where R is the concentration of USP methotrexate reference solution in milligrams per 100 ml, W_{av} is the average weight of one tablet in milligrams, and W is the weight of an aliquot of the tablet triturate taken for assay.

Table II summarizes the values of various samples obtained by Methods 1 and 2 and the cellulose column method. All three methods gave essentially similar values with light-degraded methotrexate samples and less than 70% heat-degraded methotrexate samples. Therefore, considering the relative quickness of the assay procedure by Method 1 (less than 15 min), this method is recommended for routine assays of methotrexate in commercial products. Method 2 is more important in studying the kinetics of methotrexate and for quantitating its degradation products. Also, it is essential to use Method 2 in methotrexate samples that are more than 70% heat degraded.

The sensitivity of Method 1 was determined using a 100-μl sample size to detect a concentration of 0.25 μg of methotrexate/ml of solution. An aqueous solution containing 1 μg of methotrexate/ml of solution, when analyzed by Method 1, gave a recovery of 93.1 ± 4.7% of methotrexate (average of three runs). Thus, Method 1 could be potentially useful in quantitating to a level of 1 μg of methotrexate/ml in biological fluids.

The tablet excipient dye did not interfere with the assay, as seen by the close agreement between the values obtained by Methods 1 and 2 and the cellulose column method. In the latter method, the dye could be seen as a yellow band remaining at the top of the cellulose column. The benzyl alcohol preservative present in the injectables is nonionic and eluted almost with the solvent front. Other preservatives such as methylparaben and propylparaben eluted immediately following the solvent front and did not interfere with the assay of intact methotrexate.

In all of the described assays, the amount of intact methotrexate in the unknown is calculated as a percentage of the reference solution. The explicit quantities are then calculated from the known concentration of methotrexate USP in the reference solution. However, methotrexate USP has been reported (2) to be only 85–90% pure. Therefore, explicit quantities in the assays, which are calculated by assuming methotrexate USP as 100% pure, must be interpreted accordingly. However, in the determination of relative amounts in various preparations or in the determination of percentage degradation, this consideration is obviated.

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ACKNOWLEDGMENTS AND ADDRESSES

Received July 15, 1976, from the *Pharmacy Department, The Clinical Center, Public Health Service, National Institutes of Health, Bethesda, MD 20014.*

Accepted for publication November 3, 1976.

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Vehicle Effects on Ocular Drug Bioavailability II: Evaluation of Pilocarpine

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Abstract □ The influence of vehicle composition on ocular penetration of pilocarpine was studied in the albino rabbit. Increasing the pH of a vehicle promoted increased corneal penetration for pilocarpine, in accordance with the pH–partition hypothesis, but a similar series of experiments with a nonionizable drug, glycerin, gave similar results. The extent of pH-induced lacrimation by the vehicle and its effect on precorneal drug concentration also was determined. Increased pilocarpine absorption at neutral to slightly alkaline pH was due primarily to its peculiar solubility characteristics coupled with less irritation and lacrimation rather than a direct pH effect on the molecule. Incorporation of pilocarpine into a petrolatum-based ointment vehicle resulted in increased aqueous humor pilocarpine levels above those provided by an equivalent dose of aqueous solution. The mechanism of this increase was determined to be a higher effective concentration of pilocarpine in the ointment vehicle coupled with an increase in contact time of the dose. The ointment system also exerted an unusual form of vehicle control in that it promoted the corneal penetration of pilocarpine while impeding uniform mixing of the dose with tears and thereby imposed a restriction on the amount of pilocarpine available to the ocular tissues.

Keyphrases □ Pilocarpine—effect of vehicle composition and pH on ocular bioavailability, rabbits □ Vehicles—effect of composition and pH on ocular bioavailability of pilocarpine, rabbits □ Bioavailability, ocular—pilocarpine, effect of vehicle composition and pH, rabbits □ Ocular bioavailability—pilocarpine, effect of vehicle composition and pH, rabbits □ Ophthalmic cholinergic agents—pilocarpine, effect of vehicle composition and pH on ocular bioavailability, rabbits

The first report in this series dealt with vehicle influence on the ocular bioavailability of the relatively water-insoluble steroid fluorometholone (1). In that study, mechanisms of vehicle effects on ocular bioavailability were based on the interplay of the steroid and vehicle properties with the physiological and physicochemical nature of the drug administration site, *i.e.*, the precorneal portion of the eye. The present study extended this work to include the important water-soluble antiglaucoma drug pilocarpine. Thus, this examination of a water-soluble drug, along with earlier work on the relatively water-insoluble steroid, provides considerable perspective on factors that should be considered when formulating an ophthalmic drug for topical delivery to the eye to obtain maximum drug benefit.

BACKGROUND

During the past 30 years, numerous vehicles have been screened for various drugs to improve the overall intraocular penetration of topically applied drugs. Because of an inadequate understanding of the interplay between drug–vehicle–precorneal area properties, the primary thrust of much research has been directed toward prolonging the presence of the instilled dose in the precorneal area, usually referred to as increasing the contact time. Most often, these formulations rely mainly upon the viscosity character of the vehicle and its subsequent rheological effect on drainage loss of the dose *via* the nasolacrimal duct. Thus, most topically applied ocular vehicles have included viscous polymers such as hydroxypropylcellulose (2–4), methylcellulose (5–12), and polyvinyl alcohol (2–4, 6–8, 13–15).

Detailed studies of the drainage loss rate of these vehicles also have been performed (3, 5, 13, 16, 17) to permit selection of vehicles exhibiting

the best retention characteristics. In addition, the chemical nature of the vehicle, such as buffer composition and pH (18–22), has been modified in hopes of presenting the most favorable form of the drug to the eye for optimum corneal uptake and permeation.

While most studies provided some qualitative and semiquantitative information concerning corneal drug penetration, the primary drawback has been a consistent lack of correlation between the physicochemical characteristics of the drug and the properties of the chosen vehicle. The contact time of the vehicle is all too often assigned primary importance, whereas release of the drug from the vehicle and its subsequent corneal permeation mechanism are rarely considered.

For an ophthalmic dosing system to provide optimum ocular drug penetration, a balance must be achieved between the requirements of both the drug and the vehicle, given the constraints of the corneal pocket. The only suitable means to attain this goal is to determine the mechanism(s) of vehicle effects and to relate them quantitatively to the mechanism of ocular penetration of the drug. The effect of vehicles on the transcorneal permeation of pilocarpine was examined in this study, and a mechanistic interpretation of the interactions among the drug, the vehicle, ocular fluids, and transport dynamics is given.

EXPERIMENTAL

Materials—Tritiated pilocarpine alkaloid (specific activity of 4.1 Ci/mmole) in ethanol and ^{14}C-glycerin (specific activity of 10 mCi/mmole) in sterile aqueous solution were obtained commercially[1]. The pilocarpine was purified by vacuum distillation immediately prior to each series of experiments to prevent errors due to tritium exchange (23). The ointment vehicle was a commercially available, sterile, petrolatum-based product[2]. All other chemicals were either reagent or analytical grade and were used as received.

Male albino rabbits[3], 1.8–2.4 kg, were fed a regular diet with no restrictions on the amounts of food or water consumed.

Pilocarpine and Glycerin Solutions—The pilocarpine solutions were prepared fresh before each experiment by adding a solution of 10^{-2} *M* pilocarpine alkaloid in the desired buffer to the purified labeled material. It was determined that 0.25 mCi of tritiated pilocarpine alkaloid/ml of final solution was sufficient to ensure good counting efficiency, *i.e.*, 150,000–175,000 counts/min/μl. The glycerin solutions were prepared by adding an aqueous aliquot of the isotopic species to a 0.14 *M* solution of glycerin in the appropriate buffer. The final solution had an activity of 20,000 counts/min/μl.

The small amount of labeled material added to the drug solutions did not alter the molarity of the final solutions. Unless otherwise specified, all solutions were isoosmotic with 0.9% sodium chloride.

Pilocarpine Ointment—A small quantity of distilled water, *i.e.*, 0.05 ml/g of ointment, containing 2.08 mg of pilocarpine alkaloid/0.05 ml of solution was added to a sufficient amount of the purified tritiated material so that the final activity was 0.25 mCi/g of ointment. This solution then was mixed carefully with the ointment vehicle, yielding a 10^{-2} *M* pilocarpine ointment containing 5% water. Final counts in the ointment were 160,000 counts/min/mg.

For the experiment in which the percent water was increased, the ointment was prepared by simply adding more water in the first step. Homogeneity of the final product was verified by the reproducibility of measured radioactivity among weighed samples of ointment. Ointments were prepared fresh prior to each experimental run.

Removal of Corneal Epithelium—Two drops of 0.5% tetracaine hydrochloride solution were instilled into the eyes of the animals, and the epithelial layer was removed *in toto* by scraping with a scalpel. The eye was then blotted with moist tissue to remove any loose epithelium fragments from the precorneal area. Dosing was initiated after 1 hr to allow the effect of the local anesthetic to diminish.

Aqueous Humor Drug Concentration–Time Profiles—*Pilocarpine and Glycerin Solutions*—The basic experimental techniques used for monitoring aqueous humor drug levels after topical dosing with solutions in both intact and abraded eyes were described previously (1, 24). Unanesthetized animals were used, and a standard 25-μl dose was instilled in all experiments. Standard liquid scintillation counting techniques were used for analysis of the aqueous humor samples.

Pilocarpine Ointment—Individual doses of ointment were weighed on an analytical balance. The weighed dose was transferred carefully to a microspatula and then placed inside the center of the lower cul-de-sac, with care being taken not to irritate the eye or to touch the corneal surface. The lower lid was gently moved across the cornea to spread the dose evenly and was then released. No further mechanical action was performed, and the remainder of the run was carried out using the technique previously mentioned.

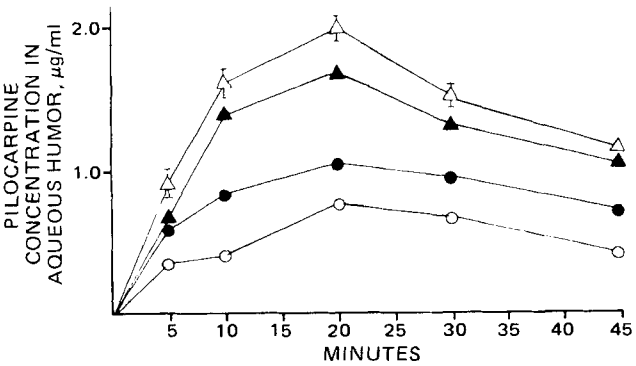

Figure 1—*Aqueous humor drug concentration–time profiles after topical dosing with 10^{-2} M pilocarpine solutions. Key: ○, pH 5.0; ●, pH 6.24; ▲, pH 7.0; and △, pH 8.0. All points represent an average of at least six eyes, and error bars represent the standard error of the mean. Where no error bar is indicated, the standard error of the mean is smaller than the size of the symbol used.*

Measurement of Glycerin Concentration in Tears *versus* Time as a Function of Instilled Solution pH—Unanesthetized rabbits were placed into wooden restraining boxes in a normal upright posture. Isotonic 0.14 *M* ^{14}C-glycerin solutions were prepared using Sorenserts buffer at pH 5 and 8, and 25-μl doses were topically instilled as in the aqueous humor studies. Tear samples were removed at 0, 1, 2, 3, 4, and 5 min postinstillation using 1-μl disposable capillary pipets[4]. The zero-time sample was removed from the eye within 10 sec after the dose was instilled. Eyes were used only once, and both buffers were used for each animal. Left and right eyes were alternated for the two buffers, and extreme care was exercised not to irritate the eye during sample removal.

Only the tip of the capillary tube was placed into the tear pool in contact with the cornea along the lid margin, and the filled pipets were transferred to sample vials for liquid scintillation counting. The raw counts per minute data were converted to a microgram per microliter basis using a standard 1-μl sample withdrawn from the solution prior to dosing. Errors due to the loss of volume and drug from sample removal were neglected on the basis of the small sample size and number and the relatively greater volume of the precorneal pool during the sampling interval.

RESULTS

Effect of Vehicle pH on Aqueous Humor Pilocarpine Levels—Previous studies with pilocarpine suggested that raising the dosing solution pH enhances its corneal penetration (25). Although such increases were reported (18–22), the data have not been sufficient to allow a precise mechanistic interpretation. The current studies were performed to provide a more complete analysis of this effect.

The aqueous humor concentration *versus* time profiles for pilocarpine at four vehicle pH values, using isotonic Sorensen's phosphate buffer, are presented in Fig. 1. As the pH was raised from 5 to 8, there was a two- to threefold increase in the amount of pilocarpine reaching the anterior chamber. The peak aqueous humor drug concentration occurred at 20 min after dosing, and subsequent drug elimination from the aqueous humor followed first-order kinetics with an associated rate constant of 0.017–0.024 min^{-1}.

Since the peak time in the aqueous humor did not appear to change for the four initial pH experiments, additional pH data were obtained at 20 min postinstillation using other buffer combinations. In this way, a broad range of pH values was examined to estimate the overall pH influence. Data at pH 4 and 5 were obtained using a phthalate buffer; data at pH 9, 10, and 11 were obtained with the Atkins–Pantin buffer.

Both buffers were moderately hypertonic. However, as a precaution against tonicity effects, an overlapping pH experiment was performed

[1] New England Nuclear, Boston, Mass, and ICN, Isotope and Nuclear Division, Cleveland, Ohio.
[2] Lacri-Lube, Allergan Pharmaceuticals, Irvine, Calif.
[3] Klubertanz, Edgerton, Wis.
[4] Drummond Microcap, Scientific Glass Apparatus Co., Bloomfield, N.J.

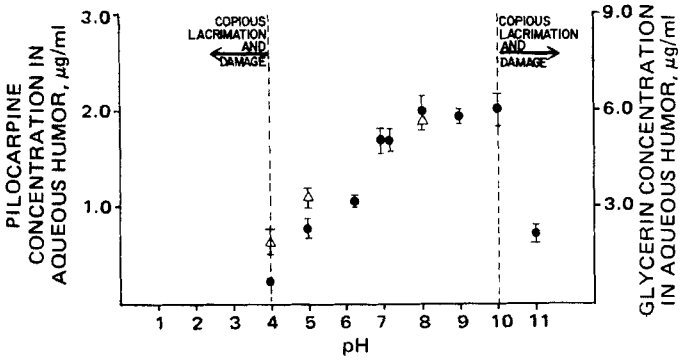

Figure 2—*Influence of vehicle pH on the aqueous humor concentration of pilocarpine and glycerin. Key:* ●, 10^{-2} M *pilocarpine solutions; and* △, *0.14* M *glycerin solutions. All samples were obtained at 20 min postinstillation and represent a minimum of six eyes.*

in both the acidic and alkaline regions. For example, both the Sorensen phosphate buffer and the phthalate buffer were used for pH 5, and the Sorensen and Atkins–Pantin buffers were used at pH 8. Since no differences in aqueous humor drug levels were detectable for the buffer pairs, tonicity effects were assumed negligible for the pH range studied. Further support for this assumption comes from the results of corneal perfusion studies (26) in which the epithelial permeability did not change over the tonicity range of 0.9–10% sodium chloride.

The results obtained at 20 min postinstillation for all pH values are summarized in Fig. 2. The extent of corneal penetration by pilocarpine gradually increased as the vehicle pH was raised. The exception to this behavior occurred at pH 11. However, excessive lacrimation and reddening of the eyes were evident at both extremes of the pH range. Maurice (26) also found ocular damage outside the range of pH 4–10 (indicated as vertical dotted lines in Fig. 2), and this finding appears to be consistent with the visual appearance of ocular irritation in the present studies.

Effect of Vehicle pH on Aqueous Humor Glycerin Levels—Glycerin is a nonionizable drug. According to the pH–partition hypothesis, the direct influence of pH on the extent of absorption of such a molecule should be negligible. An examination of the effect of vehicle pH on the corneal penetration of glycerin would make possible a separation of the direct and indirect effects of pH on an ionizable molecule such as pilocarpine.

The results of the glycerin experiments are shown in Fig. 2. All values were obtained at 20 min after dosing. The effect of pH on glycerin penetration was nearly identical to that observed for pilocarpine. Increasing the vehicle pH from 5 to 8 produced a twofold increase in the amount of glycerin penetrating to the anterior chamber. Also, as in the pilocarpine experiments, lowering the pH to 4 caused obvious irritation to the eye and a corresponding decrease in aqueous humor drug levels. The implications of this behavior relative to the mechanism of the pH effect observed with pilocarpine will be discussed subsequently.

Effect of Vehicle pH on Lacrimation and Precorneal Glycerin Concentration—The precorneal dynamics of a topically instilled dose of pilocarpine were extensively discussed previously (27–29). Drainage of the dose *via* the nasolacrimal duct removes only an amount of drug from the precorneal pocket and has no direct effect upon the drug concentration. The precorneal drug concentration can only be reduced by the loss of drug without an attendant loss of solution volume or by addition of tears to the precorneal pool *via* the lacrimal apparatus. Accordingly, the instilled drug concentration is decreased by initial mixing of the dose with the normal resident precorneal tear volume, by drug loss *via* absorption into the ocular tissues or systemic circulation, and by dose-induced lacrimation as well as normal tear turnover.

The data presented in Fig. 3 show the effect of vehicle pH on the precorneal concentration of glycerin as a function of time. The pH 5 solution induced a greater degree of lacrimation than the pH 8 solution. The first-order decline of precorneal glycerin concentration was twice as fast for the acidic solution, being 0.87 min^{-1} for pH 5 and 0.40 min^{-1} for pH 8. Of course, concentration changes can also arise due to drug absorption into ocular tissues, but this influence was presumed to be small since only a small percentage of the total dose was absorbed. Thus, lacrimation is the predominant factor responsible for the fall in precorneal drug concentration.

Table I presents the percent reduction of precorneal glycerin concentration *versus* time for the two pH values. The fall in tear concentration was markedly accelerated with the acidic solution. The percent reduction

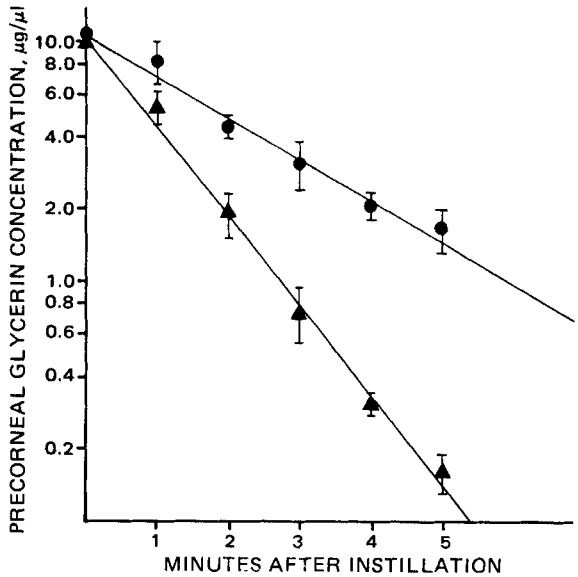

Figure 3—*Precorneal concentration of glycerin in tears* versus *time as a function of vehicle pH. Key:* ●, *pH 8.0; and* ▲, *pH 5.0. All points represent a minimum of six determinations.*

for the zero-time samples, taken within 10 sec after instillation, was 20% for both pH values. This value corresponds very well to the calculated 23% reduction in initial concentration when the 25-μl dose is mixed with the 7.5-μl resident tear volume. Therefore, aqueous solutions with viscosities similar to water equilibrate very rapidly with tears in the precorneal area.

Another important observation of the lacrimation study is that the fall in precorneal glycerin levels for the pH 8 solution could be accelerated to a rate as great or greater than the pH 5 solution by simply irritating the eye with the capillary tube during sample removal. A precipitous fall in precorneal concentration could be suddenly induced at any time after dosing as a result of reflux tearing in response to the mechanical stimulus.

Mishima *et al.* (30) showed that tear flow can increase suddenly due to a variety of factors, and even very subtle stimuli produce a dramatically increased tear turnover rate in a matter of seconds. In studies with humans, it was noted that the turnover rate could increase by a factor of 8–10 without being noticed by the subject; such variations in turnover did not cause an overflow of tears from the cul-de-sac.

Aqueous Humor Levels of Pilocarpine after Dosing with 10^{-2} *M* Ointment—*Intact* versus *Abraded Eyes*—The results obtained after instilling 25-mg doses of pilocarpine ointment into intact and abraded eyes are shown in Fig. 4. In the intact eye, pilocarpine reached a peak concentration in the aqueous humor in 20 min. This peak time corresponded to that obtained for an equivalent dose of solution in intact eyes, but the peak concentration obtained with the ointment was approximately three to four times higher than that with the solution. When the same ointment was applied to abraded eyes, the drug reached peak levels in the anterior chamber within 5 min, consistent with the abraded solution data.

However, in contrast to results with intact eyes, the aqueous humor drug levels in an abraded eye with the ointment were substantially less than the abraded eye solution data. In fact, with the exception of the shift in peak time, the amount of pilocarpine penetrating to the aqueous humor from the ointment was nearly the same for both intact and abraded eyes. This behavior is markedly different from that seen with the solution. Elimination of the drug from the anterior chamber remained unchanged in each case, with an attendant first-order rate constant of approximately 0.02 min^{-1}.

Table I—Percent Reduction of Precorneal Glycerin Concentration *versus* Time as a Function of Instilled Solution pH

pH	Minutes after Instillation					
	0	1	2	3	4	5
5	20	59	85	94	98	99
8	20	35	66	76	84	87

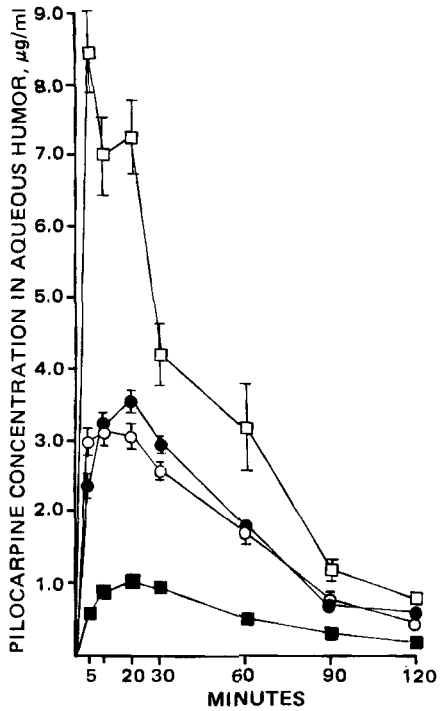

Figure 4—*Aqueous humor levels of pilocarpine after dosing with 10^{-2} M ointment and solution in intact and abraded eyes. Key: ●, 25 mg of ointment, intact eyes; ○, 25 mg of ointment, abraded eyes; ■, 25 μl of solution, intact eyes; and □, 25 μl of solution, abraded eyes. All points represent an average of eight to 16 eyes.*

Variation of Dose—Since incorporation of pilocarpine into a viscous oleaginous vehicle may increase the contact time of a dose with the precorneal area, the effect of increasing the dose was investigated. In this way, it was anticipated that any beneficial effects associated with increased contact time could be magnified.

Figure 5 presents the data obtained with various doses of ointment. The amount of pilocarpine reaching the anterior chamber increased as the dose was increased. In addition, the peak time shifted to later times for the larger doses, occurring at 20 min for the 25-mg dose and at 30 min for the 50-mg dose. No definitive statement regarding the peak time for the 10-mg dose can be made since the 10- and 20-min points were not statistically different. The areas under the curve for the 25- and 50-mg studies were consistent with the doses, showing a twofold increase for the higher dose. The 10-mg dose also was reasonably consistent with the area, being one-half that of the 25-mg dose. The first-order rate constants for elimination for all cases were 0.017–0.022 min^{-1}.

Variation of Percent Water—To determine if the amount of water incorporated into the ointment had any effect on the availability of pilocarpine from the vehicle, an ointment was prepared using 10% water rather than 5%. There was no statistically significant difference between the profiles for 5 and 10% water (Fig. 4). Higher percentages of water were attempted, but serious problems with bleeding of the water from the oleaginous vehicle occurred and a satisfactory ointment could not be prepared.

DISCUSSION

Effect of Vehicle pH on Corneal Penetration of Pilocarpine—The vehicle pH has long been considered to be an important parameter for the passive diffusion of ionizable compounds into and through the cornea (31). The pH–partition hypothesis predicts that maximum penetration will occur when an alkaloidal drug is presented to a membrane as the uncharged free base. This form is generally more lipid soluble than the ionized species and will readily penetrate a lipophilic tissue such as the corneal epithelium. Since the epithelium is the primary barrier to corneal drug uptake, such compounds should show marked increases in corneal penetration as the vehicle pH is raised, reaching a maximum at pH's above values corresponding to their pK's.

The pK_1 for pilocarpine is 7.15, and previous studies (18–22) indicated that corneal penetration does increase as the pH of the dosing solution is increased. For example, Ramer and Gasset (18) observed an approximate doubling of corneal penetration when the vehicle pH was raised

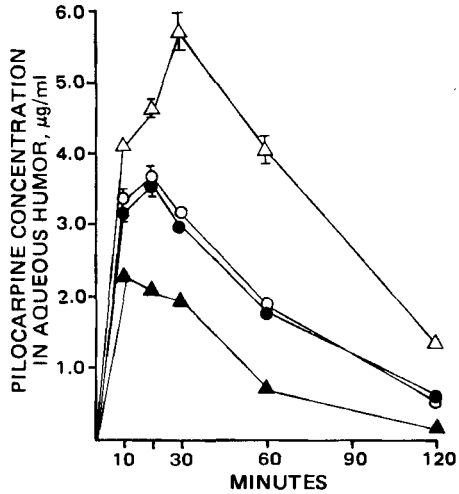

Figure 5—*Aqueous humor levels of pilocarpine after topical dosing with 10^{-2} M ointment in intact eyes. Key: ●, 25 mg of ointment, 5% water; ○, 25 mg of ointment, 10% water; ▲, 10 mg of ointment, 5% water; and △, 50 mg of ointment, 5% water.*

from 5 to 8 and attributed this increase to a predominance of the free base form at the higher pH. However, while such results are certainly consistent with the pH-related event, they are not conclusive.

The results of the current studies (Fig. 2) also show a two- to threefold increase in corneal penetration between pH 5 and 8, as well as the expected sigmoidal shape, but an important aspect of pilocarpine's physical character must be considered before a mechanistic judgment is made. Pilocarpine is somewhat unique in the sense that the free base form of the drug is more soluble in water than are any of the salts. Pilocarpine is freely soluble in water, whereas the solubilities of the hydrochloride and the nitrate are 1 g/0.3 ml and 1 g/4 ml, respectively (32). The preferential aqueous solubility of the free alkaloid is responsible for the diminished absorption sensitivity of pilocarpine to changes in vehicle pH. However, the true impact of this solubility effect only becomes apparent when the results of the glycerin study (Fig. 2) are considered.

Glycerin is a nonionizable compound that should not derive any direct benefit from pH manipulation for its corneal penetration. Nevertheless, the results of the glycerin study show that a twofold increase is also apparent when the pH is varied between 5 and 8. Such behavior strongly suggests that some mechanism other than a direct pH effect on the drug molecule is operating to produce this increase and that this same mechanism must also be responsible for the increase observed for pilocarpine. The existence of another mechanism is also implied by the data for pilocarpine since the inflection point for the pilocarpine data in Fig. 2, corresponding to the pK_1 for pilocarpine, occurs nearly 1 full pH unit lower than expected.

The results obtained from the lacrimation study (Fig. 3 and Table I) show that pH-induced lacrimation is responsible. The magnitude of the difference in aqueous humor levels at pH 5 and 8 corresponds to the observed changes in precorneal glycerin concentration for the two solutions. The greater amount of drug reaching the anterior chamber when the pH 8 vehicle is used is simply the result of a smaller pH-induced lacrimation response in the precorneal area. These results are consistent with previous work that indicated that the eye is better able to tolerate a basic pH than an acidic pH (33).

The data in Fig. 2 for the pH 11 solution provide further support for pH-induced lacrimation. As the pH of the vehicle became sufficiently alkaline to irritate the eye, the level of pilocarpine in the anterior chamber fell to a value similar to the pH 5 solution. During these experiments, both the pH 5 and 11 solutions caused eye irritation and copious lacrimation, as indicated by an abnormally high blink rate and eye wetness after instillation of the dose.

The curves presented in Fig. 1 also are remarkably similar to previous data when the pilocarpine dose was rinsed away from the precorneal area with saline at various times after instillation (24). This rinsing process, which essentially accelerates the drainage process to a maximum and thereby represents the upper limit of lacrimation, creates an identical variation in the magnitude of the levels of pilocarpine in the anterior chamber. An extensive discussion of the effects of normal and accelerated loss of drug from the precorneal area was presented previously (24, 34).

The current results indicate that the highest pH influence on corneal

penetration will be observed for a drug with a pKa greater than 6 or 7, since above neutrality the extent of pH-induced lacrimation, which opposes a direct pH effect on the drug molecule, is diminished. However, while this should be the case with pilocarpine since its pK_1 is 7.15, it does not occur because of the preferential aqueous solubility of this molecule throughout the pH spectrum.

A major point of concern in any discussion of vehicle pH in the eye is how fast the eye is able to return to a normal physiological pH. Although the precise buffer capacity of the ocular fluids has not been determined, a few general considerations support the contention that an instilled buffer is not readily altered by the eye. Since the normal resident tear volume in the rabbit is about 7.5 μl (30), instillation of 25 μl of a moderately strong buffer would be expected to overwhelm momentarily the ocular fluids and to establish the buffer pH in the eye. Although tear turnover in the rabbit occurs at the rate of 0.66 μl/min, it does not become significant until drainage has removed the bulk of instilled solution *via* the nasolacrimal duct (27). However, by the time the drainage process is completed, permeation of drug into the cornea is also nearly at an end (24), so the instilled solution pH can be expected to remain constant for at least as long as the majority of the absorption process. Therefore, the possibility of an altered solution pH after instillation can be neglected. Further support for this approach comes from recent work in which the precorneal pH remained equal to the buffer pH for a considerable time after dosing[5].

The effect of pH on the corneal penetration of pilocarpine is substantially different than previously believed. While pH partitioning or some other mechanism (35) may contribute to some small extent, the primary overriding influence is pH-induced lacrimation. Thus, the observed effect of pH is, in reality, largely an indirect one, influencing both ionizable and nonionizable drugs to a similar extent and by similar means. The magnitude of this effect must be considered when evaluating the effect of vehicle pH on the ocular penetration of any drug from an instilled solution.

Effect of Vehicle on Corneal Penetration of Pilocarpine—One primary disadvantage of a topically applied ophthalmic solution is the rapid drainage loss that occurs in the precorneal area. Attempts to increase the corneal drug penetration often begin by increasing the viscosity of the vehicle to delay this loss. Previous work with pilocarpine showed that viscous polymer solutions (5, 9, 13) are only moderately successful in enhancing its corneal penetration. The intent of the present series of experiments was to formulate an explanation for these observations within the context of the mechanism of pilocarpine's transcorneal permeation as detailed previously (24).

The data in Fig. 4 show that the ointment vehicle used in the present study was superior to an aqueous solution. Equivalent doses of each vehicle, containing the same amount of pilocarpine, were delivered into intact eyes, and the ointment gave three to four times higher aqueous humor drug levels. Before a precise discussion of this observed effect, it will be beneficial to consider some important general aspects of the corneal penetration of a drug from its vehicle.

Figure 6 depicts some theoretical profiles of corneal drug penetration as a function of time. This figure illustrates two primary absorption parameters associated with transcorneal dynamics: increased concentration of the dosing system and extended contact time of the instilled dose.

In Fig. 6, profiles A, B, and C represent successively higher concentrations of a drug in a given vehicle (*e.g.*, a solution). As the concentration is increased, the amount of drug reaching the aqueous humor increases also so that higher levels are evident throughout the profile. However, the peak time does not change since the absorption mechanism remains unchanged.

Profiles D and E illustrate the case in which the contact time of the dose is extended (*e.g.*, the use of a viscous polymer solution rather than a simple aqueous solution). Increasing the contact time allows for an extension of the time permitted for the absorption process. As a result, the aqueous humor levels continue to increase beyond those obtained for the simple solution, the peak time is shifted to a later time, and the magnitude of the peak shift is determined by the degree to which drainage loss is delayed. The most important aspect of this case is that the early portion of the profile remains essentially unchanged; that is, increasing the contact time simply extends the profile obtained for the original dosing solution.

The reason for this behavior is that the peak time obtained for a topically applied drug solution is determined by the rapid parallel first-order drainage process occurring in the precorneal area (34). Rapid loss of drug

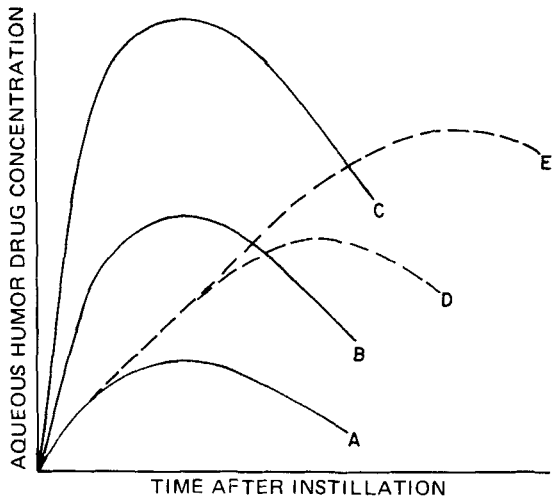

Figure 6—*Graphical representation of aqueous humor drug concentration, showing the influence of increasing concentration and extended contact time of the dosing system. Key: curves A, B, and C, effect of increasing concentration; and curves A, D, and E, effect of extended contact time.*

from the absorption depot creates an artificial peak in ocular tissues and results in subsequent overestimation of the absorption rate constant. Thus, any process that prolongs the presence of the drug at the absorption site extends the absorption process and shifts the apparent peak time to the right. The limit of this effect is, of course, the true peak time of the drug, beyond which a plateau is observed and only the times at which elimination of drug from the aqueous humor parallels those of a simple solution are shifted. This effect can be readily recognized in recent work with pilocarpine where the drainage ducts of rabbits were plugged prior to dosing with a solution (29).

In light of this discussion, it is easy to recognize why past attempts to increase the corneal penetration of pilocarpine through the use of viscous solutions met with only limited success. The maximum benefit that can be derived from such endeavors is the provision of more pilocarpine to the anterior chamber at later times after dosing. However, even this aspect has realized only limited potential, even in the extreme case where the drainage apparatus has been totally blocked (29). Increasing the aqueous humor pilocarpine levels at early times above those provided by a simple aqueous solution can only be achieved by increasing the concentration of the instilled solution or by altering the absorption mechanism.

It can now be recognized from the data in Fig. 4 that the ointment vehicle exhibits characteristics similar to those obtained when the applied pilocarpine concentration is increased. The peak time in the aqueous humor is the same as for the solution. Also, the pilocarpine level in the anterior chamber is raised, but the elimination rate is unchanged. This behavior is characteristic of a "pulsed-dose" mechanism (36), in which the cornea is simply presented with a greater amount of drug for a brief time, rather than any type of sustaining mechanism.

What must now be considered is the question of how a dose of ointment containing the same total amount of pilocarpine as an equivalent dose of solution could behave as though a higher drug concentration was being applied to the eye. The explanation lies in the physical composition of the ointment.

When pilocarpine is placed into an oleaginous vehicle, it is introduced into a medium unfavorable to its solubility. In the current studies, the alkaloid did not possess sufficient oil solubility to prepare a true solution of the drug in the ointment vehicle alone. To demonstrate this point, a 25-ml sample of 10^{-2} *M* tritiated pilocarpine alkaloid solution was shaken with an equal volume of light liquid petrolatum, and the two phases were analyzed by liquid scintillation techniques for pilocarpine content. The results showed that the water–oil ratio of pilocarpine was approximately 1500:1.

To achieve solution of the alkaloid, it was decided to dissolve the drug in a minimum amount of water (5%) and to incorporate it into the hydrophilic vehicle. The pilocarpine would be expected to remain in the water when mixed with the vehicle. Therefore, the ointment consisted of two phases: the bulk oleaginous phase containing little or no pilocarpine and the aqueous phase containing essentially all of the drug. A 25-mg dose of ointment would have all of its pilocarpine dissolved in only 1.25 μl of

[5] N. Keller, Alza Research Corp., Palo Alto, Calif., personal communication.

water, in contrast to the same amount of pilocarpine dissolved in a 25-μl dose of solution. As a result, the effective concentration of pilocarpine in the ointment was 20 times more than the solution. For this reason, the ointment behaved as though a higher concentration of drug was applied to the eye and gave corresponding increases in the aqueous humor drug levels at all times in the profile.

It might also be expected that the 20-fold difference in effective concentration should have produced even higher levels of pilocarpine than were actually observed, since previously corneal penetration of pilocarpine was linear over a wide concentration range (23). However, the results can be easily rationalized on the basis of the mixing problems existing between the oleaginous ointment and the aqueous tears.

To test the effective concentration mechanism, a 10% water vehicle was prepared and applied to intact eyes. However, the results were inconclusive. Although doubling the water content reduced the effective concentration by 50%, the amount of water in the ointment film in contact with the cornea was doubled; the differences were apparently offsetting at such a low percentage of water. Unfortunately, higher percentages of water could not be satisfactorily tested to prove the proposed mechanism.

Another important aspect of the ointment system can be seen by referring to Fig. 5. While the 25-mg dose apparently obeyed an increased concentration type of mechanism, the overall behavior when the dose was varied between 10 and 50 mg was somewhat different. The peak time shifted to a later time, and there were increased levels of pilocarpine at all times throughout the profile. Therefore, the ointment vehicle also extended the contact time of the dose. This effect becomes recognizable as the dose is increased, and the greater volume of the larger doses undoubtedly plays a role in prolonging the contact time.

The overall behavior of the ointment can be discussed in relation to three hypothetical mechanisms: (*a*) the ointment system is well behaved and exhibits classical vehicle control characteristics, (*b*) the ointment forms an occlusive nonrenewable film over the corneal surface, and (*c*) the ointment exhibits surface mixing only, and drug within the bulk is unavailable to the ocular fluids.

Each alternative can now be discussed in terms of supportive and nonsupportive data from the present experiments.

Figure 4 depicts a rather interesting result in that the ointment produced similar aqueous humor levels in both intact and abraded eyes. The area under the curve (*AUC*) was the same for each case, so it must be concluded that this *AUC* represents some limiting amount of pilocarpine available from the ointment vehicle.

It might be tempting to attribute this behavior to vehicle control in the ointment system. This is not possible, however in a classical sense, since the peak time is essentially unchanged from that obtained when using a solution and the aqueous humor levels are increased at times immediately following dosing rather than decreased. Therefore, the ointment mechanism is not true vehicle control in the sense of delayed release or partitioning of drug from the vehicle.

The behavior of the pilocarpine ointment is in direct contrast to results from a similarly conducted study with the steroid fluorometholone (1). In that study, the steroid in the same oleaginous vehicle exhibited true classical vehicle control. Aqueous humor drug levels were decreased for the first 60 min, and the peak time was not achieved until 3 hr after dosing, as compared to 30 min for a suspension or a solution. In addition, increasing the dose of fluorometholone ointment from 25 to 50 mg did not increase aqueous humor drug levels, which further demonstrates the effect of vehicle control. A comparison of the results from the two studies can be made by referring to Fig. 7. The behavior of the steroid was not unexpected, since the relatively water-insoluble steroid had preference for its oleaginous vehicle; in the present study, the solubility character of pilocarpine makes its solubilization or retention in the vehicle thermodynamically unfavorable.

The results of the steroid study also prove that the ointment vehicle is indeed retained in the eye for a considerable time, since aqueous humor steroid levels were maintained for up to 7 hr after dosing as compared with 2 hr for a solution. This evidence, coupled with the previously described similar behavior of pilocarpine ointment in both intact and abraded eyes, gives strong support to mechanism (*b*). In this case, a stagnant ointment film is postulated to exist at the corneal surface. The film is not renewed, even under a mechanical influence such as blinking. As a result, permeation of pilocarpine into the cornea would stop as soon as pilocarpine is depleted from the water in the film. This mechanism would adequately account for the observed similarity with intact and abraded eyes; in each case, the film is rapidly depleted of drug and further penetration is inhibited by the occlusive film. However, this film would not present itself as an absolute barrier to the penetration of a drug such as

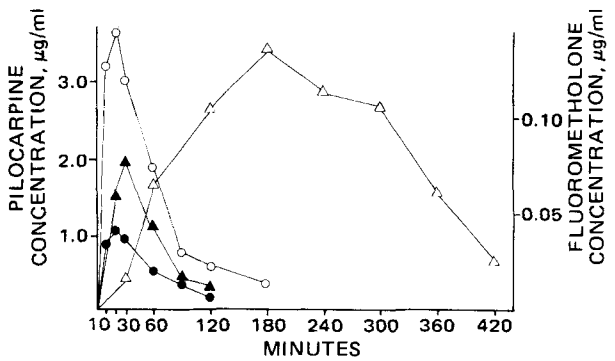

Figure 7—*Aqueous humor levels after dosing with pilocarpine and fluorometholone in ointment and aqueous solution (1, 24). Key:* ●, 10^{-2} M *pilocarpine solution, 25 μl;* ○, 10^{-2} M *pilocarpine ointment, 25 mg;* ▲, *saturated fluorometholone solution, 50 μl; and* △, *0.1% fluorometholone ointment, 50 mg.*

fluorometholone, since the steroid's lipid solubility permits slow diffusion through the vehicle to replenish the concentration of drug in the film adjacent to the corneal surface.

To determine if this mechanism is reasonable, a calculation was performed to determine the amount of pilocarpine present in a film of ointment covering the corneal surface. By assuming an average corneal diameter of 15 mm (37), it can be easily shown that a layer of ointment 10 μm thick contains 3.3 μg of pilocarpine. From analysis of the *AUC* for the ointment, it can be determined that the amount of drug penetrating the cornea to the aqueous humor is 3.2 μg. If 10 μm is accepted as a reasonable thickness for the film, the amount of drug reaching the aqueous humor would be the same as that present in the initial film. This assumption does not appear to be unreasonable, since the original tear film covering the cornea is also about 10 μm thick (38).

Although these calculations appear to confirm the existence of a nonrenewable ointment film at the corneal surface, two major contradictions reject this mechanism. The first, and most obvious, is the observation that larger doses of ointment give increased corneal penetration of pilocarpine. It should be clear that a stagnant film at the corneal surface would not be influenced by the remainder of the dose in the cul-de-sac. If such a mechanism is operative, increasing the size of the dose should have no effect. Second, an additional study showed that predosing the cornea with drug-free ointment vehicle had no effect upon subsequent doses of 10^{-2} *M* pilocarpine ointment or solution. If an occlusive barrier is formed by the vehicle, little or no drug penetration would be possible under such conditions. However, the observed unimpeded penetration of pilocarpine from both dosing systems clearly demonstrates that the ointment film *per se* is not a barrier.

At this point, it is clear that mechanism (*c*) is the only postulate consistent with all data. In this mechanism, pilocarpine is only available from the water present at the ointment surface. This amount of drug, fixed by the size of the dose, mixes with the tears and creates the precorneal drug concentration that is the driving force for corneal drug uptake. This approach easily explains why the same amount of drug penetrates both intact and abraded eyes. A homogeneous dose of ointment of a given size will present the same limited amount of drug, *via* surface mixing, without regard to the condition of the corneal surface. Once this amount has been absorbed or lost *via* the parallel elimination pathways (34), penetration into the cornea ceases, since drug entrapped within the bulk of the ointment is unavailable to the ocular fluids which cannot penetrate the oleaginous vehicle. Increasing the dose will have a positive effect, since the larger surface area allows more drug to mix with the fixed resident tear volume. This creates a higher precorneal drug concentration and subsequent increased corneal penetration.

The overall mechanism of the ointment can thus be described as being composed of combined influences, wherein an increased effective concentration is operating in conjunction with a limited extension of contact time. The ointment exerts a somewhat unusual form of vehicle control inasmuch as it impedes uniform mixing of the entrapped pilocarpine with the ocular fluids. At the same time, it serves to prolong the contact time, particularly with larger doses. The end result is a complex sum of positive and negative effects, with the ointment ultimately achieving superiority over the solution at all times after dosing. However, many characteristics of the solution are retained by virtue of the water contained within the ointment.

Whenever pilocarpine is presented to the cornea in an immediately

available form (*i.e.*, in aqueous solution), it will penetrate to the anterior chamber. The implication of this behavior is that sustained release of pilocarpine to the ocular tissues is only possible if the release of the drug from its vehicle is rate limiting. Simply increasing the contact time (viscosity) of the vehicle is not sufficient, since this process merely results in pulse-dose behavior rather than sustained release. However, the release characteristics must also be such that the dosing system can overcome the constraints of the precorneal pocket in terms of fluid dynamics and nonproductive absorption.

SUMMARY

Several important findings emerged from the present study relative to behavior of pilocarpine in ocular vehicles and subsequent corneal absorption characteristics. The corneal absorption of pilocarpine is increased in neutral to alkaline solutions as compared to acid solutions, in keeping with observations of others. However, the mechanism of this improved absorption does not appear due to the specific pH–partition behavior usually attributed to this phenomenon but rather to the nonspecific lacrimation that occurs at acid pH. Thus, all drugs will show an improved absorption pattern, irrespective of whether they are ionized or not, in the neutral to alkaline range simply because there is less lacrimation in this region and, hence, less drug loss. This observation is important; before a claim of significant pH effect can be made with other drugs, the baseline of lacrimation effect must be considered.

Earlier studies established that polymer solutions would provide only a moderate improvement in the corneal absorption of pilocarpine at the low levels of viscosity generally used for ocular solutions. An ointment system for pilocarpine, based on improved contact time, was also expected to show an improved fraction of dose absorbed and a sustaining effect. However, when using essentially a lipophilic ointment vehicle, no sustaining effect for pilocarpine was observed and only a moderate improvement in the fraction of dose absorbed across the cornea occurred. These observations were contrary to what was observed when the identical ointment was employed with the relatively water-insoluble steroid fluorometholone. This difference was ascribed to the difference in the solubility of the two drugs in the ointment.

Since pilocarpine was insoluble in the vehicle, it was first dissolved in a small amount of water and then incorporated into the ointment to generate a dilute water-in-oil emulsion. This system was unable to provide drug uniformly from the ointment, and only that drug in immediate contact with the tear film became available to eye tissues. The conclusion from the ointment study is that the drug solubility in the ointment dictates the success or failure of this drug delivery system in terms of improved bioavailability and sustaining effect. Extension of the pilocarpine ointment study suggests that an appropriate ointment vehicle for this drug should be water soluble or perhaps an oil-in-water emulsion.

Although definitive guidelines for the formulation of topical ocular drug systems are not complete, we do have considerable quantitative data and theories on two drugs with extremes in water solubility, namely, pilocarpine and fluorometholone. Merely placing a drug in a stable favorite vehicle is inappropriate for ocular drug delivery systems. One must consider the properties of the drug and the vehicle in combination with the unusual characteristics of the precorneal pocket to formulate a drug delivery system with optimum delivery properties. A comprehensive picture of the interplay among drug, dosage form, and site of ocular delivery is emerging.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 22, 1976, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706.*

Accepted for publication October 6, 1976.

Supported by Allergan Pharmaceuticals, Irvine, CA 92664, and Grant EY-01332 from the National Institutes of Health.

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