Reports

The effect of topical pilocarpine on intraocular pressure and pupil size in the normotensive and glaucomatous beagle.

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Topical 1, 2, and 4 percent pilocarpine were evaluated in the normotensive and glaucomatous beagles, comparing intraocular pressure (IOP), pupillary size, and time. In normotensive beagles pilocarpine produced maximal decreases of 25 percent (5.5 mm. Hg) at 1 percent concentration; 34 percent (7.26 mm. Hg) at 2 percent concentration; and 25 percent (6.9 mm. Hg) at 4 percent concentration. The maximum reductions in IOP after pilocarpine instillation in the glaucomatous beagle were 30 percent (9.1 mm. Hg) at 1 percent concentration; 44 percent (14.92 mm. Hg) at 2 percent concentration; and 31 percent (10.89 mm. Hg) at 4 percent concentration. The glaucomatous beagles responded with a greater reduction of IOP than did the normotensive beagles.

For the investigation of the effect of a number of commonly used clinical and experimental drugs for the treatment of glaucoma, a problem arises with the availability of a satisfactory animal model. The ocular normotensive and hypertensive albino rabbit has been studied most frequently. Buphthalmia in albino rabbits exhibits a decreased facility of aqueous outflow but is also associated with a semilethal gene, producing less healthy rabbits and a high rate of stillborns and offspring mortality.[1, 2] Lack of pigment in albino animals and man can significantly affect the duration of topical ophthalmic medications.[3-5] Water-loading procedures and artificially induced alterations of aqueous outflow channels have resulted in increased intraocular pressure (IOP).[6, 7] These procedures are either transient in nature or modify intraocular structures in such a way as to make them less than ideal in studying the effect of pressure-reducing agents in the glaucomatous eye.

An inherited open-angle glaucoma has been described in the beagle dog.[8] The disease is associated with decreased outflow of the aqueous humor as the disease progresses and is responsive to topical adrenergic agents.[9, 10] Additional studies are necessary for further evaluation of the response of normotensive and glaucomatous beagles to other pressure-altering agents. This strain of glaucomatous beagles may represent a useful pharmacologic model for studying the effect of antiglaucomatous drugs on aqueous formation as well as aqueous outflow.

The purpose of this study is to evaluate the effects of various concentrations of pilocarpine in normotensive and glaucomatous beagles with respect to IOP, pupil size, and time.

Materials and methods. Adult male and female beagles were used. Ten beagles with open-angle glaucoma and five normotensive beagles were used throughout the study. All animals were examined with a slit lamp and gonioscopic lens prior to testing. In all animals used, iridocorneal angles were open and normal in appearance. Beagles with a luxated or subluxated lens were not used.

Pilocarpine was used in concentrations of 1, 2, and 4 percent. The drugs were prepared by mixing the powder form with sterile saline (pH 7.4). The solution was then filtered through a 0.22 μ Millipore filter to assure sterility and refrigerated.

Baseline values for pupil size and IOP were obtained from 3 days of consecutive measurements prior to drug administration for each concentration. The drug was instilled topically (0.05 ml.) once daily in one eye of each dog immediately after the first tonometric reading. The untreated contralateral eye served as the control. Each concentration was studied for 5 days of daily instillations. The dogs were rested for 1 week between each study.

IOP and pupil size were measured every 15 min. for the first hour, then hourly for 6 hr. All tests were begun at the same time of each day (8:30 A.M.). IOP was measured by MacKay-Marg tonometry after application of 1 drop of 0.4 percent benoxinate hydrochloride (Dorsacaine; Dorsey Laboratories, Lincoln, Neb.). After 10 sec. the excess anesthetic was washed from the conjunctival sac with physiologic saline. The tonometer was calibrated daily. Pupil size was measured by calipers under uniform artificial illumination in the horizontal plane.

Degrees of significance were based on the variation of IOP and pupil size from baseline values to values obtained after various concentrations of topical pilocarpine. The results from the drug-treated eyes and untreated eyes were analyzed separately as to significance or nonsignificance, with the use of the paired t test. Results were tabulated and presented as the mean $\pm$ standard error of the mean. The tonometric recordings between the treated and untreated eyes at each pilocarpine concentration and time period

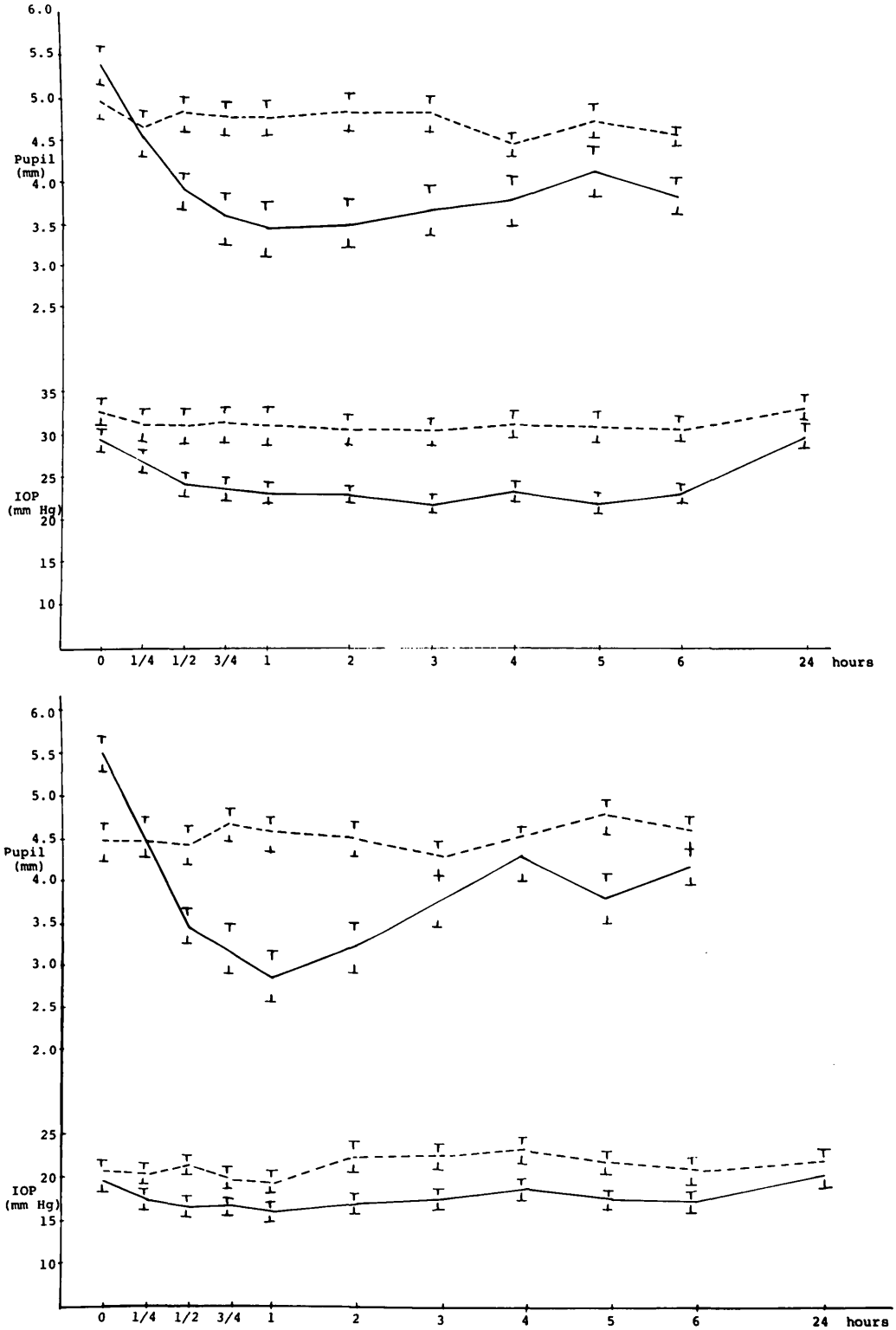

Fig. 1. Mean time course of pupillary and IOP responses of treated eyes to topical 1 percent pilocarpine in the glaucomatous (upper) and normotensive (lower) beagle. - - - -, Predrug values; ——, postdrug values.

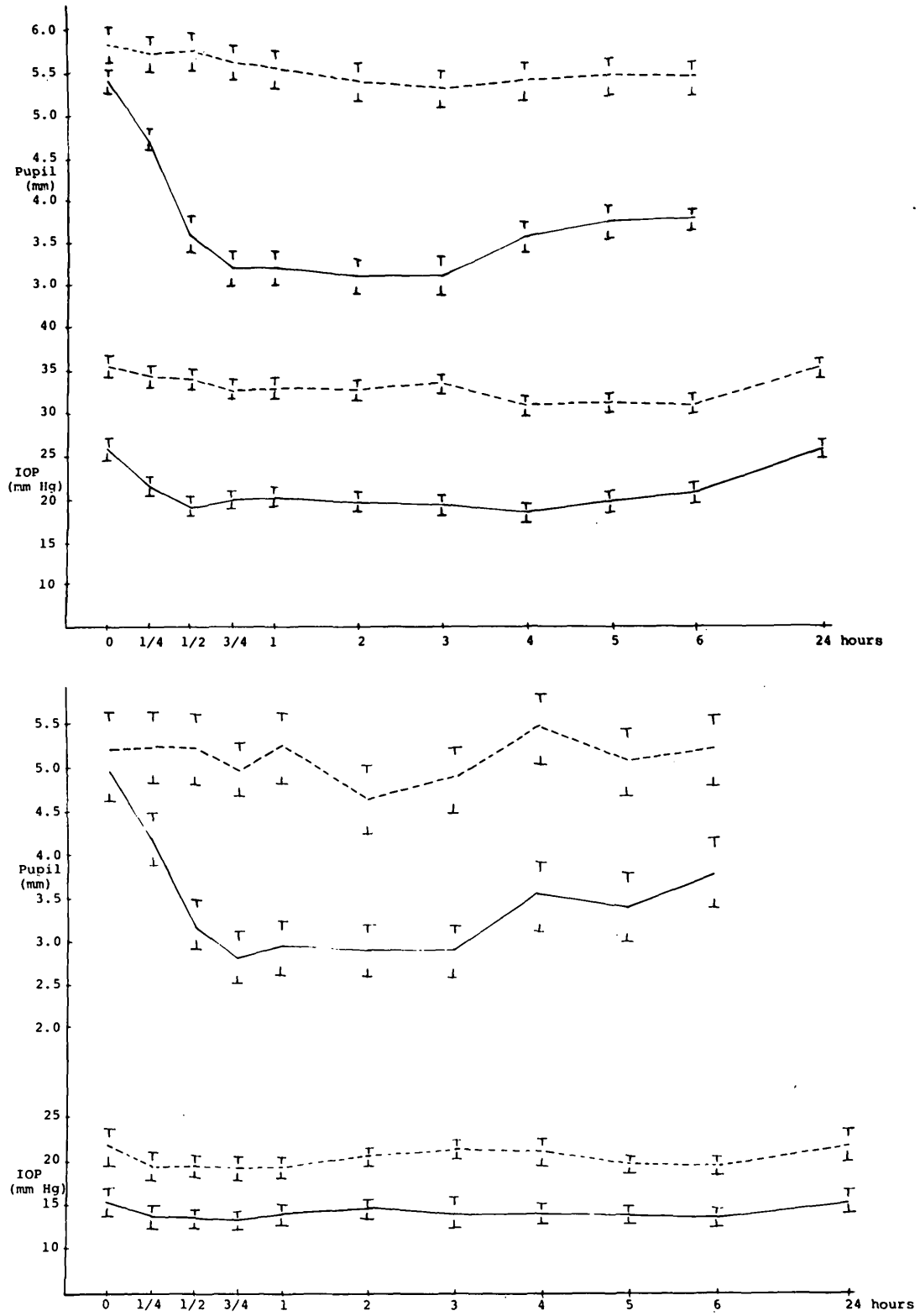

Fig. 2. Mean time course of pupillary and IOP responses of treated eyes to topical 2 percent pilocarpine in the glaucomatous (upper) and normotensive (lower) beagle. - - - -, Predrug values; ——, postdrug values.

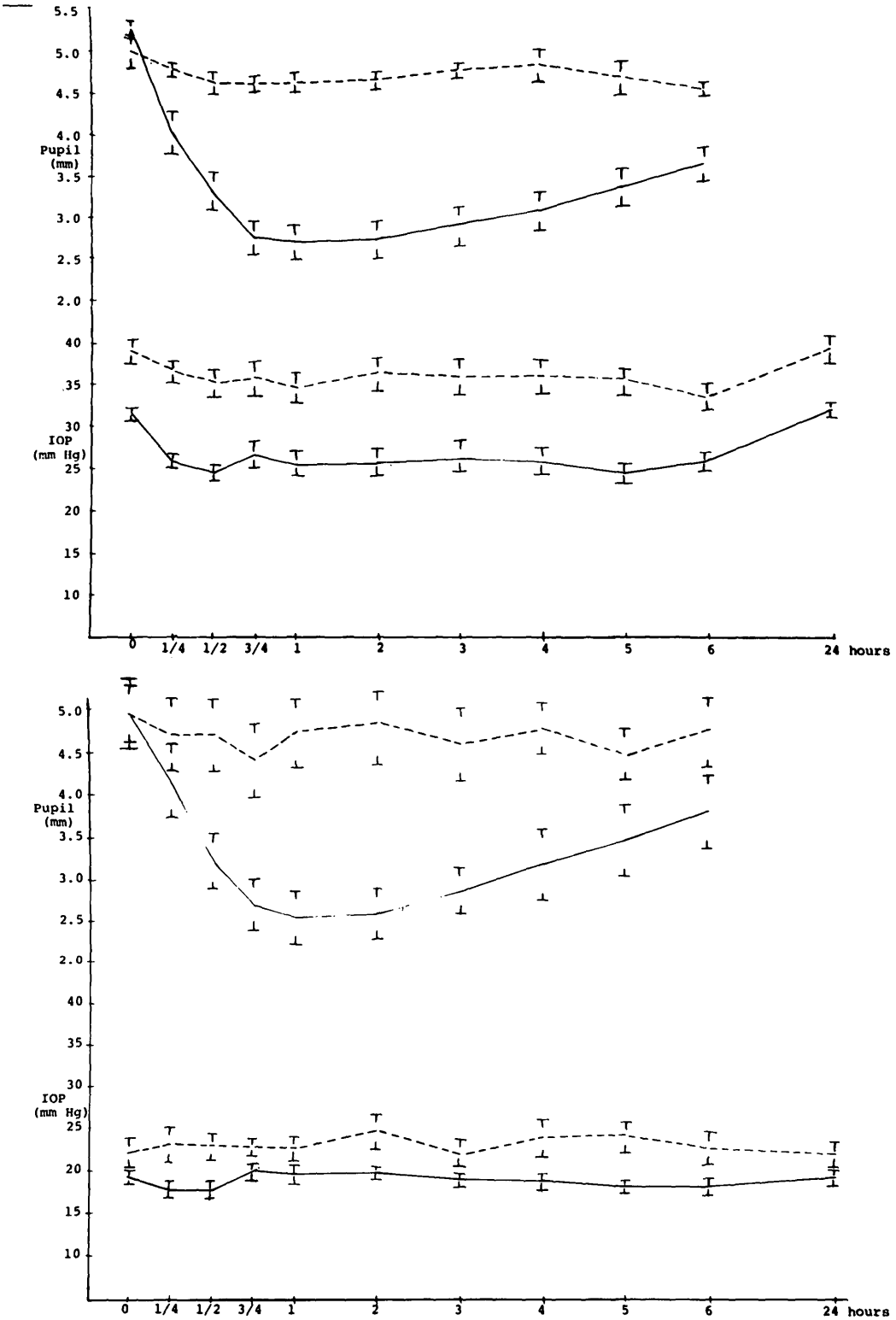

Fig. 3. Mean time course of pupillary and IOP responses of treated eyes to topical 4 percent pilocarpine in the glaucomatous (upper) and normotensive (lower) beagle. - - - -, Predrug values; ——, postdrug values.

were also compared satistically with the paired t test for the normal and glaucomatous dogs.

Results

1 percent pilocarpine. The mean time courses of intraocular pressure and pupillary responses to topical 1 percent pilocarpine in the normotensive and glaucomatous beagle are summarized (Fig. 1).

Decrease in IOP of treated eyes in glaucomatous dogs was significant ($p < 0.001$), with a maximal decrease of 9.1 mm. Hg at 5 hr. after drug instillation. Significant changes occurred 30 min. after instillation ($p < 0.001$) and were significant throughout the 6 hr. ($p < 0.001$). IOP 24 hr. later was still less than baseline values, but the difference was not significant ($p > 0.2$). The 1 percent pilocarpine did not cause a significant decrease in the contralateral untreated eye ($p > 0.97$). In normotensive dogs, 1 percent pilocarpine application resulted in a significant decrease in IOP ($p < 0.001$), with a maximal decrease of 5.5 mm. Hg at 2 hr. after drug instillation. Significant changes occurred 30 min. after drug instillation ($p < 0.001$) and remained significant through the 5 hr. reading ($p < 0.002$). A significant decrease in IOP did not occur in the untreated eye ($p > 0.4$). When the treated and untreated eyes were compared for both the normotensive and glaucomatous beagles given 1 percent pilocarpine, a significant difference in IOP was present ($p < 0.001$).

Miosis in the treated eyes of glaucomatous dogs was significant ($p < 0.005$), with maximal miosis of 1.4 mm. at 2 hr. Miosis in the untreated eyes was not significant ($p > 0.1$). Miosis in the treated eyes of normotensive dogs was maximal at 1 hr. (1.8 mm.), which was significant ($p < 0.001$). Pupil size was not significantly altered in the untreated eyes ($p > 0.1$).

2 percent pilocarpine. The mean time courses of IOP and pupillary responses in treated eyes to topical 2 percent pilocarpine in the normotensive and glaucomatous beagles are indicated in Fig. 2.

IOP in glaucomatous beagles decreased in a maximum of 14.9 mm. Hg from baseline values 30 min. after drug instillation. IOP was 9.4 mm. Hg below baseline values 24 hr. after drug instillation. Values obtained at all time periods were highly significant ($p < 0.001$). The over-all mean reduction in IOP was 12.4 mm. Hg. A significant decrease in IOP also occurred in the untreated glaucomatous eyes ($p < 0.001$), with a maximal decrease of 6.8 mm. Hg at 15 min. after drug instillation. At 24 hr. after drug administration (0 hr.), the IOP in the untreated eye was 5.8 mm. Hg below baseline values. Comparison of the treated and untreated eyes of glaucomatous beagles given 2 percent pilocarpine revealed a significant difference ($p < 0.001$) even though the untreated eye had shown a significant decrease in pressure. The IOP in normotensive beagles was significantly decreased ($p < 0.001$), with a maximal reduction of 7.3 mm. Hg 3 hr after drug administration. At 24 hr., the IOP was still significantly lowered by 6.3 mm. Hg from baseline values ($p < 0.004$). A significant reduction ($p < 0.001$) occurred in the untreated eye with 2 percent pilocarpine, with an average decrease of 5.0 mm. Hg. In the normal dogs given 2 percent pilocarpine, comparison of the treated and untreated eyes indicated a less significant difference ($p < 0.02$) concurrent with the significant lowering of IOP in the untreated eye.

Miosis in the treated eye of glaucomatous dogs was maximal 45 min. following drug administration, with 2.4 mm. of pupil constriction. After 3 hr. the pupil began to increase in size. A significant miosis did not occur in the untreated eye ($p > 0.05$).

In normal dogs, miosis of the treated eye was significant ($p < 0.005$), with a maximal response by 1 hr. of 2.3 mm. of pupil constriction. After 3 hr. pupil size began to increase. No significant change in pupil size occurred in the untreated eye of the normal dog ($p > 0.1$).

4 percent pilocarpine. The mean time courses of IOP and pupillary responses to topical 4 percent pilocarpine in the normotensive and glaucomatous beagle are summarized in Fig. 3.

A significant decrease in IOP ($p < 0.001$) occurred in the treated eyes of glaucomatous beagles, with a maximal decrease at 5 hr. of 10.9 mm. Hg. A significant reduction ($p < 0.001$) of IOP (7.3 mm. Hg) was present at 24 hr. after drug instillation. The untreated eyes were not altered significantly ($p > 0.15$). The normotensive dogs responded with a significant lowering of IOP ($p < 0.001$), which was maximum at 5 hr. with a 6.0 mm. Hg decrease. The 24 hr. decrease was not significant ($p > 0.1$). IOP was not significantly altered in the untreated eye ($p > 0.07$). When the treated and untreated eyes were analyzed for both normotensive and glaucomatous beagles given 4 percent pilocarpine, a significant difference in IOP was present ($p < 0.001$).

Miosis in treated glaucomatous eyes was significant at 1 hr. ($p < 0.001$), with maximum pupillary constriction of 1.9 mm. The untreated eye was not significantly affected ($p > 0.1$). In normal dogs, the treated eyes responded with maximum miosis at 2 hr. with 2.3 mm. of pupillary constriction which was significant ($p < 0.001$). The untreated eyes did not respond significantly ($p > 0.05$).

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In the comparing of glaucomatous beagles to normotensive beagles with regards to reduction of IOP following 1, 2, and 4 percent pilocarpine, a significant difference was found at all concentra-

tions ($p < 0.02$). Pilocarpine, 2 percent, in normotensive and glaucomatous beagles was found to reduce IOP to a greater extent than 4 percent pilocarpine ($p < 0.10$) or 1 percent pilocarpine ($p < 0.05$), whereas 1 percent pilocarpine and 4 percent pilocarpine did not differ significantly ($p > 0.02$).

Discussion. All concentrations (1, 2, and 4 percent) of pilocarpine reduced IOP significantly in both glaucomatous and normotensive dogs. The time course in decreasing IOP was variable but was maximum between 2 and 5 hr. For each concentration, the glaucomatous beagle had a greater order of reduction in IOP than did the normotensive dogs. The greatest ocular hypotonic response occurred with 2 percent pilocarpine. Not only did the 2 percent pilocarpine continue to significantly reduce IOP for the full 24 hr. period in both groups of dogs, but also 2 percent pilocarpine had significant pressure-lowering effects in the contralateral untreated eyes in glaucomatous and normotensive dogs. In the untreated eyes with 2 percent pilocarpine the IOP remained low over the 24 hr. period with little variation suggesting systemic effect.

Maximal pupillary constriction preceded maximal reductions of IOP by several hours in most cases. Miosis was greatest between 45 min. and 2 hr., then slowly returned to pretreatment levels.

The response of decreasing IOP and miosis in the canine appears similar to man with regard to amount of response and time course. The mode of action of pilocarpine in the canine has not been reported and will be evaluated in future studies. The maximal response with 2 percent pilocarpine was not anticipated, since the maximal dose response in other species is usually much higher. This may be associated to the anatomical differences present in the canine iridocorneal angle, specifically the ciliary musculature and absence of a scleral spur, or to an increased sensitivity to cholinergic agents. Also, irritation at higher doses was considerable and may interfere with the action of pilocarpine. Other concentrations of pilocarpine will be evaluated to establish a dose-response for the dog. This study indicates the glaucomatous beagle is a useful model for the study of the effects of cholinergic agents.

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Key words: open-angle glaucoma, animal model, pilocarpine, cholinergic, dog, intraocular pressure.

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Lack of levamisole effect on experimental herpes keratitis.

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Levamisole, which is an anthelminthic, can restore depressed cell-mediated immunity (CMI) under some circumstances. In a controlled trial of experimental herpetic keratitis in rabbits, levamisole was found to have no significant effect on acute herpetic keratitis or its recurrence rate. This is consistent with previous findings that other nonspecific CMI stimulation had no effect on recurrences of experimental herpes keratitis. Because of the known tendency of levamisole to produce agranulocytosis, we believe it should not be used in man unless proven effective in a carefully controlled double-blind study.

The role of cell-mediated immunity (CMI) in herpetic keratitis, both acute and recurrent, is uncertain. It seems unlikely that CMI plays a