Ocular Evaluation of Methylcellulose Vehicle in Albino Rabbits

SUKHBIR S. CHRAI* and JOSEPH R. ROBINSON ˣ

Abstract □ Viscous solution drainage from the eyes of unanesthetized albino rabbits was determined and related to drug bioavailability. When using methylcellulose as the viscosity-inducing polymer, it was shown that the rate of solution drainage is related to viscosity and decreases with increasing viscosity. Over a range of 1–15 cps viscosity of the instilled solution, there is a threefold change in the drainage rate constant and a further threefold change over the viscosity range of 15–100 cps. The decline in precorneal drug concentration was determined to be first order in concentration, and the rate of decline was proportional to the viscosity of the instilled solution. When using pilocarpine nitrate as the test drug in both miosis and aqueous humor drug concentration studies, it was shown that, over a range of 1–15 cps solution viscosity, there is a linear relationship between the first-order drainage rate constant and both the miotic activity and aqueous humor drug levels attained. Comparison of the present study in rabbits to reported behavior in humans suggests that the vehicle viscosity influence is qualitatively similar but quantitatively different in both species, with the quantitative difference being due, in part, to the difference in rate of blinking and precorneal solution movement in rabbits as compared to humans.

Keyphrases □ Ophthalmic vehicle viscosity—effect on ocular drug bioavailability, pilocarpine nitrate in methylcellulose, rabbits, relationship to effect in humans □ Methylcellulose—effect as vehicle on ocular drug bioavailability of pilocarpine nitrate, rabbits, relationship to effect in humans □ Viscosity effect of ophthalmic vehicle (methylcellulose)—bioavailability of pilocarpine nitrate, rabbits

It has long been assumed that a substantial increase in ocular drug bioavailability can be achieved by placement of the drug in a high viscosity vehicle prior to instillation into the eye. Presumably, the highly viscous solution would prolong contact of the drug with eye tissues, resulting in a greater drug absorption into the appropriate eye area. Over the years, this belief has been strengthened by qualitative and semiquantitative studies on vehicle viscosity effects in both humans and experimental animals. A recent report (1) challenged this belief and concluded that increasing vehicle viscosity does not prolong ocular contact time nor markedly improve ocular drug bioavailability in humans. Specifically, these researchers showed that aqueous humor levels of fluorescein increased only slightly with relatively large increases in solution viscosity. This small increase was attributed to an elevated initial concentration of fluorescein in the tear film resulting from less initial loss of drug solution from the precorneal area following topical instillation.

This earlier work (1), while shedding considerable light on the influence of vehicle viscosity in humans, has not provided a complete quantitative picture of the influence of solution viscosity on ocular drug bioavailability, nor has it explained contradictory results in animals. The present study attempts to provide a quantitative picture of ophthalmic vehicle viscosity effects in albino rabbits and its relationship to behavior in humans.

EXPERIMENTAL

Materials—USP grade pilocarpine nitrate[1] was used as received, but the tritiated pilocarpine nitrate[2] was subjected to further purification (2) by repeated vacuum evaporation. Usually, three such evaporations were required to remove tritiated solvent, which was present as a result of tritium exchange. In each evaporation the solution was reduced to a very small volume and then brought back to volume by addition of water. The purification end-point was reached when the measured radioactivity reached a constant value and purity was established by TLC. The final isotopic activity in the drug solution was approximately 50,000 counts min^{-1} μl^{-1}. All other chemicals were either USP or reagent grade.

Adult, male, albino rabbits[3], weighing 1.8–2.4 kg, were used without any special pretreatment diets.

Solution Preparation—The preparation and composition of technetium sulfur colloid solutions, pilocarpine nitrate solutions, and anesthetic solutions were described earlier (2–5). Methylcellulose–technetium solutions were prepared by mixing equal volumes of an appropriate concentration of methylcellulose solution and technetium colloid solution. The methylcellulose solutions were prepared by addition of hot water to methylcellulose 100, followed by cooling of the resultant mixture until solution was effected. The final volume of the solution was then adjusted and the flask was equilibrated to the appropriate temperature prior to use. Methylcellulose–pilocarpine nitrate solutions were prepared in the same manner as the methylcellulose–technetium solutions, with the modification that Sørensen's pH 7.38 phosphate buffer solution was used instead of water in preparation of the methylcellulose solution. All solutions were prepared fresh.

Procedures—*Viscosity Measurements*—Solution viscosity was determined with a viscometer[4] at 25 ± 0.1°.

Drainage Studies—A complete description of the nonsampling isotopic technique for measuring solution drainage was reported previously (3). Unanesthetized, preconditioned rabbits were used for all experiments.

Miosis–Time Studies—Unanesthetized, preconditioned rabbits were used as described previously (6). No animal was used more than once, and at least five separate determinations were made for each experiment. Twenty-five microliters of 1×10^{-2} *M* pilocarpine nitrate solution, containing various concentrations of methylcellulose, was instilled into one eye of an experimental animal; the other eye served as a control. Pupillary diameter measurements commenced immediately postinstillation of drug and continued until the pupil size returned to its normal value (4).

Aqueous Humor Drug Studies—Experimental details of aqueous humor–drug concentration studies were reported previously (2). Unanesthetized rabbits were used in all experiments.

RESULTS

Effect of Vehicle Viscosity on Drainage Rate—Several methylcellulose–technetium solutions of varying viscosity were prepared and instilled into the eyes of unanesthetized rabbits, fol-

[1] Merck and Co., Rahway, N.J.
[2] New England Nuclear, Boston, Mass.
[3] Klubertanz, Edgerton, Wis.
[4] Brookfield Engineering Labs., Stoughton, MA 02072

Table I—Fractional Amount of Technetium Tracer Remaining in the Precorneal Pocket as a Function of Time for 25 μl of Solutions of Varying Viscosity

Seconds	Fractional Amount of Drug Remaining					
	100[a] (6)[b]	40 (4)	12.5 (6)	4.2 (6)	1.8 (8)	1.3 (7)
50	0.97 (0.03)[c]	0.86 (0.05)	0.78 (0.05)	0.72 (0.04)	0.59 (0.06)	0.51 (0.05)
100	0.91 (0.04)	0.74 (0.05)	0.71 (0.05)	0.55 (0.04)	0.47 (0.05)	0.42 (0.05)
150	0.87 (0.02)	0.64 (0.04)	0.63 (0.03)	0.48 (0.04)	0.42 (0.06)	0.36 (0.06)
200	0.85 (0.04)	0.57 (0.04)	0.55 (0.03)	0.43 (0.02)	0.36 (0.04)	0.32 (0.06)
250	0.81 (0.06)	0.54 (0.04)	0.48 (0.03)	0.39 (0.04)	0.35 (0.05)	0.29 (0.06)
300	0.79 (0.08)	0.52 (0.05)	0.45 (0.04)	0.37 (0.03)	0.34 (0.05)	0.28 (0.04)
350	0.76 (0.12)	0.51 (0.06)	0.43 (0.05)	0.36 (0.04)	0.34 (0.03)	0.26 (0.04)
400	0.74 (0.15)	0.49 (0.06)	0.41 (0.03)	0.35 (0.05)	0.32 (0.03)	0.25 (0.04)
450	0.73 (0.14)	0.48 (0.06)	0.40 (0.06)	0.35 (0.03)	0.30 (0.02)	0.24 (0.03)
500	0.71 (0.13)	0.47 (0.06)	0.40 (0.02)	0.34 (0.03)	0.30 (0.02)	0.23 (0.03)
550	0.72 (0.14)	0.46 (0.06)	0.40 (0.03)	0.34 (0.04)	0.30 (0.03)	0.23 (0.03)
600	0.71 (0.14)	0.45 (0.05)	0.37 (0.03)	0.34 (0.04)	0.30 (0.03)	0.23 (0.03)

[a] Viscosity of the instilled solution in centipoises. [b] Number of determinations. [c] Standard deviations are shown in parentheses.

lowed by measurement of technetium radioactivity in the tear film at different time intervals. In all cases, the volume of solution instilled was kept constant at 25 μl.

The fractional amounts of technetium tracer remaining in the tear film as a function of time are shown in Table I. Except for the 100-, 150-, and 200-sec points of the 40- and 12.5-cps runs, there is a statistically significant difference for all other values with 90% confidence limits and, in most cases, with 95% confidence limits. The data in Table I are presented graphically as smooth curves in Fig. 1.

Cessation in the decline of radioactivity occurred at about 600–700 sec for all viscosities (Table I and Fig. 1). In the case of the 100-cps viscosity solution, a large standard deviation is associated with the values obtained after 300 sec. This is due to the fact that in a few runs there was no decline in radioactivity after 250 sec and in others the decline was continuous, thus widening the gap between experimental values.

A possible explanation for the plateau region is based on the rate of blinking of the experimental animal. Since the rabbit blinks infrequently, the decrease or cessation in the decline of radioactivity is interpreted as due to formation of a relatively stable film. The fact that the curves for fraction of radioactivity remaining against time for 1.3- and 1.0-cps solutions are superimposable for the first 500 sec and then bifurcate—1.3 cps being almost a plateau and 1.0 cps continuing to decline—lends support to the postulate of a relatively stable film. When the animal blinked, this film appeared to lose its integrity and there was a further decrease in radioactivity. Thus, the plateau region in Fig. 1, from 600 to 1000 sec, is probably due to a lack of blinking by the animal together with perhaps temporary blockage of the puncta and/or formation of a film.

The thickness of the layer as physically observed at about 600 sec appeared to be proportional to the viscosity of the instilled solution. Since the film did not appear to expand over 5–15 min, it may be assumed that tear production is inhibited during this period. Mueller and Deardorff (7) felt that polymer solutions might decrease reflex lacrimation, so normal tear turnover might be inhibited. This possibility of tear inhibition appears unlikely in view of the results of Adler *et al.* (1) as well as the calculation of precorneal drug concentrations at various times postinstillation, as will be discussed. Thus, the plateau in the fractional amount remaining against time plot is ascribed to a lack of blinking by the rabbit. Destruction of the film by blinking or forcing the pool solution out by blinking results in a further decline of radioactivity. The rate of decline of radioactivity after rupture of the film at long time periods postinstillation is not close to the rate of turnover of tears. It was not possible to carry out the experiments long enough to determine when the rate of decline in isotopic activity could be ascribed to normal tear turnover.

The initial decline in radioactivity (Fig. 1) appears to be proportional to the viscosity of the instilled solution. Nonproductive drug loss by adsorption or absorption into eye tissues is expected to be minimal, since both methylcellulose and technetium sulfur colloid are not absorbed from the conjunctival sac. Adler *et al.* (1), in their vehicle studies in humans, reported that polymer solutions such as methylcellulose did not increase corneal contact time. This is clearly not the case in rabbits, as judged by the results shown in Fig. 1 as well as by the work of others (8–11). This inconsistency can perhaps be ascribed to the difference in blinking rate of humans *versus* rabbits as well as to the difference in tear turnover rate between these species (3).

First-order drainage rate constants were determined from the slope of the line of plots of logarithm of the volume remaining against time for the first few minutes postinstillation as shown in Eq. 1 (4):

$$V_r = V_0 e^{-k_{dv} t} \qquad \text{(Eq. 1)}$$

where k_{dv} is the drainage rate constant for viscous instilled solutions.

Table II shows the drainage rate constants for various viscosity solutions and clearly indicates that the drainage rate is significantly retarded as the viscosity of the solution is increased. For a 100-fold change in viscosity, there is an overall 10-fold decrease in the drainage rate constant. From the standard deviations pre-

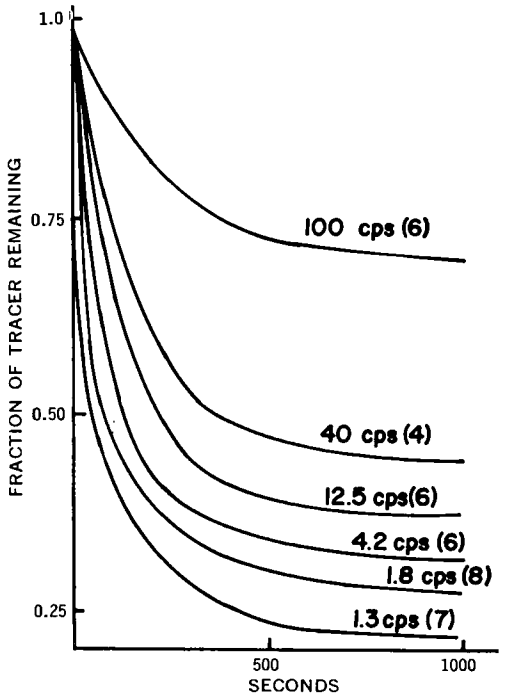

Figure 1—*Fractional amount of drug remaining as a function of time for 25 μl of solutions of varying viscosity. The number in parentheses indicates the number of runs conducted at each instilled volume, and the lines represent the mean values for these runs. Each line was generated from a minimum of 20 points, many of which are shown in Table I.*

Table II—First-Order Rate Constants for Drainage of Instilled Solution of Varying Viscosities[a]

Viscosity of Instilled Solution, cps	Drainage Rate Constant, k_{dv}, $sec^{-1} \times 10^3$
1.0	9.1
1.3	9.1
1.8	7.7
4.2	6.0
12.5	3.0
40.0	2.4
100.0	0.88

[a] Volume of the solution instilled was 25 μl.

sented in Table I, the calculated rate constants are probably accurate to ±5–10%.

To determine if there is a direct relationship between the drainage rate constant and vehicle viscosity, the calculated first-order drainage rate constants were plotted against the viscosity of the instilled solution (Fig. 2). Although a thorough investigation was not performed, it appears that the distribution of the experimental points is biphasic. The first phase is a relatively rapid decrease in the drainage rate constant with a small increase in viscosity, and the second phase is a small decrease in the drainage rate constant with a large increase in viscosity. The two lines appear to intersect each other at about 15–20 cps, which may be said to be the optimum viscosity of an instilled solution of methylcellulose for rabbits. A slight increase in viscosity above this optimum viscosity does not lead to a proportional improvement in drainage property.

The range of viscosity employed in this study was restricted at the lower end to a simple aqueous solution and at the upper end by the maximum viscosity the eye can tolerate without rapid blockage of the drainage duct. With the highest viscosity used, 100 cps, great care was needed in instilling the drug solution onto the cornea. In cases where the drug solution was inadvertently instilled into the cul-de-sac instead of onto the cornea, the animal would close its eye very tightly and keep the viscous solution from

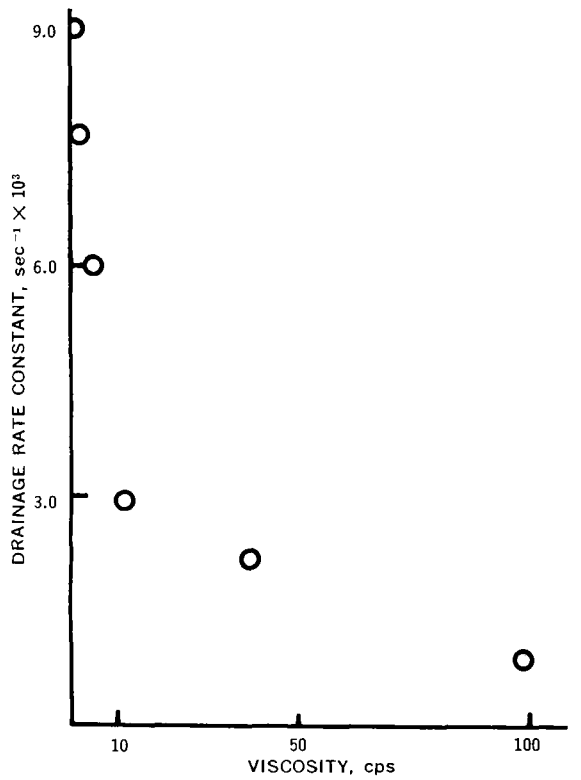

Figure 2—*Drainage rate constants,* k_{dv}, *as a function of the viscosity of the instilled methylcellulose–technetium solution. The volume instilled in all cases was 25 μl.*

Table III—Average Values for the Change in Pupillary Diameter at Random Times for Different Viscosity Solutions

Minutes	Average Change in Pupillary Diameter, mm		
	1[a] (6)[b]	1.8 (5)	4.2 (5)
20	1.87 (0.13)[c]	2.20 (0.16)	2.28 (0.15)
30	2.12 (0.11)	2.37 (0.06)	2.58 (0.14)
40	2.05 (0.12)	2.20 (0.16)	2.50 (0.17)
90	1.10 (0.20)	1.35 (0.21)	1.60 (0.20)

[a] Viscosity of the solution instilled in centipoises. [b] Number of experimental runs. [c] Standard deviations are shown in parentheses.

mixing and draining. Such holding of the solution in the pocket was observed in a few runs and these runs were discarded. There was no decline in radioactivity in such cases for the first few minutes postinstillation.

Miosis–Time Study to Demonstrate Effect of Vehicle Viscosity on Drug Activity—To determine the influence of viscosity of the instilled solution on drug activity, several methylcellulose solutions containing 1×10^{-2} *M* pilocarpine nitrate were prepared and instilled into the eyes of unanesthetized rabbits. The volume of solution instilled was maintained at 25 μl. If there is a change in the drainage rate for these solutions, it would be expected that the extent of drug absorption and hence the peak height, area under the miosis–time curves, and duration of the miotic effect would be different and proportional to solution viscosity.

The change in pupillary diameter due to the miotic effect of pilocarpine nitrate was determined at approximately 25–35 time points, representing the full range of drug activity (4) for several viscosity solutions. Table III illustrates the magnitude of the miotic effect and experimental reproducibility for a few random time points. There is a statistically significant difference with a 90% confidence limit between the results of the three viscosities used at all time points; for some time points, such as the values at the peak height, there is a difference with a 95% confidence limit.

Table IV provides the area under the miosis–time curves, duration of the miotic effect, and first-order rate constants for elimination and absorption. The first-order rate constants for elimination were determined from the terminal slope of the lines of logarithm change in pupillary diameter against time plots. The first-order rate constants for absorption were determined by graphical analysis using the feathering technique. From the standard deviations presented in Table III, the experimental or calculated values for the different parameters presented in Table IV are probably accurate to ±5–10%.

The areas under the miosis–time curves, the peak heights, and the duration of the miotic effect of pilocarpine nitrate are presumably proportional to the concentrations of drug reaching the target area which, in turn, is proportional to the concentrations of drug present in the tear fluid. Since all of these solutions initially had the same concentration, the increased miotic effect observed as the viscosity of the instilled solution is increased is due to the fact that the fraction of the instilled solution remaining in the tear film for different viscosity solutions is different. The time to reach a maximum in the miosis–time profile remained constant for all solutions tested, thus tending to rule out a vehicle-controlled availability. Figure 3 shows plots of the first-order drainage rate constants, k_{dv}, against the peak height of miotic effect, the area under the miosis–time curves, and the duration of miotic effect. Excellent linearity is observed in all three cases, supporting the idea that increased ocular drug bioavailability is due to a decreased rate of drainage.

Determination of Effect of Viscosity of Instilled Solution on Aqueous Humor Drug Concentration—Several solutions of 1×10^{-2} *M* pilocarpine nitrate of varying viscosity were prepared and instilled into the eyes of unanesthetized rabbits to determine the influence of drug solution viscosity on the aqueous humor drug concentration attained. Aqueous humor samples were withdrawn at 20 and 30 min postinstillation of drug, and the pilocarpine nitrate concentrations were determined. These particular time periods were chosen because they represent the time periods for maximum drug concentration in aqueous humor (2). The average concentrations of pilocarpine nitrate reaching the anterior

Table IV—Effect of Viscosity of Instilled Solution on Miotic Effect of Pilocarpine Nitrate in Rabbits

Viscosity of Instilled Solution, cps	Peak Height[a], mm	Area under Curve[b], cm^2	Duration of Miotic Effect[c], min	First-Order Rate Constant for Absorption, min^{-1}	First-Order Rate Constant for Elimination, min^{-1}
1	2.12	92	172	0.077	0.012
1.8	2.37	109	202	0.077	0.016
4.2	2.58	132	235	0.076	0.011

[a] The peak height is the average of maximum change in pupillary diameter and is taken as the change in pupillary diameter at 30 min. [b] Units are millimeters per minute. On the vertical axis, 1 cm equals 0.20 mm; on the horizontal axis, 1 cm equals 10 min. [c] Time for pupil to return back to normal, *i.e.*, the time when change in pupillary diameter is zero.

chamber, along with the number of runs and standard deviations, are presented in Table V. Since some of the standard deviations shown in Table V appear high, a rejection test was performed on the individual values. At the 90% level, no value could be rejected. In addition, the values at both 20 and 30 min were significantly different with a 90% confidence limit, except for the 1.8- and 4.2-cps values in the 30-min runs.

From Table V, it is clear that higher aqueous humor drug levels are achieved with increasing solution viscosity, indicating that the extent of drainage loss is not the same for each solution. There is approximately a twofold increase in aqueous humor drug levels for a 100-fold increase in solution viscosity.

The increase in aqueous humor drug concentrations might be ascribed to a slow release of drug from the vehicle at higher vehicle viscosities, *i.e.*, vehicle control. If this rate of release of drug from the vehicle is the controlling factor, it would be expected that the peak concentration of the aqueous humor drug concentration would shift toward the right and that the degree of shift would be proportional to the viscosity of the instilled solution. Careful examination of the results indicates that, at 30 min postinstillation, there is a twofold increase in aqueous humor drug concentration with a 100-fold increase in viscosity, the same as was observed for the 20-min study. Furthermore, the ratio of aqueous humor drug concentration at 20 min postinstillation to aqueous humor drug concentration at 30 min postinstillation for different viscosity solutions is similar within experimental error. This finding, when combined with the results from the miosis study, appears to rule out the possibility that the rate of release from the vehicle is the controlling factor.

These aqueous humor drug concentration data agree with earlier speculation concerning the biphasic nature of drainage as a function of viscosity (Fig. 2). There was a 1.6-fold increase in aqueous humor drug concentration at 20 and 30 min postinstillation for a 12.5-fold increase in the viscosity of the instilled solution below the "optimum" viscosity, and there was only a 1.2-fold increase in aqueous humor drug concentration for an 87.5-fold increase in the viscosity of the instilled solution above the optimum viscosity.

The increase in aqueous humor drug concentration is presumably due to higher drug concentrations in the conjunctival sac as a result of slow drainage. Figure 4 shows the relationship between drainage rate constants and aqueous humor drug concentrations for drug solutions of varying viscosities. A linear relationship is observed for both the 20- and 30-min postinstillation aqueous humor drug concentrations and the slope of both lines appears similar. The parallel linearity supports the idea that the aqueous humor drug concentration is a function of higher concentration of drug in the conjunctival sac and is *not* controlled by the rate of release of drug from the vehicle.

The area under the response–time profile for any biological response usually reflects the tissue concentration of the drug. By the same token, the area under the miosis–time curve due to pilocarpine nitrate solution should be a reflection of the aqueous humor concentration of the drug. Figure 5 shows that a linear re-

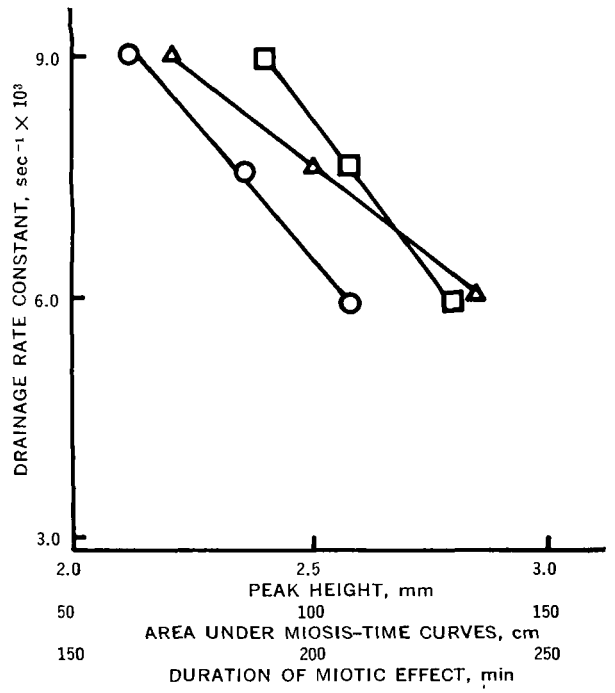

Figure 3—*Effect of the drainage rate constants on the peak height* (○), *area under the miosis–time curves* (□), *and duration of miotic effect* (△) *for pilocarpine nitrate.*

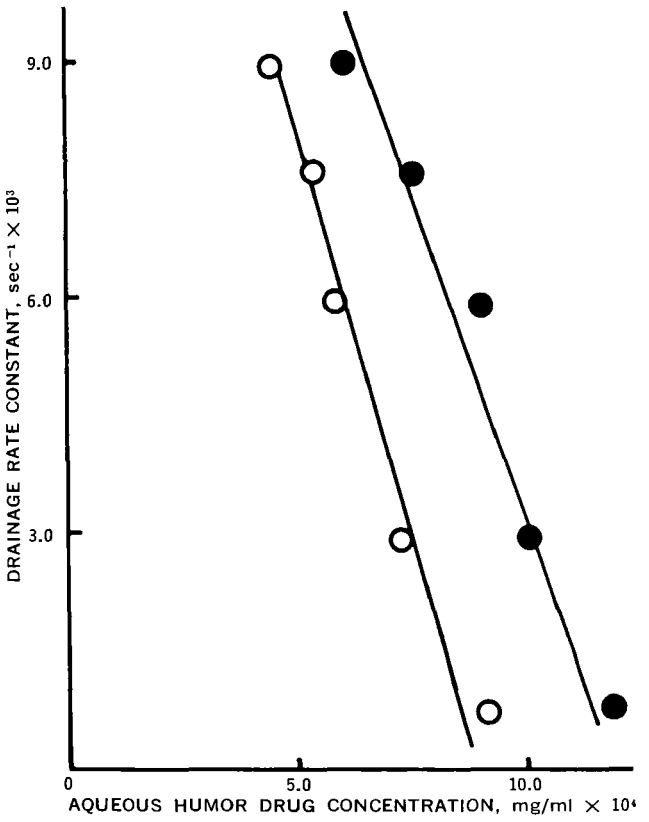

Figure 4—*Relationship between the drainage rate constants and the aqueous humor drug concentrations of pilocarpine nitrate attained at 30 min* (○) *and 20 min* (●) *postinstillation.*

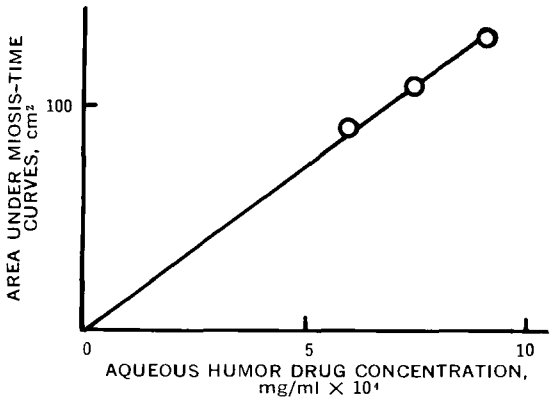

Figure 5—*Relationship between area under the miosis–time curves and aqueous humor drug concentration at 20 min postinstillation of pilocarpine nitrate.*

lationship exists between the area under the miosis-time curve and the aqueous humor drug concentration at 20 min postinstillation of 1×10^{-2} *M* pilocarpine nitrate solution of different viscosities. Naturally, there is also a linear relationship between aqueous humor drug concentration and duration of miotic effect as well as the maximum change in pupillary diameter, *i.e.*, peak height.

DISCUSSION

The quantitative studies on ocular vehicle effects in rabbits reported are in agreement with previously reported observations (8–11). For example, Blaug and Canada (8) observed in rabbits that an increase in contact time of methylcellulose ophthalmic vehicle was approximately proportional to the viscosity of the solution in the lower viscosity region, *i.e.*, 5–25 cps, and levels off at 55 cps or above.

The results presented in this report on the influence of vehicle viscosity on ocular drug bioavailability in rabbits are in some respects similar and in some respects different from those found in humans. Clearly, the fact that there is only a relatively small increase in aqueous humor drug level for a large increase in solution viscosity is common to both humans and rabbits. Higher levels are achieved in rabbits than humans, presumably due to the slower drainage rate of instilled solutions in rabbits as compared to humans (2).

Adler *et al.* (1) found no prolongation of the drug solution in the tear film with viscous drops, and they attributed the increase in aqueous humor drug level to a higher zero time concentration of the drug in the conjunctival sac due to less loss of unmixed drug solution on instillation. To the contrary, studies in rabbits show a substantial prolongation of contact time with viscous drops. Moreover, the increased aqueous humor drug levels in rabbits cannot be completely attributed to higher zero time concentration of the drug in the tear film. If drug concentration at zero time is the driving force, then the zero time concentration in the present study should be double for the 100-cps solution compared to the 1-cps solution. Earlier studies (3) with nonviscous solutions showed that the loss of unmixed drug solution for 1-cps solutions is about 3.5 μl when the instilled volume is 25 μl. When assuming that there is no loss of unmixed solution for a 100-cps solution and a loss of 3.5 μl for a 1-cps solution, the zero time concentration for instillation of 25 μl of 1% drug solution will give zero time concentrations of 0.77 and 0.66%, respectively, *i.e.*, $C_{100} = (25 \times 1)/32.5 = 0.77$ and $C_1 = (21.5 \times 1)/32.5 = 0.66$. These calculations are based upon a lacrimal fluid volume of 7.5 μl. The only way the zero time concentration for a 1-cps solution can be half of the zero time concentration for the 100-cps solution is if the loss of unmixed 1-cps solution is about 13 μl or the lacrimal fluid volume is about 40 μl, both of which are intuitively unacceptable values.

Table V—Aqueous Humor Drug Concentration after Instillation of 25 μl of 1×10^{-2} *M* Pilocarpine Nitrate Solutions of Varying Viscosities

Viscosity of Instilled Solution, cps	Aqueous Humor Drug Concentration at 20 min Postinstillation[a], mg/ml × 10^4	Aqueous Humor Drug Concentration at 30 min Postinstillation[a], mg/ml × 10^4
1	6.0 ± 0.5 (8)	4.5 ± 0.4 (12)
1.8	7.5 ± 1.6 (6)	5.3 ± 1.2 (6)
4.2	9.0 ± 1.2 (8)	5.7 ± 1.7 (6)
12.5	10.0 ± 1.5 (8)	7.2 ± 1.5 (6)
100	11.8 ± 1.7 (7)	9.3 ± 1.6 (6)

[a] Numbers in parentheses represent the number of samples of aqueous humor, *i.e.*, number of eyes.

Table VI—Calculated Concentration of Drug in the Conjunctival Sac after Instillation of Drug Solution[a] of Various Viscosities

Minutes	Concentration[b] in Conjunctival Sac[c], % w/v					
	100[d]	40[d]	12.5[d]	4.2[d]	1.8[d]	1.0[d]
0[e]	0.77	0.77	0.77	0.77	0.77	0.77
1	0.745	0.743	0.739	0.737	0.729	0.722
2	0.719	0.712	0.705	0.695	0.681	0.671
3	0.693	0.677	0.667	0.647	0.630	0.617
4	0.667	0.642	0.626	0.599	0.581	0.560
5	0.641	0.605	0.586	0.551	0.531	0.506

[a] Concentration of solution before instillation 1% w/v. [b] Concentration of the drug solution, assuming the turnover rate of tear fluid to be 0.5 μl/min (3). [c] Values to calculate volume remaining, V_r, were taken from Fig. 1. [d] Viscosity of instilled solution, centipoises. [e] Zero time concentration is the concentration after dilution with lacrimal tear fluid. The lacrimal tear fluid is assumed to be 7.5 μl, and it is further assumed that no loss of unmixed drug solution occurs (3).

An alternative explanation for higher aqueous humor drug levels is a slow decrease in precorneal drug concentration due to a decrease in the drainage rates. Table VI shows a hypothetical case, listing the concentration of drug in the precorneal pocket, calculated with the use of Eqs. 2 and 3, for the first 5 min postinstillation of drug for various viscosity solutions. For a tear turnover rate of 0.5 μl/min, dilution of the drug solution in the conjunctival sac will be different for different viscosity solutions because of the different drainage rates. The difference in concentration for different solutions will widen with time. At 1 min, the difference in concentration for solutions of 1 and 100 cps is 2%, whereas the difference widens to 14% at 5 min postinstillation for the same two solutions.

After instillation of a known concentration of drug solution into the eye, there will be a dilution effect due to the presence of the lacrimal fluid volume. The new concentration, which is also the concentration of the drug solution at time zero, $C_t = 0$, can be calculated by the use of Eq. 2 (4):

$$C_{t=0} = \frac{(C_0)(V_i)}{V_i + V_l} \qquad \text{(Eq. 2)}$$

The concentration of drug solution in the conjunctival sac at any time can be calculated by use of Eq. 3 (4):

$$C_t = \frac{(V_r - nF)}{V_r + nF} C_{t-n} \qquad \text{(Eq. 3)}$$

where C_t is the concentration to be calculated at time t, F is the turnover rate, and C_{t-n} is the known concentration at time $t - n$. This equation is valid only if $n < t > 0$. The slope of a plot of logarithm concentration against time for the first few minutes postinstillation provides the first-order rate constant, k_{con} min^{-1}, for the decline in concentration. Plots of the determined aqueous humor drug concentration against the calculated first-order rate constant for the decline in the concentration shows a linear relationship for both 20 and 30 min postinstillation (Fig. 6). Inspection of the values shows that there is approximately a twofold increase in concentration with a twofold decrease in the rate constant. This suggests that the increase in aqueous humor drug concentration with viscous solution may be partly due to the increased zero time concentration of drug in the conjunctival sac but is principally due to a decrease in the rate of decline of concentration for the first few minutes postinstillation.

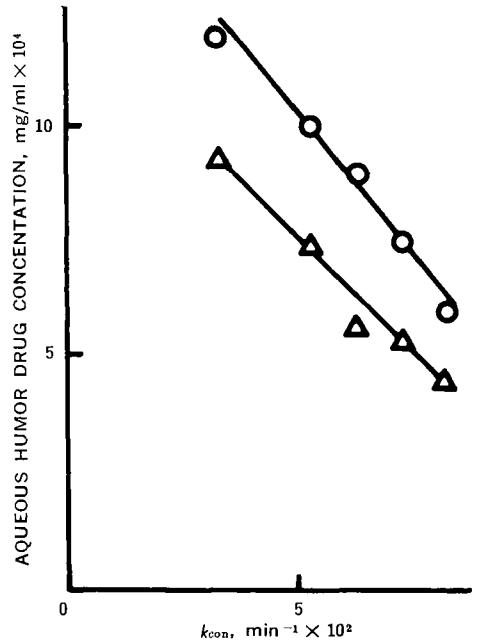

Figure 6—*Relationship between aqueous humor drug concentration and first-order rate constant for the decline of drug concentration in the cul-de-sac. Circles and triangles represent the aqueous humor drug concentrations at 20 and 30 min post-instillation, respectively.*

Considerable reservations may be felt about comparing results from rabbits with those from humans because of the differences between the physiology of tear flow and mixing and general anatomy. Nevertheless, the rabbit is the principal experimental animal in ophthalmology, so comparisons are needed.

Mueller and Deardorff (7), in human studies, found an increased cycloplegia and mydriasis from homatropine in 1% methylcellulose (about 60-cps solution) at all doses due to increased contact time. Their data suggest that double the quantity of drug would penetrate the cornea with increased viscosity, which is in agreement with the results of this study in rabbits. Furthermore, a 0.1% methylcellulose (about 1.4-cps solution) solution of drug gave a pharmacological response that was comparable to that of the nonviscous aqueous vehicle. This is also in agreement with results of the present study. Since both 1 and 0.1% methylcellulose in aqueous solution lowers surface tension to about 50 dynes/cm, the difference in effect of two solutions rules out an increased effect due to surface tension. The response is probably due to an increase in contact time, as suggested by the authors (7).

There has been a reported study (12) in humans of an enhanced pharmacological response to pilocarpine when applied in a methylcellulose vehicle. However, a comparison of that study to the present study could not be made because the viscosity of the solutions was not reported.

Linn and Jones (13) studied the rate of lacrimal excretion of various ophthalmic vehicles by determining the length of time necessary for various vehicles to pass through the drainage apparatus. Solutions of dye were instilled into the eyes of test subjects, and the dye loss was followed by placing a moistened cotton-tipped applicator in the inferior meatus of the nose and measuring the length of time for the dye to appear. This test determined the excretion time, which was defined as the length of time from instillation into the eye until the solution stained the cotton-tipped applicator. The reported result was 60 sec for an aqueous solution; for a viscous solution the results were as follows: 0.25%, 90 sec; 0.5%, 140 sec; 1.0%, 210 sec; and 2.5%, 255 sec. The corresponding viscosity of these solutions will be approximately 1, 3, 8, 65, and 33,000 cps. Qualitatively, these results agree with results in the present study; namely, that there is a large decrease in excretion rate with a small change in viscosity in the lower viscosity region and there is a small change in drainage rate with large changes in viscosity in the higher viscosity region. The fact that the results of the present study in rabbits correspond well to those in humans suggests that the mechanism of vehicle effect may be the same in both humans and rabbits. However, we are unable to offer an explanation of the finding by Adler *et al.* (1) of no increased contact time with viscous vehicles in humans.

The present study on viscosity vehicle effect in rabbits can be summarized as follows. Instillation of the drug solution of varying viscosities decreases the rate of drainage as the viscosity of the instilled solution increases. This increases the concentration, both at zero time and subsequent time periods, of the drug in the precorneal film, which results in a higher aqueous humor drug concentration. However, the magnitude of the increase in aqueous humor drug concentration is small over a 100-fold change in solution viscosity, in agreement with the observation in humans by Adler *et al.* (1).

Since there is more loss of unmixed nonviscous drug solution from the eye and a faster rate of drainage for larger volumes, it is postulated that the effect of vehicle viscosity will be greater in larger instilled volumes than in smaller instilled volumes. This is an important consideration since smaller drops will probably be used in the future and viscosity influence is expected to be less.

REFERENCES

(1) C. A. Adler, D. M. Maurice, and M. E. Paterson, *Exp. Eye Res.*, **11**, 34(1971).

(2) S. S. Chrai and J. R. Robinson, *Amer. J. Ophthalmol.*, **77** (5), 1974.

(3) S. S. Chrai, T. F. Patton, A. Mehta, and J. R. Robinson, *J. Pharm. Sci.*, **62**, 1112(1973).

(4) S. S. Chrai, Ph.D. thesis, University of Wisconsin, Madison, Wis., 1973.

(5) T. J. Mikkelson, Ph.D. thesis, University of Wisconsin, Madison, Wis., 1971.

(6) S. S. Chrai, M. C. Makoid, S. P. Eriksen, and J. R. Robinson, *J. Pharm. Sci.*, **63**, 333(1974).

(7) W. H. Mueller and D. L. Deardorff, *J. Amer. Pharm. Ass., Sci. Ed.*, **45**, 334(1956).

(8) S. M. Blaug and A. T. Canada, Jr., *Amer. J. Hosp. Pharm.*, **22**, 662(1965).

(9) A. A. Swanson, D. J. Jeter, and C. R. Gregor, *Ophthalmologica*, **156**, 425(1968).

(10) N. Krishna and F. Brow, *Amer. J. Ophthalmol.*, **57**, 99(1964).

(11) A. A. Swanson, D. J. Jeter, and P. Tucker. *Ophthalmologica*, **160**, 265(1970).

(12) J. S. Haas and L. Merrill, *Amer. J. Ophthalmol.*, **54**, 21(1962).

(13) M. L. Linn and L. T. Jones, *ibid.*, **65**, 76(1968).

ACKNOWLEDGMENTS AND ADDRESSES

Received January 31, 1974, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706*

Accepted for publication March 18, 1974.

Supported by a grant from Allergan Pharmaceuticals, Irvine, CA 92664, and a grant from the Graduate School, University of Wisconsin, Madison, WI 53706

* Present address: Schering Corp., Bloomfield, NJ 07003

ˣ To whom inquiries should be directed.