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CLINICAL ARTICLE

The effect of a topical antioxidant formulation including N-acetyl carnosine on canine cataract: a preliminary study

David L. Williams and Patricia Munday

Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, CB3 OES, England, UK

Address communications to:

D. L. Williams

Tel.: +44 (0) 1223 232977
Fax: +44 (0) 1223 232977
e-mail:
doctordlwilliams@aol.com

Abstract

Objective To determine the efficacy of a topical antioxidant formulation including N-acetyl carnosine in the treatment of canine cataract in a preliminary nonplacebo, controlled, unmasked study.

Animals studied Thirty dogs of varying breeds and ages with a spectrum of lens opacities ranging from nuclear sclerosis to total mature cataract.

Methods Dogs were treated three times daily with topical 2% N-acetyl carnosine in a buffered vehicle containing the antioxidants glutathione, cysteine ascorbate, L-taurine and riboflavin (Ocluvet™, Practivet, Phoenix, AZ, USA). Dogs were examined prior to treatment and at 2, 4 and 8 weeks during treatment, by direct and indirect ophthalmoscopy and slit-lamp biomicroscopy after pharmacologic pupil dilation. Photographic documentation of lens opacity was achieved by retroillumination photography, with three photographs taken at each examination time-point. A lens opacification index (LOI), determined by integration of the grayscale level of each pixel across the image, was evaluated by computerized image analysis of digitized images. Alteration in mean LOI was determined for each animal, having normalized the initial LOI.

Results Fifty-eight eyes of 30 dogs were evaluated, 22 with mature cataract, 13 with immature cataract, 9 with cataract associated with other intraocular disease such as uveitis and 14 with nuclear sclerosis alone. One dog was unilaterally anophthalmic after previous enucleation and one had a phthytic eye after previous uveitis-induced glaucoma. Image analysis showed a reduction in mean LOI in all cataract groups (mean resolution in opacity of 2.3 ± 0.33% for all cataracts), although this was only statistically significant in those eyes with immature cataract (mean resolution of opacity 4.5 ± 0.33%) or nuclear sclerosis (mean decrease in opacity 5 ± 0.37%). Reduction in lens opacity was seen in eyes with mature cataract (0.5 ± 0.4%) and in miscellaneous cataract associated with intraocular inflammation (1.3 ± 0.4%), but these changes were not statistically significant. Owner evaluation of visual capability, however, suggested improvement in vision in 80% of cases by the end of the study.

Conclusion This study demonstrates some marginal reduction in lens opacification in a substantial number of cases of canine cataract with the use of a topical nutritional antioxidant formulation including N-acetyl carnosine. Lens opacification was improved with treatment in eyes with immature cataract or nuclear sclerosis while in eyes with mature cataract or cataract with associated intraocular inflammatory pathology less reduction was seen.

Key Words: antioxidant, cataract, dog, N-acetyl carnosine

INTRODUCTION

Since the earliest development of operations to remove opaque lenses, surgery has been considered the only treatment for cataracts. A number of spurious reports have suggested otherwise, but physical removal of the lens, whether by intracapsular, extracapsular or phacoemulsication techniques, has been the only method of ameliorating vision in eyes with cataracts. Dietary antioxidants have been demonstrated to retard cataractogenesis[1] but application of antioxidants, whether topically or systemically, has never been shown to reduce the density of opacification of a cataractous lens.

Thus it was with a considerable degree of scepticism that the ophthalmic world viewed reports from Russia of a topical medication that could ameliorate the effects of cataract on a person's vision.[2,3,4] *In vitro* work,[5] however, has shown that N-acetyl carnosine can disaggregate crystallins aggregated by glycation, a chemical reaction postulated to contribute to cataractogenesis *in vivo*.[6] In addition it reduces inactivation of enzymes by glycation in a standard assay designed to model the changes occurring in cataractogenesis.[7] Carnosine (beta-alanyl-L-histidine), a compound naturally occurring in brain, innervated tissues and the lens at concentrations up to 20 mM in humans,[8] has been shown to act as a powerful antioxidant in other body systems.[9] It delays senescence of cultured human fibroblasts showing that carnosine could have a role retarding aging changes.[10] Carnosine has antioxidant and oxygen free-radical scavenging activities and also reacts with deleterious aldehydes to protect susceptible macromolecules. Carnosine inhibits nonenzymatic glycosylation and cross-linking of proteins induced by reactive aldehydes, such as aldose and ketose sugars, certain triose glycolytic intermediates and malondialdehyde (MDA), a lipid peroxidation product. Carnosine inhibits formation of MDA-induced protein-associated advanced glycosylation end products (AGEs) and formation of DNA-protein cross-links induced by compounds such as acetaldehyde. It is only in its acetylated form (N-alpha acetyl carnosine (NAC)), however, that transcorneal penetration occurs after stromal hydrolysis, allowing intraocular penetration.[11]

Although prior to the study it was considered unlikely by the present authors that NAC would have a substantial ameliorative effect on cataractous lenses in dogs, we aimed to perform a series of studies to assess whether this is indeed the case. Our initial study, reported here, is an unmasked trial without a placebo-controlled element. Changes in lens opacification have been evaluated by retroillumination ophthalmic photography with image analysis to determine the percentage of the lens opacified and the density of opacification. We propose that if any evidence of reduction in cataract density is demonstrated this will in itself be worthy of note, prior to a full placebo-controlled double-blind trial currently in preparation.

MATERIALS AND METHODS

Animals

Thirty-six dogs of various breeds, genders and ages with lens pathology manifest in both eyes were recruited into the trial. Three dogs (each with bilateral mature cataracts) were diabetic. Inclusion criteria (dogs of any age with any lens opacification ranging from nuclear sclerosis to mature cataract) were intentionally wide as it was aimed, in this preliminary study, to examine drug effects on lens pathologies from nuclear sclerosis to mature cataract. Thirty dogs completed at least 2 months' duration of treatment with at least four examinations during the study period. Two dogs were withdrawn from the trial because elderly owners found difficulty with three-times-daily drop administration. One dog died during the trial of a condition unrelated to its ocular condition.

Antioxidant formulation administration

One drop of Ocluvet™ (Practivet, Phoenix, AZ, USA, patent pending) containing 2% N-acetyl carnosine compounded with additional riboflavin monophosphate, L-taurine, cytseine ascorbate and glutathione was topically applied to each eye three times daily. The compounded product was kept in individual plastic dropper bottle containers at between 5 and 10 °C out of sunlight.

Ophthalmic examination

Animals were examined in the Queen's Veterinary School Hospital, Department of Veterinary Medicine, University of Cambridge and also at five first opinion 'general practice' clinics regularly visited by the primary author (DLW) in an ambulatory ophthalmic clinic. All dogs were examined at each time-point by the senior author (DLW). Informed consent was obtained from all owners prior to the commencement of the study. All dogs were examined by the primary author (DLW) before and during treatment with direct and indirect ophthalmoscopy and slit-lamp biomicroscopy 15 min, after pharmacologic pupil dilation with 0.5% tropicamide. Intraocular pressure was documented prior to pupil dilation using a Toxopen XL applanation tonometer (Mentor, Norwell, CT, USA). Photographic documentation of lens opacification was made at each examination using retroillumination provided by a Kowa RC-2 ophthalmic camera (Kowa, Tokyo, Japan) at +10 D and using the same illumination and flash setting at each examination.

Image analysis

Standard 35-mm slide photographs obtained at each examination were digitized and area of opacification and average pixel density evaluated by image analysis using image analysis software (Image J, Microscopy Info, Harvard, MA, USA). The color photographic image was transformed to a grayscale image and each pixel of the image automatically assigned one of 256 grayscale levels. Integration of the gray

Table 1. Signalment of dogs included in trial

Case	Breed	Sex	Age	Lens opacity OD	Lens opacity OS
1	Lurcher	me	0.4	ic	ns
2	Cross-breed	mn	13.5	ic	ns
3	West Highland White Terrier	mn	13	ic	ic
4	West Highland White Terrier	mn	8.5	ic	ic
5	Bichon Frise	mn	8.5	ic	ic
6	Lhasa Apso	fn	11	ic	ic
7	Lhasa Apso	fn	11	ic	mc
8	Labrador Retriever	me	0.8	mc	mc
9	Collie	me	15	imc	mc
10	Cross-breed	me	12	mc	an
11	Spitz	me	6.5	mc	mc
12	Jack Russell Terrier	mn	7.9	mc	mc
13	Jack Russell Terrier	fn	16	mc	mc
14	Yorkshire Terrier	fn	14	mc	mc
15	English Cocker Spaniel	fn	4	mc	mc
16	Dachshund	mn	13.4	mc	mc
17	Border Terrier	me	12	mc	mc
18	Standard Poodle	fn	14	mc	an
19	Bishon Frise	mn	9	mc	ns
20	Collie-cross	fn	14	mc	ns
21	Collie	fn	11.3	ns	ns
22	Cross-breed	mn	14.4	ns	ns
23	Cross-breed	mn	10	ns	ns
24	West Highland White Terrier	mn	12	ns	ns
25	English Springer Spaniel	mn	10	ns	ns
26	Collie	mn	10	mi	mi
27	Greyhound	mn	11	mi	mi
28	English Springer Spaniel	fn	14	mi	mi
29	Bichon Frise	fn	14	mi	mi
30	German Short-haired Pointer	mn	5	mi	mi

me, intact male; mn, neutered male; fe, intact female; fn, neutered female; mc, mature cataract; ic, immature cataract; mi, miscellaneous cataract (associated with uveitis or post-trauma); ns, nuclear sclerosis; an, anophthalmic (post enucleation or with phthisis bulbi).

scale level for each pixel across the image yielded a single number, combining the area of opacification and the density of grayscale for each pixel in a similar manner to that employed in previous studies of human cataract.[12]

Owner subjective assessment

Owners were questioned at each examination concerning their subjective estimation of their pet's visual acuity, and their comments on changes in the animal graded from +2 (substantial improvement) to –2 (substantial deterioration).

Statistics

As the correlation between the two eyes in each animal was low (correlation coefficient 0.24 see below) left and right eyes were considered as independent data points in the statistical analysis.[13] Differences between cataract density at time-points during treatment and cataract density before treatment were evaluated using the Wilcoxon signed rank test, with a significance reached at $P = 0.05$.

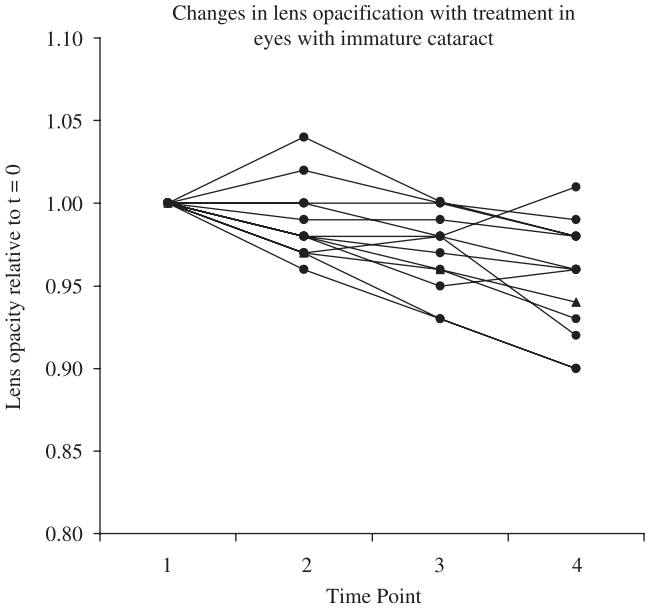


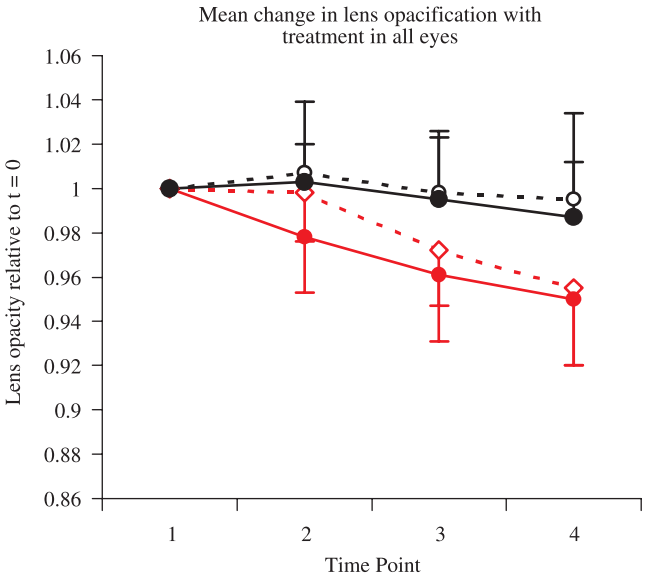
Figure 1. Change in lens opacification compared with pretreatment opacity in 13 eyes with immature cataract.

RESULTS

Signalment of the animals examined in this study is given in Table 1 together with details of lenticular opacity in each eye. Full data sets for each dog are available online at http://www.davidlwilliams.org.uk/research.php. Fifty-eight eyes of 30 dogs were evaluated, 22 with mature cataract, 13 with immature cataract, 9 with cataract associated with other intraocular disease such as uveitis, and 14 with nuclear sclerosis alone. One dog was unilaterally anophthalmic after previous enucleation and another had one phthytic eye after previous uveitis-induced glaucoma. Tonometry showed all eyes to have an intraocular pressure within 10 to 20 mmHg (data not shown). Correlation in degree of lens opacification between the two eyes was low with a correlation coefficient between LOI of left and right eyes of 0.24.

Image analysis showed a reduction in mean LOI in all cataract groups (mean decrease in opacity of $2.3 \pm 0.33\%$ for all cataracts), although this was only statistically significant in those eyes with immature cataract (mean decrease in opacity: $4.5 \pm 0.33\%$; $P = 0.002$) or nuclear sclerosis (mean decrease in opacity: $5.0 \pm 0.37\%$; $P = 0.003$). Decreases in opacity in mature cataract ($0.5 \pm 0.4\%$; $P = 0.43$) and in miscellaneous cataract associated with intraocular inflammation or post trauma ($1.3 \pm 0.4\%$; $P = 0.26$) were not statistically significant. Owner evaluation of visual capability, however, suggested improvement in vision in a number of cases; in 80% of cases owners noted an improvement in visual acuity, as judged by behavioral parameters and activities such as ball catching.

Reduction in area of lens opacification during treatment as determined by retroillumination photography is graphically



represented for each of 13 eyes with immature cataract in Fig. 1. Mean ± standard deviation of changes in lens opacity in eyes with mature and immature cataracts, eyes in dogs with cataract and associated intraocular inflammatory disease and eyes with nuclear sclerosis alone are shown in Fig. 2. Visual and numerical representation of changes in cataract opacity for case #1 is shown in Fig. 3.

In three dogs (cases 6, 11 and 26) there was some conjunctival hyperemia associated with early use of the drops but this was not associated with ocular irritation and resolved within 3 days. Yellowing of the periocular hair was noted in white dogs; this was of cosmetic but not clinical importance.

Figure 2. Mean change (± standard deviation) in lens opacification compared with pretreatment opacity in eyes with immature cataract (red filled symbol unbroken line), nuclear sclerosis (red open symbol broken line), mature cataract (black filled symbol unbroken line), and cataract associated with intraocular disease (black open symbol broken line).

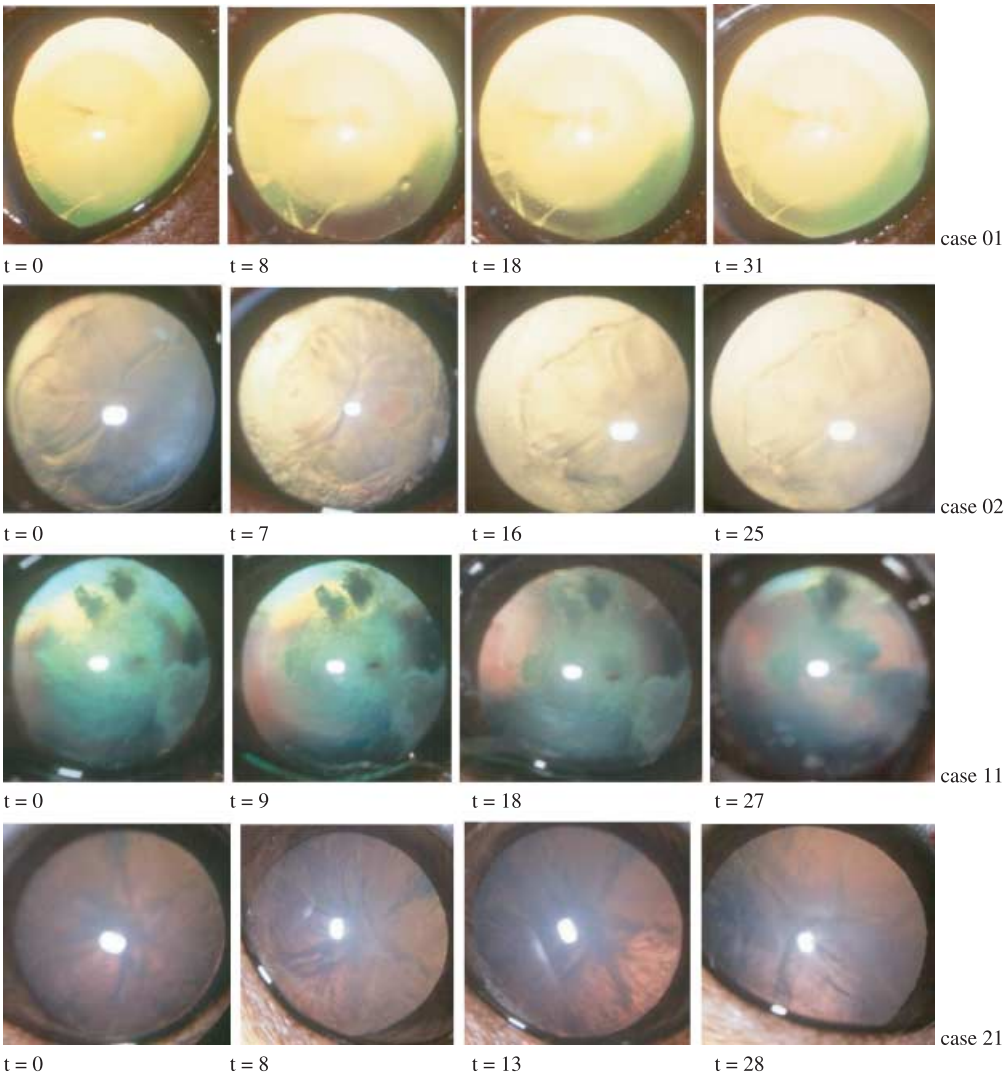


Figure 3. Cataract images before and at three time-points after varying days of treatment duration in four dogs.

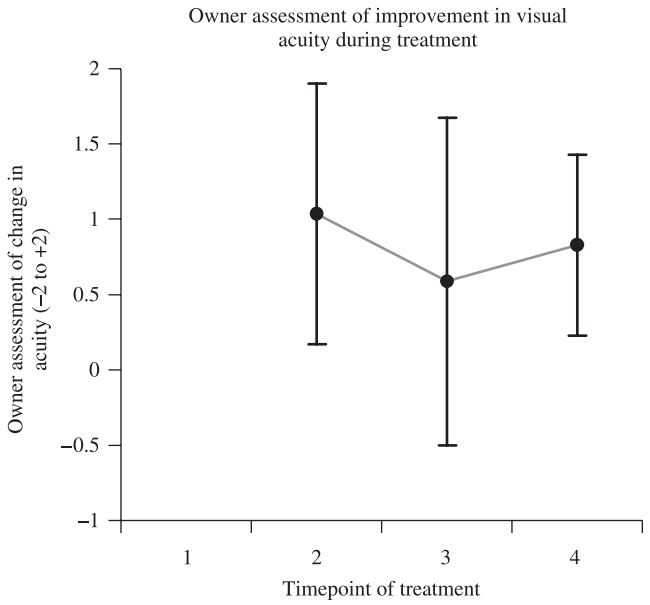


Figure 4. Client evaluation of visual capabilities during treatment period.

Of 90 observations made by the owners of dogs included in the trial, 78% noted an improvement in vision, 14% could not determine a difference and 8% suggested deterioration in vision. Of owners with sighted dogs with nuclear sclerosis or immature cataract, 75% of observations suggested an improved visual acuity, 18% saw no change and 7% noted a deterioration in vision. In bilaterally blind dogs with mature cataracts 84% of observations suggested an improvement in acuity, 8% detected no improvement and 8% noted deterioration in vision. Mean ± standard deviation of alterations in client subjective assessment is shown in Fig. 4.

DISCUSSION

This preliminary survey has several deficits which will be discussed further below, but has yielded some interesting and surprising results, given the general consensus that no medical treatment is likely to yield any improvement in lens opacity in cases of canine or human cataract.

The first deficit is clearly that this is an unmasked and nonplacebo-controlled trial. Yet, as noted above, any improvement in lens opacification in such a study would be worthy of note. Clearly the subjective owner evaluation is readily open to a placebo effect but the photographic documentation provided in this trial allows for a considerable degree of objective assessment of changes in the lens. Having said that, the inability to standardize the orientation of gaze in the dog for each photograph does mean that the images at each examination are not precisely comparable. Averaging the image analysis data for several photographs of each animal overcomes these problems to a considerable degree if not completely. Obtaining images with a digital slit lamp or Schleimpflug camera would have allowed a much more rigorous evaluation of changes in lens opacity, but financial constraints and the practical difficulties of moving such large pieces of equipment to the animals made this impossible in this preliminary study. Even with a more rigorous study, the problems associated with failure to standardize globe position – which is possible in humans by fixation on a target – will always render evaluation of cataract less precise than in humans.

With regard to owner evaluation of visual acuity, there was a wide variation in perception of improvement in visual function. The majority of owners reported an improvement in their dog's visual capacity, even in animals with mature cataracts where fundoscopy was not substantially facilitated by treatment. In retrospect, an owner questionnaire, filled in at each examination and detailing specific behavior traits such as following a hand, catching a thrown ball and negotiating furniture, would have given a more precise and rigorous assessment of the owner's perception of their pet's visual capability. Such a questionnaire is included in the placebo-controlled, blinded trail currently in progress.

It might be argued that the small improvements in cataract density are likely to be clinically insignificant. Owner observations, however, suggest that even small reductions in cataract density can make a significant difference to a dog's vision. Interestingly, the proportion of dogs showing an improvement in cataract density and/or vision are similar to those in a wider multicenter open-label study without placebo control, performed by the distributors of Ocluvet™ but as yet unpublished. In their study 87% of dogs and 83% of eyes showed an improvement in cataract density or owner-assessed visual acuity. Such studies are, we would contend, more subjective than the present investigation and show the importance of performing serial photography to be able to achieve a more objective assessment of changes in lens pathology with the agent.

It should be noted that the other compounds in the preparation, namely ascorbate, riboflavine and glutathione, have potentially anticataractogenic effects, as discussed in a review of oxidation, antioxidants and cataractogenesis by the senior author (DLW) in the current issue of *Veterinary Ophthalmology*.[1] While compounds such as glutathione clearly have an important role in intralenticular redox status, topical administration has not been shown to be beneficial in human cataract.[14] Similarly, dietary ascorbate has beneficial anticataractogenic effects in humans and experimental animals,[1] but no work shows it to be effective by topical application. These antioxidants are used in the current preparation to protect the N-acetyl carnosine from unwanted oxidation in solution and not as cataractoprotective agents in their own right.

Finally, can we postulate a mechanism of action for these drops in preventing and even resolving early lens opacities? As we have noted above, N-acetyl carnosine has been found to protect lens proteins from oxidation[7] and even to disaggregate aggregated crystallins.[5] We would suggest that this latter effect may be responsible for the partial resolution of

early lens opacification in these dogs. It should be noted that in more severe and longstanding cataracts this effect was not noted here, although anecdotal reports have suggested a more pronounced effect of the formulation on advanced lens opacities in the dog.

Even given all these reservations, use of the topical nutritional antioxidant formulation including N-acetyl carnosine does appear to have a positive effect in cataract opacification in dogs with nuclear sclerosis and with immature cataract, and a beneficial effect on owner-observed visual capacity in the majority of dogs with any type of lens opacification. Further evaluations in a placebo-controlled double-blinded trial, with digital photography facilitating standardization of image acquisition, are currently underway. A final comment must, however, be that dogs with cataracts should always be referred to a qualified veterinary ophthalmologist for evaluation and that, while it does demonstrate an effect of the product tested on cataract opacity, this study does not show that N-acetyl carnosine in this formulation is a cure for canine cataract in the same way in which phacoemulsification can restore vision to a dog previously blinded by lens opacification.

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