dogs developed bilateral cortical opacities dense enough to result in a decrease in the tapetal reflex after being fed the galactose-containing diet for 16 weeks. Administration of the ARI arrested further development of cataract formation. In contrast, cataracts in the vehicle-treated dogs progressed over the 10-week period to the mature stage. Evaluation of the isolated lenses after 26 weeks of galactose feeding indicated that lenses from treated dogs were significantly less optically dense than lenses from control dogs. Lenticular myo-inositol concentration was significantly higher in the treated than in the control dogs.

Conclusions and Clinical Relevance—Results suggest that topical application of a proprietary ARI formulation may arrest or reverse the development of sugar cataracts in dogs fed a diet high in galactose. This suggests that this ARI formulation may be beneficial in maintaining or improving functional vision in diabetic dogs with early lens opacities. (*Am J Vet Res* 2006;67:1783–1787)

Abbreviations

ARI	Aldose reductase inhibitor
HPLC	High-pressure liquid chromatography

In dogs, diabetes mellitus is characterized by the rapid appearance of bilateral sugar cataracts. Over the past 30 years, the incidence of canine diabetes mellitus has increased 3-fold.[1,2] Currently, nearly 1 in 3 dogs with cataracts is also diabetic. Since cataracts lead to vision loss that can currently only be treated by surgery, a medical treatment that preserves vision and prevents the need for surgery in diabetic dogs would be beneficial.

Cataracts can be experimentally produced in animals by inducing diabetes mellitus or feeding a diet high in lactose or galactose, with the rate of cataract formation proportional to the blood glucose or galactose concentration.[3-5] Studies in rats suggest that galactosemic "sugar" cataracts undergo histologic and biochemical changes similar to those seen with diabetic "sugar" cataracts[3,6-8] and that clinical progression is similar for galactosemic and diabetic cataracts.[9,10]

Oxidative stress, redox changes, altered membrane permeability, glycation, and production of advanced glycation end products all contribute to the formation of diabetic cataracts,[11] but extensive studies[5,12,13] have shown that lenticular aldose reductase activity is the primary factor in cataract development. Specifically, biochemical changes in the lens that ultimately lead to cataract formation are initiated by the intracellular accumulation of sorbitol or galactitol. Aldose reductase reduces glucose to sorbitol and reduces galactose to galactitol. Sorbitol, in turn, is oxidized by sorbitol dehydrogenase to fructose, but galactitol is not further metabolized.

The critical role of aldose reductase in cataract formation has been confirmed in diabetic animals and animals fed a diet high in galactose. For example, hyperglycemic mice and mice fed a diet high in galactose do not develop cataracts because they have low aldose reductase concentrations in their lenses. In contrast, transgenic mice that express high lenticular aldose reductase concentrations rapidly form diabetic and galactosemic cataracts.[14] Furthermore, diabetic cataract formation is enhanced when sorbitol dehydrogenase activity is also deleted in transgenic mice with high lenticular aldose reductase concentrations. Similarly, diabetic cataracts are uncommon in cats, compared with dogs, even though incidences of diabetes mellitus in cats and dogs are similar because concentrations of aldose reductase are much lower in the lenses of cats than in the lenses of age-matched dogs.[15,16] Further proof of the role of aldose reductase in the development of cataracts comes from studies[17,18] showing that ARIs can arrest cataract formation, even

Received December 15, 2005.
Accepted April 28, 2006.
From the College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68198 (Kador, Blessing, Randazzo); Therapeutic Vision Inc, 11778 Whitmore S, Omaha, NE 68142 (Kador, Betts, Wyman); the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA 50011 (Betts); and the Department of Comparative Ophthalmology, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210 (Wyman).
Supported by start-up funds provided by the University of Nebraska Medical Center.
Drs. Kador, Betts, and Wyman have financial interests in Therapeutic Vision Incorporated.
Address correspondence to Dr. Kador.

though they do not decrease lens glycosylation or formation of advanced glycation end products, and arrest the biochemical changes associated with oxidative stress in the lenses of diabetic animals and animals fed a diet high in galactose.[13,19,20]

Several studies[6,21,22] have shown that ARIs can prevent the development of cataracts in animals when given at the onset of galactosemia or diabetes. In addition, studies[23-26] have shown that ARIs can reverse the formation of cataracts in rats, but only if given during the early vacuolar stage of cataract formation. In many dogs, however, diabetes mellitus is diagnosed only after the owner has brought the dog to a veterinarian because of apparent lens changes. Thus, in most diabetic dogs, it is likely that substantial biochemical changes have already occurred in the lens at the time diabetes mellitus is diagnosed. To be useful clinically, therefore, ARIs must be able to arrest the progression of cataracts or reverse their development in dogs that already have clinical evidence of cataract formation. The purpose of the study reported here, therefore, was to determine whether a new topical formulation of an ARI would arrest or reverse the development of cataracts in dogs fed a diet high in galactose.

Materials and Methods

Dogs—Ten 6-month-old purpose-bred male Beagles[a] were used in the study. Dogs were housed in individual runs for the duration of the study. For all dogs, results of complete physical and ophthalmologic examinations performed prior to the study were normal. All procedures were performed in accordance with the *Guide for the Care and Use of Laboratory Animals*.

Experimental protocol—To induce cataract formation, dogs were fed a diet containing 30% galactose[b] for the duration of the study, with each dog receiving 450 g of the diet at approximately 8 AM each day. After this diet had been fed for 16 weeks, 6 dogs were randomly assigned to the treatment group and the remaining 4 dogs were assigned to the control group. Dogs in the treatment group were treated with a proprietary topical formulation of an ARI,[c] whereas dogs in the control group were treated with a placebo consisting of vehicle alone. For both groups, treatment consisted of topical application of 2 drops of the drug formulation or placebo in each eye, administered 10 minutes apart, at 8 AM and 4 PM. Treatments were administered for 10 weeks.

At the end of the study (ie, week 26), dogs were euthanized. Both eyes were enucleated from each dog, and the lenses were carefully removed by means of a posterior approach. All lenses were evaluated for density. Myo-inositol and galactitol concentrations were determined in the lens from 1 eye of each dog, and ARI concentration was determined in the lens of the contralateral eye.

Ophthalmic evaluations of lens changes—Ophthalmic examinations were conducted at the onset of the study (ie, week 0) to establish that all dogs were free from lens opacities and retinal lesions. Subsequent follow-up ophthalmic examinations, including indirect ophthalmoscopy and slit-lamp biomicroscopy, were performed at approximately 4-week intervals by veterinary ophthalmologists blinded to treatment group assignments of the dogs. Dogs were not anesthetized during these examinations; mydriasis was induced prior to examination by means of topical administration of 1% tropicamide hydrochloride. Lens changes were documented by means of fundus photography[d] at the onset of the study and during weeks 16 and 25.

Clinicopathologic testing—For all dogs, a CBC and serum biochemical profile were performed at the onset of the study (ie, week 0) and at the end of the 10 weeks of treatment (ie, week 26). The serum biochemical profiles include measurement of serum glucose, urea nitrogen, creatinine, sodium, potassium, chloride, calcium, albumin, globulin, and total bilirubin concentrations and serum aspartate transaminase, alanine transaminase, and alkaline phosphatase activities. Tests were performed by a commercial laboratory.[e]

Glycosylated hemoglobin concentration was measured at the onset of the study and during week 26 by means of HPLC. Testing was performed by a commercial laboratory.[f]

Concentrations of galactose and galactitol in RBCs and serum were measured at the onset of the study and during weeks 12 and 25. For these analyses, venous blood samples were collected into evacuated tubes containing EDTA. Blood samples were washed twice with 2 mL of saline (0.9% NaCl) solution and centrifuged at 1,800 × *g* for 15 minutes. Supernatants obtained after each centrifugation were combined and mixed with 2 mL of HPLC-grade water containing 3 µmol of xylose as an internal standard. The mixture was then deproteinized with 1 mL of 0.3N zinc sulfate and 1 mL of 0.3N barium hydroxide. Similarly, the precipitate containing RBCs was transferred to a glass homogenizer tube with 2 mL of HPLC-grade water containing 3 µmol of xylose as an internal standard, and the homogenate was deproteinized with 1 mL of 0.3N zinc sulfate and 1 mL of 0.3N barium hydroxide. Deproteinized RBC and plasma samples were centrifuged at 10,000 × *g* for 15 minutes, and a 400-µL aliquot of each sample was evaporated.[g] The dried residue was dissolved in 900 µL of pyridine and then derivatized with 900 µL of phenyl isocyanate at 55°C for 60 minutes. After cooling in an ice bath, the reaction was halted with cold methanol, and the sample was again heated for 5 minutes. Samples were then evaluated by means of HPLC, as described.[27]

Lens density—Density of the individual lenses was determined by placing the lenses on the lit surface of a digitizing slide scanner.[h] Color digital images of each lens were obtained with and without a grid. Lens opacity was determined from the images obtained without a grid by use of standard software.[i] Briefly, each lens image was inverted, and color ranges for the red, green, and blue spectrum were set from 0 to 255. The intensity of each photograph was calibrated by means of pixels at the lens equator, where the black ciliary processes attached. Next, the incident concentration of each photograph was calibrated by selecting pixels outside of the lens and away from the ciliary process. Multiple rectangular areas of interest were constructed until most (approx 80%) of the lens area was covered. The weighted average of the integrated optical density for all areas of interest was then obtained. Total lens clarity was defined as an integrated optical density of 0.0.

Lenticular myo-inositol and galactitol concentrations—In the lens, myo-inositol concentration decreases as sorbitol or galactitol accumulates. For determination of lens myo-inositol and galactitol concentrations, each lens was homogenized in a glass homogenizer with 2 mL of HPLC-grade water containing 3 µmol of xylose as an internal standard. Homogenate proteins were removed by centrifugation[j] overnight at 8°C. Filtrates were dried,[g] and dried residues were dissolved in 900 µL of pyridine and then derivatized with 900 µL of phenyl isocyanate at 55°C for 60 minutes. After cooling in an ice bath, the reaction was halted with cold methanol, and the sample was again heated for 5 minutes. Samples were then evaluated by means of HPLC, as described.[27] Briefly, samples were analyzed with an automated instrument[k] equipped with a diode array detector. Samples (5 µL) were injected onto a 150 × 4.6-mm column[l] containing a

3.2 × 15-mm guard column at 35°C and were eluted isocratically with 20mM potassium phosphate–acetonitrile (35:65 vol%; pH, 7.0) at a flow rate of 1.0 mL/min and detection at 235 nm. Sample concentrations were quantified by comparison with standard curves for glucose, galactose, sorbitol, galactitol, myo-inositol, and xylose (0.008 to 6.0 µmol).

Lenticular ARI concentration—For determination of lens ARI concentration, lenses were homogenized with HPLC-grade water containing sorbinil, an ARI, as an internal standard. The homogenates were deproteinated by treatment with sodium fluoride, then acidified with hydrochloric acid and extracted with diethyl ether. The ether layer was washed with 0.25M phosphate buffer (pH, 7.0). The ether layer was then evaporated, and the residue was dissolved in HPLC-grade methanol. Concentrations of ARI were measured on a reverse-phase, 5-µm C18 column[m] equipped with a 3.2 × 15-mm guard column by eluting with an isocratic mixture of 55% HPLC-grade methanol and 45% HPLC-grade water at a flow rate of 0.5 mL/min. Chromatography was conducted at room temperature, and the compound was detected at 220 nm over a linear range of 0 to 70 µg.

Statistical analysis—Data are given as mean ± SD. For analysis of lens density data, the mean value for the 2 lenses from each dog was used. The independent 2-sample *t* test was used to compare values between the treatment and control group. All analyses were performed with standard software.[n] Values of $P < 0.05$ were considered significant.

Results

For all dogs, results of CBCs and serum biochemical profiles performed at the onset (ie, week 0) and termination (week 26) of the study were unremarkable, and no clinically important abnormalities were found. All dogs were equally galactosemic as demonstrated by the 25- to 26-week blood samples, in which no significant differences between the treatment and control group were found in regard to plasma galactose concentrations (436 ± 106µM vs 544 ± 63.4µM), plasma galactitol concentrations (9.9 ± 3.2µM vs 16.4 ± 10.4µM), RBC galactose concentrations (108 ± 42.4µM vs 95.7 ± 30.2µM), and glycosylated hemoglobin concentrations (4.9 ± 0.1% vs 5.0 ± 0.1%). However, week 26 RBC galactitol concentrations were significantly lower among dogs in the treatment group (17.0 ± 8.50µM), compared with dogs in the control group (112.7 ± 36.3µM).

As the study progressed, suture accentuation was the earliest observed lens change and was apparent after dogs had been fed the high-galactose diet for 4 weeks. After the diet was fed for 8 weeks, vacuoles were apparent, and by 12 weeks, superficial cortical opacities were apparent in all dogs. At 16 weeks, bilateral cortical opacities were present in all dogs, and minimal tapetal reflex of the flash of the fundus camera was evident because of the density of the lens opacities (**Figure 1**). Among dogs in the treatment group, the amount of tapetal reflex appeared subjectively to increase over time following the initiation of treatment with the ARI formulation, suggesting that the density of lens opacities had decreased or lens clearing had occurred. In contrast, among dogs in the control group, the amount of tapetal reflex subjectively appeared to decrease further as the study progressed, and at the termination of the study, all control dogs had dense, mature cataracts.

When images of the individual lenses were obtained with a grid, it appeared subjectively that more of the grid was visible through the lenses obtained from treatment group dogs than from control dogs. Analysis of digitized images obtained without a grid indicated that integrated optical density of lenses from control dogs ($3.9 \pm 2.2 \times 10^{-3}$ pixel density × mm^2) was significantly greater than density of lenses from treatment group dogs ($0.47 \pm 0.34 \times 10^{-3}$ pixel density × mm^2), even though the percentage of lens area analyzed for control dogs (81.6 ± 3.8%) was not significantly different from the percentage for treatment group dogs (80.3 ± 2.8%).

Lenticular myo-inositol concentration was significantly higher in treatment group dogs (311.0 ± 89.4µM) than in control dogs (105.0 ± 18.0µM; **Figure 2**). In contrast, lenticular galactitol concentration in treatment group dogs (34.7 ± 14.2mM) was not significantly different from concentration in control dogs (50.9 ± 7.0mM). For dogs in the treatment group,

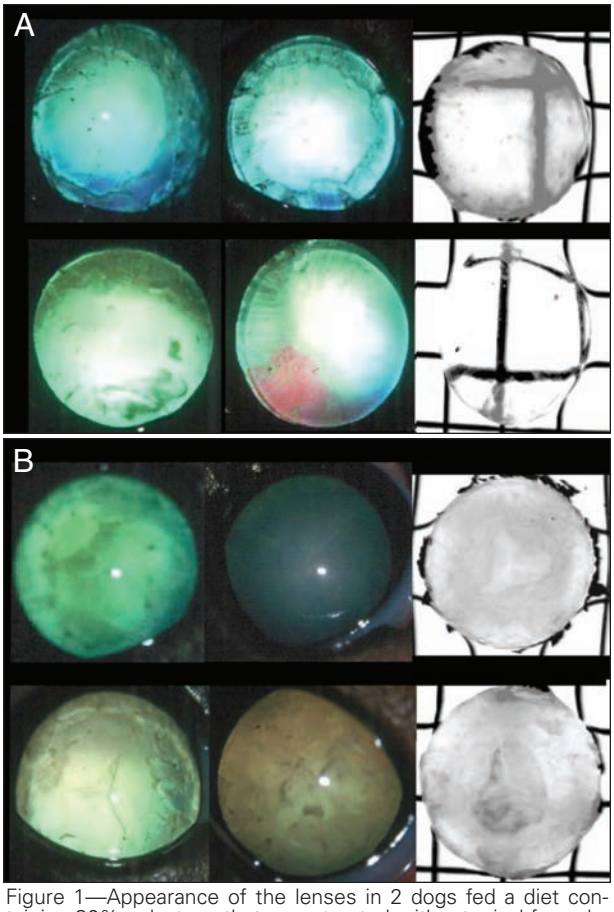

Figure 1—Appearance of the lenses in 2 dogs fed a diet containing 30% galactose that were treated with a topical formulation of an ARI (A) and in 2 dogs fed the same diet that were treated with a placebo (B). In each series, the image on the far left represents the appearance of the eye after the high-galactose diet had been fed for 15 weeks (1 week prior to the start of ARI or control treatment); the image in the center represents the appearance of the same eye after 9 weeks of treatment (ie, week 25); and the image on the far right represents the appearance of the isolated lens, obtained after euthanasia of the dog 1 week later, photographed over a grid. Notice the absence of a tapetal reflex of the flash of the fundus camera in the eyes of the control dogs, particularly after the high-galactose diet had been fed for 25 weeks.

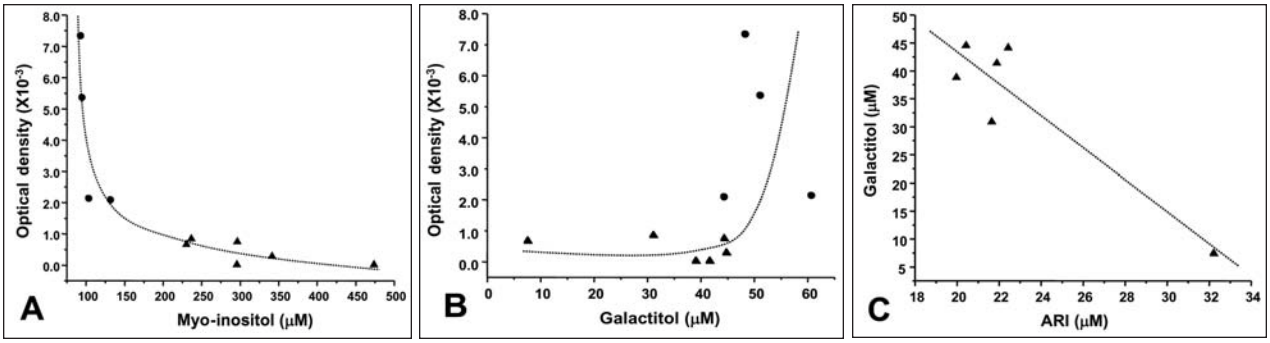

Figure 2—Scatterplots of weighted average integrated optical density versus lenticular myo-inositol (A) and lenticular galactitol (B) concentrations in 10 dogs fed a high-galactose diet that were treated with a topical ARI formulation (n = 6; triangles) or a placebo (4; circles) and of lenticular galactitol concentration versus lenticular ARI concentration in treated dogs (C). The dotted line in part C represents the least squares fit (r^2 = 0.84).

there was a significant negative relationship (r^2 = 0.84) between lenticular ARI concentration and lenticular galactitol concentration.

Discussion

Results of the present study suggest that topical application of a proprietary ARI formulation may arrest or reverse the development of sugar cataracts in dogs fed a diet high in galactose. This suggests that this ARI formulation may be beneficial in maintaining or improving functional vision in diabetic dogs with early lens opacities.

The initial lens changes associated with cataract formation in dogs fed a diet high in galactose are similar to those that occur in diabetic dogs,[28] with cataract formation directly linked to aldose reductase-catalyzed accumulation of galactitol and sorbitol, respectively.[9,15,16,22] In dogs with diabetes, glucose is converted to sorbitol, which, in turn, is oxidized to fructose by sorbitol dehydrogenase. Therefore, lenticular sorbitol accumulation is regulated both by inhibition of aldose reductase activity and by sorbitol dehydrogenase activity. In contrast, in dogs fed a diet high in galactose, galactitol accumulation can only be controlled by inhibition of aldose reductase activity because galactitol is not further metabolized, with the net result being that galactitol accumulates more rapidly and at higher concentrations in dogs fed a diet high in galactose than sorbitol accumulates in diabetic dogs. As a result, cataract formation is more rapid and more severe in dogs fed a diet high in galactose than in diabetic dogs. Therefore, dogs fed a diet high in galactose are often used to evaluate the efficacy of ARIs in cataract prevention because more robust inhibition is required.[3]

In dogs, lenticular aldose reductase activity is age dependent, with activity decreasing to plateau concentrations in adult animals. As a result, the onset and severity of cataract formation are also age dependent.[29] In the present study, we elected to use 6-month-old dogs because results of a previous study[29] indicated that cortical opacities develop by 12 weeks and mature cataracts develop by 26 weeks when dogs of this age group are fed a diet containing 30% galactose.

Previous studies[3,4,6] of the efficacy of ARIs have focused on prevention, with the drugs administered at the onset of diabetes or galactosemia, and these studies have established that ARIs are effective in preventing cataract formation in diabetic and galactosemic rats. Similarly, a previous study[22] demonstrated that the onset and progression of cataracts in 9-month-old dogs fed a diet high in galactose can be inhibited in a dose-dependent manner with ARIs. In contrast, studies[3,23-26] investigating whether ARIs can arrest or reverse the progression of cataract development once cataracts have formed have been limited. It has generally been thought that opacities resulting from lens fiber degeneration are irreversible and that only those opacities resulting from early vacuolation can be reversed. In the present study, however, we found that opacities resulting from fiber degeneration could be reversed, with overall density of the lens significantly reduced, by use of a topical ARI formulation. This decrease in lens density was associated with a subjective improvement in the apparent tapetal reflex. Taken together, these results suggest that, compared with control dogs, functional vision in dogs treated with the ARI improved or was maintained.

Results of biochemical analyses in the present study also supported the conclusion that ARI treatment reduced cataract formation. In general, lenticular concentrations of sorbitol and galactitol initially increase during cataract formation, but then decrease because of increased lens permeability as the cataract becomes more severe. In contrast, lenticular myo-inositol concentrations are inversely proportional to lenticular concentrations of sorbitol and galactitol. In the present study, lenticular myo-inositol concentrations were significantly higher in dogs treated with the ARI than in control dogs, suggesting that aldose reductase activity was indeed being inhibited in the treated dogs.

Although no adverse effects resulting from long-term oral administration of ARIs have been published, it has been suggested that some ARIs may modify select hepatic enzymes associated with oxidative defense or P_{450} induction.[30,31] In contrast, this has not been observed with topical administration.[32] Thus, topical administration may be preferred, particularly because hepatic function may already be compromised in diabetic dogs. However, the finding that RBC galactitol concentration was significantly decreased in treatment group dogs, compared with control dogs, in the present study indicates that there were some minor systemic effects following topical application of this ARI.

a. Marshall Farms USA Inc, North Rose, NY.

b. Bio-Serve, Frenchtown, NJ.
c. Kinostat, Therapeutic Vision Inc, Omaha, Neb, and the University of Nebraska Medical Center, Omaha, Neb.
d. FS-3 fundus camera, Nikon, Tokyo, Japan.
e. Regional Pathology Service, Nebraska Medical Center, Omaha, Neb.
f. Associated Regional and University Pathologists Inc, Salt Lake City, Utah.
g. Savant SpeedVac, Thermo Electron Corp, Waltham, Mass.
h. FOTOVIX, Tamron USA Inc, Commack, NY.
i. Image-Pro Plus, Media Cybernetics, Silver Spring, Md.
j. Microcon YM-10 centrifugal filter device, Millipore Corp, Burlington, Mass.
k. ChemStation 1100, Hewlett Packard, Agilent Technologies, Palo Alto, Calif.
l. TSKgel ODS-80Tm, Tosoh Bioscience LLC, Montgomeryville, Pa.
m. LUNA, Phenomenex, Torrance, Calif.
n. Origin, version 7.0, OriginLab Corp, Northampton, Mass.

References

1. Feldman EC, Nelson RW. *Canine and feline endocrinology and reproduction*. Philadelphia: WB Saunders Co, 1996;187–255, 339–394.
2. Guptill L, Glickman L, Glickman N. Time trends and risk factors for diabetes mellitus in dogs: analysis of veterinary medical data base records (1970–1999). *Vet J* 2003;165:240–247.
3. Kador PF, Kinoshita JH. Diabetic and galactosemic cataracts. *Ciba Found Symp* 1984;106:110–123.
4. Kinoshita JH, Kador PF, Datiles MD. Aldose reductase in diabetic cataracts. *JAMA* 1981;246:257–261.
5. Kador PF. Biochemistry of the lens: intermediary metabolism and sugar cataract formation. In: Viola E, Dowling J, eds. *Principles and practice of ophthalmology. Volume basic sciences*. Philadelphia: WB Saunders Co, 1994;146–167.
6. Datiles M, Fukui H, Kuwabara T, et al. Galactose cataract prevention with sorbinil, an aldose reductase inhibitor: a light microscopic study. *Invest Ophthalmol Vis Sci* 1982;22:174–179.
7. Sakuragawa M, Kuwabara T, Kinoshita JH, et al. Swelling of the lens fibers. *Exp Eye Res* 1975;21:381–394.
8. Kuwabara T, Kinoshita JH, Cogan DG. Electron microscopic study of galactose-induced cataract. *Invest Ophthalmol* 1969;8:133–149.
9. Sato S, Takahashi Y, Wyman M, et al. Progression of sugar cataract in the dog. *Invest Ophthalmol Vis Sci* 1991;32:1925–1931.
10. Monti F, Bellan B, Peruccio C, et al. The clinical picture of diabetic retinopathy in the dog. *Folia Vet Lat* 1976;6:249–274.
11. Bron AJ, Brown NA, Harding JJ, et al. The lens and cataract in diabetes. *Int Ophthalmol Clin* 1998;38:37–67.
12. Lee AY, Chung SS. Contributions of polyol pathway to oxidative stress in diabetic cataract. *FASEB J* 1999;13:23–30.
13. Obrosova IG, Fathallah L. Evaluation of an aldose reductase inhibitor on lens metabolism, ATPases and antioxidative defense in streptozotocin-diabetic rats: an intervention study. *Diabetologia* 2000;43:1048–1055.
14. Lee AY, Chung SK, Chung SS. Demonstration that polyol accumulation is responsible for diabetic cataract by the use of transgenic mice expressing the aldose reductase gene in lens. *Proc Natl Acad Sci U S A* 1995;92:2780–2784.
15. Salgado D, Reusch C, Spiess B. Diabetic cataracts: different incidence between dogs and cats. *Schweiz Arch Tierheilkd* 2000; 142:349–353.
16. Richter M, Guscetti F, Spiess B. Aldose reductase activity and glucose-related opacities in incubated lenses from dogs and cats. *Am J Vet Res* 2002;63:1591–1597.
17. Kador PF, Lee JW, Fujisawa S, et al. Relative importance of aldose reductase versus nonenzymatic glycosylation on sugar cataract formation in diabetic rats. *J Ocul Pharmacol Ther* 2000;16:149–160.
18. Chiou SH, Chylack LT Jr, Bunn HF, et al. Role of nonenzymatic glycosylation in experimental cataract formation. *Biochem Biophys Res Commun* 1980;95:894–901.
19. Obrosova I, Faller A, Burgan J, et al. Glycolytic pathway, redox state of NAD(P)-couples and energy metabolism in lens in galactose-fed rats: effect of an aldose reductase inhibitor. *Curr Eye Res* 1997;16:34–43.
20. Lou MF, Dickerson JE Jr, Garadi R, et al. Glutathione depletion in the lens of galactosemic and diabetic rats. *Exp Eye Res* 1988;46:517–530.
21. Datiles MB III, Fukui H. Cataract prevention in diabetic *Octodon degus* with Pfizer's sorbinil. *Curr Eye Res* 1989; 8:233–237.
22. Sato S, Mori K, Wyman M, et al. Dose-dependent inhibition of sugar cataract formation in galactose-fed dogs by the aldose reductase inhibitor M79175. *Exp Eye Res* 1998;66:217–222.
23. Beyer-Mears A, Kelly K, Cruz E. Synergism of sorbinil and normal diet on reversal of stage-II sugar cataract. *Pharmacology* 1985;31:170–179.
24. Beyer-Mears A, Cruz E, Varagiannis E. Reversal of stage-I sugar cataract by sorbinil, an aldose reductase inhibitor. *Pharmacology* 1985;31:88–96.
25. Hu TS, Datiles M, Kinoshita JH. Reversal of galactose cataract with sorbinil in rats. *Invest Ophthalmol Vis Sci* 1983;24:640–644.
26. Reddy VN, Schwass D, Chakrapani B, et al. Biochemical changes associated with the development and reversal of galactose cataracts. *Exp Eye Res* 1976;23:483–493.
27. Miwa I, Kanbara M, Wakazono H, et al. Analysis of sorbitol, galactitol, and myo-inositol in lens and sciatic nerve by high-performance liquid chromatography. *Ann Biochem* 1988; 173:39–44.
28. Wyman M, Sato S, Akagi Y, et al. The dog as a model for ocular manifestations of high concentrations of blood sugars. *J Am Vet Med Assoc* 1988;193:1153–1156.
29. Lackner PA, Rodriguez L, Sato S, et al. Age-dependent changes in galactose-fed dogs. *Exp Eye Res* 1997;64:431–436.
30. Hoyle VR, Gilbert PJ, Troke JA, et al. Studies on the biochemical effects of the aldose reductase inhibitor 2,7-difluorospirofluorene-9,5′-imidazolidine-2′,4′-dione (Al 1576, HOE 843). Detection of D-glucaric and D-glucuronic acid excretion by high resolution 1H and 13C NMR spectroscopy. *Biochem Pharmacol* 1992;44:231–241.
31. Thomas T, Rauscher F, Sanders R, et al. Effects of aldose reductase inhibitors on antioxidant defense in rat and rabbit liver. *Toxicol Sci* 2000;53:145–149.
32. Sastry SG, Sanders RA, Veltman JC, et al. Minimal effects of two aldose reductase inhibitors, AL-1576 and AL-4114, after subacute topical-ocular dosing on xenobiotic biotransformation in rabbits. *Drug Metab Dispos* 1995;23:1094–1098.

Evaluation of retinal images for identifying individual dogs

Juliet R. Gionfriddo, DVM, MS; Arthur Claire Lee, DVM, PhD; Terry A. Precht; Cynthia C. Powell, DVM, MS; Kimberly K. Marren, MS; Steven V. Radecki , PhD

Objective—To F whether vessels in the ocular fundus changed over the lifetime of Beagles and whether any changes were substantial enough to likely preclude positive identification of individual dogs by use of their retinal vascular patterns.

Animals—18 Beagles.

Procedures—Fundic photographs of both eyes of 18 Beagles taken at 1 or 3, 5, and 7 or 9 years of age were digitalized. Photographs were analyzed by use of 2 software programs. One was used to determine vessel numbers and widths and the other to determine the locations of the 3 largest vessels. Measurements were compared over time periods in the life of each dog. Only observations made at baseline (1 or 3 years of age) and again at 5 and 9 years of age were included in the statistical analysis, as these points were common to all dogs.

Results—No significant changes in numbers or locations of the blood vessels were detected over time. Widths of the vessels decreased significantly as the dogs aged.

Conclusions and Clinical Relevance—The ocular fundus of Beagles changed over each dog's lifetime in that the retinal blood vessels became smaller but did not change in number or location. Results suggest that digitalized retinal images can likely be used to identify dogs over their lifetimes. (*Am J Vet Res* 2006;67:2042–2045)

Positive identification of individual dogs is important for veterinarians, dog breeders, and animal rescue agencies. Unalterable identification of dogs via hip-joint radiography and certification by the Orthopedic Foundation of America, ocular identification and registration by the Canine Eye Registry Foundation, or semen collection would ensure that dog substitutions could not be made. Animal shelters would also benefit from a reliable and easily achieved means of animal identification.

Identification methods presently used in dogs include use of morphologic features, tattooing, and SC placement of microchips. Identification by morphologic features is an inexact means of identification because many dogs have similar appearance. Tattooing of dogs in the groin area or the auricular pinna provides a more objective means of identification, but subsequent readings of tattoos can be difficult because of fading of the tattoo ink as well as growth of hair over the tattooed area. In addition, tattoos can be altered or removed.

Microchip placement is also an objective means of identification but is not without problems. Placement of the chip requires SC injection over the shoulder-neck area with a large needle. This procedure could result in infection, or the microchip could be accidentally deposited in the thoracic cavity or lungs. The microchip can migrate from its site of insertion to many other sites (such as internal organs), which could cause associated problems as well as difficulty finding the microchip when identification by a microchip reader is attempted. Retinal morphology has been used as an accurate means of identifying individual humans.[1,2] Identification of humans by the pattern of their retinal blood vessels was first suggested in 1935,[3] and the first commercial product for human retinal blood vessel identification appeared in 1985.[3] Retinal-scanning systems have been used in the military and in financial institutions in high-security access areas.[4] A system for retinal imaging and identification of individual cows is presently in use in the United States.[5,a]

Retinal imaging may be a valid means of individual identification in dogs because, as in cows and humans, there are numerous retinal vessels emanating from the optic disk. The larger vessels are veins, and the smaller vessels are arteries. These vessels form a distinct pattern unique to each individual. Although it has never been reported, it is assumed that retinal blood vessels do not change in pattern throughout the life of the animal and that retinal blood vascular patterns are virtually unalterable. Ocular fundic diseases could potentially alter retinal vessels by making them more tortuous or making them smaller; however, these conditions do not alter the positions of the vessels within the retina.

The purpose of the study reported here was to determine whether age-related fundic changes in clinically normal Beagles were of sufficient magnitude to preclude positive identification of individuals by use of digital retinal scanning.

Materials and Methods

Photographic slides of the ocular fundi of approximately 100 Beagles were obtained by one of the authors (ACL) during a long-term study of the effects of radiation on those dogs. Dogs chosen for the study reported here were nonirradiated control dogs. Ocular photographs of 18 dogs (11 sexually intact males and 7 sexually intact females) were selected on the basis of the clarity and focus of the photographs over the time periods intended for study. The photographs were scanned and digitalized.

Received June 9, 2006.
Accepted July 25, 2006.
From the College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80528 (Gionfriddo, Lee, Powell, Marren); Technology Driven Products, 4015 S Lincoln Ave, Loveland, CO, 80537 (Precht); and Colorado State University, Fort Collins, CO 80542 (Radecki).
Supported by PetSafe Incorporated.
Address correspondence to Dr. Gionfriddo.

The dogs were housed at Colorado State University during the years 1980 to 1990. The ocular fundi of the dogs were photographed annually during their entire lives or until 10 years of age. Photographs were taken with a fundic camera[b] and 35-mm slide film. Camera settings were the same for all photographs taken. All dogs were placed on a table and manually restrained. Their pupils were maximally dilated with 1 drop of tropicamide.

All dogs remained healthy throughout the study period, and no ocular abnormalities were seen. To detect any age-related fundic changes that were substantial enough to preclude positive identification of individual dogs, 1 photograph of each eye from each dog at each age (1 or 3 years, 5 years, and 7 or 9 years) was analyzed.

Two software programs were used to examine the digitalized photographs and analyze the fundic vessel patterns in each dog. This was done to determine the number, widths (sizes), and positions (angles and lengths) of the large vessels present. To determine whether the numbers and sizes of the larger vessels in each dog's fundus changed substantially over time, each digitalized image was analyzed with software[c] that measured the widths and counted the numbers of larger vessels by detecting them where they intersected 2 concentric circles projected around the optic disk (**Figure 1**). The starting point for plotting the vessels was a line drawn from the disk to the ora ciliaris retinae at the 3 o'clock position in the fundus; this represented 0°. The 2 concentric circles were moved synchronously and placed around the optic nerve to form a ring 0.2 mm wide. The center of the circles (denoted by a dot) was situated over the optic pit, and the placement of the rings was done by the same person each time. The program analyzed the digital image and filtered it by removing the high-frequency noise coming from the imaging device. This was done by taking a moving mean value in which the high-pass frequency was set by the window size of the moving mean value. The vessels were identified by segmentation, which is the process of separating features in an image by their contrast with the background (eg, the tapetum or areas of nontapetal pigmentation). Segmentation is based on a limit threshold in which the data are examined and a threshold value is selected that gives a distinct separation of the vessels from the background. Three width measurements were used to compute a mean value. All of the major vessels were detected, and their locations were plotted on a graph and counted according to their positions (in degrees around the circles) and mean widths (in pixels; **Figure 2**).

A second software progam[d] was used to determine whether the 3 major blood vessels on each of the retinal pictures changed in position over time. This program was used to compensate for rotation around a clockwise axis in the different photographs. With this program, the angles, from a horizontal line through the center of the optic pit, subtended by the 3 vessels as they crossed the border of the optic disk, were determined at each age (**Figure 3**). In addition, the distances from a point placed in the optic pit to points placed where each of the 3 vessels crossed the edge of the optic disk were calculated. These 4 points were identified in each photograph. The initial point (point 0) was manually located in the center of the optic pit. Points 1, 2, and 3 were located in the centers of the 3 largest blood vessels closest to the 9, 12, and 3 o'clock positions, respectively, at the points where they crossed the optic disk. The distance between point 0 and each other point was measured 3 times, and these values were used to compute a mean value. These data were defined as the distance data and reported in pixels.

Statistical analysis—For each dog, the number of vessels and the mean widths (in pixels) of the vessels were calculated at each time point. Mean widths were evaluated by

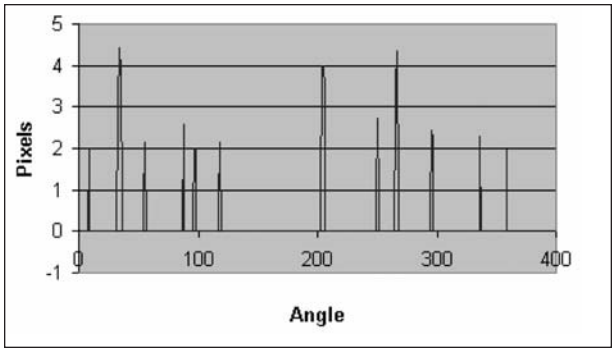

Figure 2—Plot of the vessels on the fundus of a retina in Figure 1. The horizontal axis represents the vessel position in degrees, and the vertical axis represents their widths in pixels.

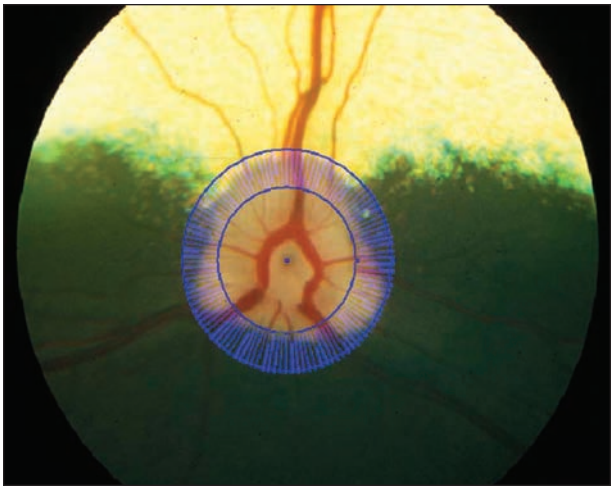

Figure 1—Photograph of the retina of a dog in a study of use of retinal images for identification of individual dogs. With the retinal measurement program used in the study, the small blue dot is placed in the center of the optic disk and 2 concentric circles are placed around the disk.

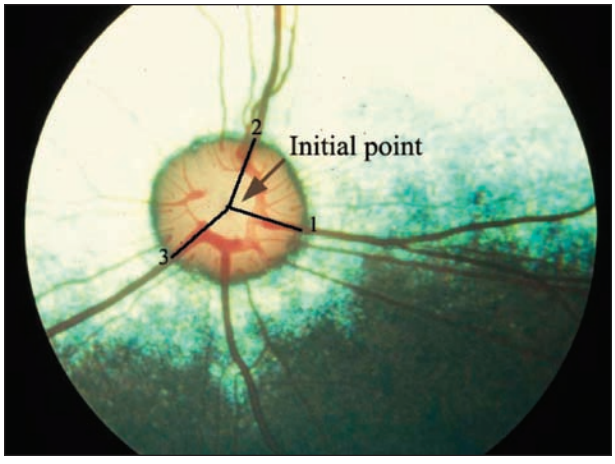

Figure 3—Photographic image of a retina of a dog, with lines applied by use of a computer software program. Four points were located: the initial point; the point in the center of the optic disk; and points 1, 2, and 3, the points at which the largest blood vessels crossed the external edge of the optic disk. Lines from the initial point to points 1, 2, and 3 were drawn, and their lengths were calculated to determine the distance data. A horizontal line through the optic pit was drawn and used as the baseline to determine the angles (a, b, and c) at which the lines crossed the edge of the optic disk.

use of ANOVA for repeated measures (repeated in time). The frequency of vessels was evaluated by use of a generalized linear mixed-model method with an assumed Poisson distribution. The statistical model included side (ie, right or left eye), sex, time, and the 2- and 3-way interactions among these 3 factors, all as fixed effects.

For evaluation of the distance from point 0 and angles measured at points 1, 2, and 3, the initial value was used as a baseline value. Relative values (percentages) were calculated as baseline values divided by subsequent values multipled by 100%. Results were used in an ANOVA appropriate for repeated measures to assess changes in time (ie, age). Each measurement was analyzed as a separate outcome. A multivariate ANOVA was used to evaluate the 3 outcomes simultaneously, with time as the only fixed effect. To maximize the power of the test, only values for which age was 5 or 9 were included. Values of $P < 0.05$ were considered significant.

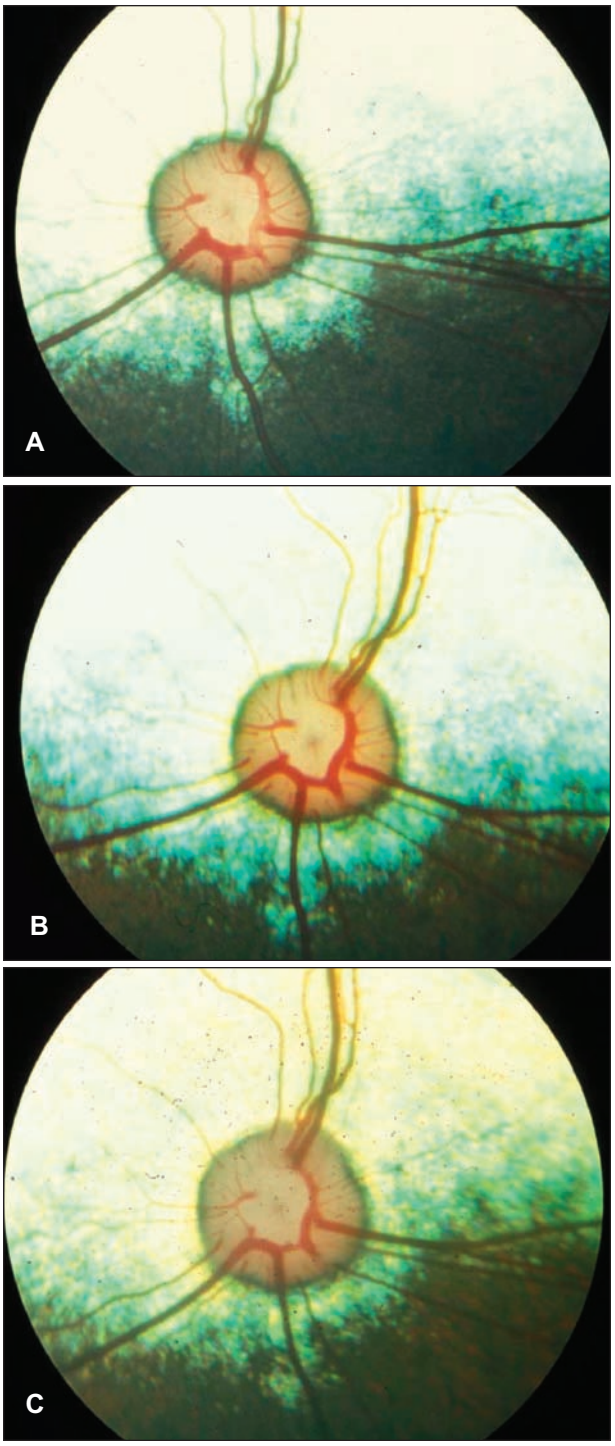

Figure 4—Photographic images of the retina of dog, obtained at 1 (A), 5 (B), and 9 (C) years of age. Notice a slight decrease in the widths of the vessels and a slight cloudiness of the image in C, although the numbers and locations of the vessels do not change with age.

Results

For all 18 dogs, all photographs from each individual dog's eyes at all time points were grossly similar (**Figure 4**). When the photographic slides were stacked on top of each other and compared, the shapes (eg, curves and branches) and numbers of blood vessels did not appear subjectively to change over time. However, the fundic image on the slides became noticeably less clear as each dog's age increased, which was attributed to changes in the optical media (eg, the cornea, lens, and vitreous) and did not affect the ability to easily delineate the blood vessels. The diameter of the blood vessels appeared to decrease considerably as each dog aged, which was confirmed by the measurement data. Mean width of vessels decreased significantly over time (1 year of age, 11.70 pixels; 5 years of age, 10.33 pixels; and 9 years of age, 6.98 pixels). Neither sex nor side was associated with this change. A significant sex effect was detected for the number of vessels per dog when both eyes were considered. Female dogs had fewer vessels (15.19/dog), compared with male dogs (20.12/dog). Age and side were not associated with the number of vessels per dog. Retinal hemorrhages were evident in one 9-year-old dog; these did not change the ability to measure the retinal vessels, so the dog was included in the study.

Age was not significantly associated with changes in distance from point 0 for points 1, 2, or 3 (**Table 1**). However, multivariate ANOVA revealed that age was

Table 1—Mean ± SD (percentage of baseline [age, 1 year]; range) values of the distances (in pixels) from the center of the optic disk to the points at which each of 3 major vessels (points 1, 2, and 3) crossed the edge of the optic disk in Beagles at 5 and 9 years of age.

Age (y)	Point	No. of observations	Mean ± SD	Range
5	1	31	100 ± 30	57–34
	2	32	105 ± 9	89–26
	3	32	103 ± 19	76–48
9	1	33	99 ± 36	0–238
	2	33	104 ± 15	84–165
	3	33	94 ± 28	14–149

Table 2—Mean ± SD (percentage of baseline [age, 1 year]; range) values of the angles of 3 major vessels as calculated from horizontal to the points at which they crossed the optic disk in Beagles at 5 and 9 years of age.

Age (y)	Point	No. of observations	Mean ± SD	Range
5	1	32	94 ± 6	85–107
	2	32	92 ± 8	79–107
	3	32	93 ± 6	81–100
9	1	33	92 ± 7	80–108
	2	33	83 ± 9	54–98
	3	33	89 ± 7	77–100

significantly associated with change in the angle of the vessels measured at points 2 and 3, but not at point 1 (**Table 2**). This significant change did not seem intuitively correct, and the digital photographs, marked with the 4 points, were carefully examined visually. It was apparent that this statistical finding was an artifact because in all of the dogs, both points 2 and 3 shifted away from point 1 in the same direction by almost the same number of pixels. When the digital scans of the photographic slides of an eye of all dogs over the 3 age ranges were superimposed on a computer screen by manually aligning the centers of the optic pits and point 1, it became subjectively obvious that points 2 and 3 had indeed shifted. This was thought to be attributable to the axis at which the initial photograph had been taken rather than to an actual shifting of the vessel.

Discussion

The fact that the retinal vascular pattern of most mammalian species does not change substantially over time seems to be accepted by most ophthalmologists, although no data that support this concept could be found. In the study reported here, the fundus did not change enough with age to likely preclude positive identification of an individual. The artifact introduced by differences in the axis at which photographs are taken will need to be taken into account when developing a software program to analyze the angle data. It was interesting that although the numbers and positions of the retinal vessels did not change over time, vessel widths became smaller, which was probably associated with a generalized decrease in circulation as seen in organs and limbs because of aging.

Digital retinal imaging is presently being used to identify cattle,[5] which is important in tracking diseases and settling ownership disputes. This technology could also be valuable as a simple and effective means of identifying dogs. Although eye diseases such as glaucoma, progressive retinal atrophy, and retinal degeneration could alter the vascular patterns enough to make them unrecognizable, these diseases are relatively rare in the general dog population. Cataracts and corneal opacities could decrease or eliminate the ability to visualize and photograph the fundus, but surgery or medical treatments could reverse the lesions enough to restore this ability. Results of the present study suggest that normal aging of Beagles will not significantly alter retinal vascular patterns and individuals might be identified by their vascular patterns throughout their lives. Although Beagles were used, it is likely that the results of this study can be applied to the other breeds and mixed-breed dogs.

a. Optibrand Inc, Fort Collins, Colo.
b. Kowa, Optimed Inc, Torrence, Calif.
c. Technology Driven Products, Loveland, Colo.
d. GNU Gimp, version 2.2.13, Boston, Mass.

References

1. Wu C. Private eyes: biometric identification is set to replace passwords and PINs. *Science News Online* [serial online]. 1998;153(14). Available at: www.sciencenews.org/pages/sn_arch98/4_4_98/bob1.htm. Accessed Apr 19, 2005.

2. Larish J. Digital photographs for security. *Adv Imaging* 2002; 1:39–41.

3. Biometrics Web site. Iris and retinal identification. Available at:www.et.wcu.edu/aidc/BioWebPages/Biometrics_Eye.html. Accessed Apr 18, 2005.

4. Computer business review. The future of recognition. Available at: www3.xls.com/cgibin/bni.exe. Accessed Apr 18, 2005.

5. Iowa Farmer Today Web site. Retina reader may replace branding for cattle. August 11, 2004. Available at: www.iowafarmer.com. Accessed Apr 19, 2005.