JOURNAL OF PHARMACEUTICAL SCIENCES

JUNE 1979
VOLUME 68 NUMBER 6

RESEARCH ARTICLES

Mechanistic and Quantitative Evaluation of Precorneal Pilocarpine Disposition in Albino Rabbits

VINCENT HON-LEUNG LEE and JOSEPH R. ROBINSON ˣ

Received July 6, 1978, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706.* Accepted for publication December 13, 1978.

Abstract □ The low ocular bioavailability of topically applied pilocarpine is attributed to extensive precorneal drug loss in conjunction with the resistance to corneal penetration. Several elements of precorneal loss were reported earlier, but a complete mechanistic understanding has not been available. The present study was designed to gain a better understanding of the mechanisms governing pilocarpine disposition in the precorneal areas as well as the relative influence of these parameters on ocular drug bioavailability. Radioactive pilocarpine and glycerin solutions were instilled into the precorneal area of the albino rabbit eye under various experimental conditions, and the drug concentration in the lacrimal lake was monitored as a function of time. The results demonstrated that nonconjunctival loss of pilocarpine, vasodilation due to the drug, and lacrimation due to vehicle formulation are additional aspects of precorneal drug disposition. The individual influence of all precorneal loss parameters on drug bioavailability was then assessed using a mathematical model formulated from experimental findings on both precorneal and intraocular drug disposition. Drainage and vasodilation, as well as nonconjunctival pilocarpine loss, exerted major influences on drug loss at the absorption site.

Keyphrases □ Pilocarpine—precorneal disposition, mechanistic and quantitative evaluation □ Precorneal area—pilocarpine disposition, mechanistic and quantitative evaluation □ Distribution, ocular—pilocarpine, mechanistic and quantitative evaluation □ Dosage forms, topical—pilocarpine, precorneal disposition, mechanistic and quantitative evaluation □ Cholinergics, ophthalmic—pilocarpine, precorneal disposition, mechanistic and quantitative evaluation

The mechanisms by which a topically applied dose of drug disappears from the precorneal area of the eye are important from the standpoint of improving ocular drug delivery systems. Pilocarpine, an important agent in glaucoma management, was selected as the probe drug for investigating this aspect of drug action.

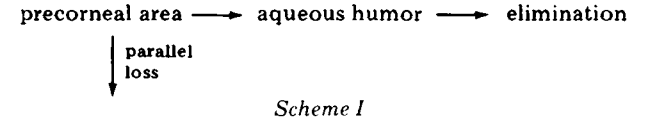
Previous studies with pilocarpine established that solution drainage (1, 2), drug protein binding (3–5), tear turnover (1, 6), and conjunctival absorption (7) compete with corneal absorption for the drug from the precorneal area. Only 1–2% or less of an instilled pilocarpine dose gains access to the internal eye structures (8, 9). While drainage removes a substantial portion of a dose within minutes of instillation, this parameter alone cannot explain the low pilocarpine bioavailability, nor can a combination of the other parameters.

In a similar manner, the large first-order rate constant, $\sim 0.6\ min^{-1}$, associated with the decline of the pilocarpine concentration in the tear film[1] for the critical first 5 min postinstillation is inconsistent with the values reported for the rate constants governing corneal absorption, tear turnover, and conjunctival absorption. Even though drainage has an associated rate constant whose value is $\sim 0.5\ min^{-1}$, it cannot be responsible for the large rate constant associated with precorneal drug loss because drainage is not expected to influence directly tear film drug concentration. In essence, this description for precorneal pilocarpine disposition implies that some as yet unidentified factors, with sizable rate constants, are present.

The purpose of the present study was to gain a better understanding of the mechanisms governing pilocarpine disposition in the precorneal area as well as of the relative influence of these parameters on ocular drug bioavailability.

MODEL

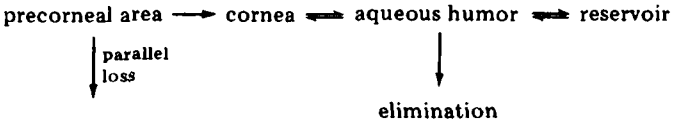
The evolution of mathematical models to describe pilocarpine disposition in the eye reflects the state of knowledge concerning the mechanisms governing its precorneal and intraocular disposition. Scheme I is an example of a commonly used model to describe the fate of pilocarpine



Scheme I

[1] The term tear film or pool is used interchangeably with lacrimal lake. It is assumed that instilled drugs are uniformly mixed with tear fluid.

0022-3549/79/0600-0673$01.00/0



Scheme II

following topical instillation. Two deficiencies of such a model are that it considers the cornea as a single membrane with no apparent role in the disposition process and that it lumps all precorneal drug disposition constants into one large rate constant.

A more recent model (10) (Scheme II) corrects for the first deficiency by including the cornea as a component. However, it does not distinguish the specific roles exercised by the corneal epithelium and corneal stroma in the movement of pilocarpine through the cornea (11).

Both models suffer from the additional drawback of not considering the effects of instilled drop size explicitly. This important precorneal factor forms a component of a recently proposed model (12) which, for simplicity, treats the cornea as a single membrane, as in Scheme I. In summary, all of these models are incomplete descriptions of the fate of pilocarpine in the precorneal area and in the various ocular tissues.

The present study began with construction of a model that was cognizant of the deficiencies mentioned (Scheme III). The model partitions the precorneal loss rate constant into its component parts for a mechanistic understanding of the processes involved. In addition, it incorporates the findings of Sieg and Robinson (11) on pilocarpine disposition: (*a*) the corneal epithelium serves as both a barrier and a depot, and (*b*) the corneal stroma and endothelium are kinetically indistinguishable from the aqueous humor. Table I details the model parameters. The major assumptions introduced while constructing the model were:

1. Instantaneous and complete mixing of instilled drug solution and tears occurs.
2. Pilocarpine metabolism in the tear fluid is negligible.
3. The tissues comprising the compartments are homogeneous.
4. The iris, ciliary body, lens, and vitreous humor constitute the reservoir.

Moreover, the present model will not explicitly consider either pilocarpine protein binding in tear fluid or ocular tissues or the resistance offered by diffusional boundary layers to membrane drug transport.

Proceeding from these assumptions, appropriate differential equations can be written to account for the rate of change in the *mass* of drug in the compartments shown in Scheme III. The volume element of disposition kinetics in the precorneal area is treated in a manner akin to that described previously (12), which is based on the concept of mass balance in open systems advanced earlier (13). Drug transfer, on the other hand, is considered to be a series of simple diffusion steps taking place simultaneously and in succession, with each step governed by Fick's first law of diffusion, in much the same manner as Teorell (14) derived formulas to describe the amount of drug in the body as a function of time. Accordingly, the amount of drug transferred per unit time, M, can be written (15):

$$M = k_p S\,\Delta C \qquad \text{(Eq. 1)}$$

where k_p is the permeability coefficient in centimeters per second, S is the effective surface area in square centimeters, and ΔC is the concentration difference in grams per cubic centimeters. Since k_p and S have not been determined, they are treated in the present model as a single coefficient denoted as p and given by $p = k_p S$ with units of cubic centimeters per second.

Equation 2 describes the rate of change in amount of pilocarpine in the precorneal area:

$$\frac{dV_A(t)[A]}{dt} = -p_p([A] - [B]) - k_d[V_A(t) - V_0][A] - Q_T(t)[A] - p_n[A] \qquad \text{(Eq. 2)}$$

Table I—Parameters of the Model Described in Scheme III

Parameter	Coefficient Associated With[a]
p_p	Transfer of pilocarpine between precorneal area and corneal epithelium
p_n	Nonproductive loss
k_d	Drainage
$Q_T(t)$	Tear flow
p_a	Transfer of pilocarpine between epithelium and corneal stroma–endothelium–aqueous chamber
p_m	Drug loss *via* metabolism in or lateral diffusion from epithelium
p_{ao}	Drug elimination from aqueous humor
p_r	Transfer of pilocarpine between corneal stroma–endothelium–aqueous chamber and reservoir
p_{ro}	Pilocarpine elimination from reservoir

[a] With the exception of k_d, the coefficients have dimensions of microliters per minute. See text for details.

where $[A]$ denotes the drug concentration in the precorneal area (or tear chamber), $[B]$ is the drug concentration in the corneal epithelium, $V_A(t)$ is the volume of fluid in the tear chamber at a given time, V_0 is the normal resident tear volume, and all other symbols are as previously described. Sink conditions are assumed to be maintained in the blood supplying the conjunctiva and related structures.

Thus, from Eq. 2, the change in amount of pilocarpine in the precorneal area is due to corneal absorption, solution drainage, tear flow, and nonproductive loss. As will be evident in the *Discussion* section, nonproductive loss is composed of conjunctival absorption, nonconjunctival loss, and the influence of pilocarpine vasodilation on several of these processes. Likewise, tear flow is composed of normal tear turnover and induced lacrimation.

Induced lacrimation is interpreted here as increased tear flow rate; consequently, it will not change the resident precorneal volume appreciably since a corresponding volume will leave the area. This appears to be the case, as there was no visually apparent fluid accumulation in the precorneal area following dosing. In essence, this treatment views induced lacrimation and instilled volume-dependent drainage as noninteracting entities. This simplification is justifiable based on an earlier study (1) in which the influence of pH on lacrimation could be estimated. The technetium sulfur colloid preparation pH was not reported in that study but was ~6.5, which was close to the pH of most pilocarpine solutions employed in the present study. Therefore, in terms of a change in precorneal fluid volume, the k_d values thus determined account not only for the contribution of instilled volume but also for that of induced lacrimation. Interestingly, the k_d value determined (1) for an instilled volume of 25 μl is consistent with that determined recently (16) by microscintigraphy for a pH 6.24 sodium pertechnetate solution. Combining this observation with the vehicle-induced lacrimation demonstrated in the present study suggests that Eq. 3 is appropriate for describing the rate of change in precorneal fluid volume:

$$\frac{dV_A(t)}{dt} = -k_d[V_A(t) - V_0] \qquad \text{(Eq. 3)}$$

Upon integration, Eq. 3 yields:

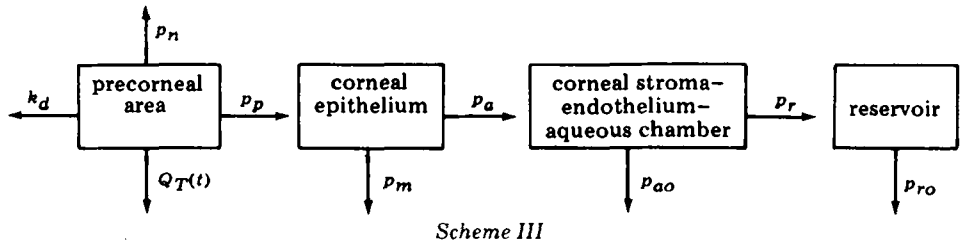
$$V_A(t) = V_0 + V_i \exp(-k_d t) \qquad \text{(Eq. 4)}$$

where V_i is the instilled volume. Equation 3 can be combined with Eq. 2:

$$\frac{d[A]}{dt} = -\left(\frac{p_p + p_n}{V_A(t)} + \frac{Q_T(t)}{V_A(t)}\right)[A] + \frac{p_p}{V_A(t)}[B] \qquad \text{(Eq. 5)}$$

In Eq. 5, $Q_T(t)$, like $V_A(t)$, is a time-dependent quantity, as shown in the empirical expression:

$$\frac{dQ_T(t)}{dt} = -k_l[Q_T(t) - Q_0] \qquad \text{(Eq. 6)}$$



Scheme III

Table II—Apparent Rate Constants for Routes of Precorneal Loss Established for Pilocarpine prior to the Present Study

Loss Route	Associated Rate Constant	Reference
Corneal absorption	0.004 min^{-1}	10, 11
Drainage, 25 μl	0.54 min^{-1}	1
Tear flow	0.66 μl/min	1
Nonproductive loss[a]	0.0217 min^{-1}	7

[a] This reported value was attributed to conjunctival absorption.

which is analogous in form to Eq. 3. Upon integration, Eq. 6 yields:

$$Q_T(t) = Q_0 + Q_i \exp(-k_l t) \qquad \text{(Eq. 7)}$$

According to this equation, the tear flow rate immediately following dosing (*i.e.*, $t = 0$) is the sum of the normal tear flow rate, Q_0, and that due to the instillation of a dose, Q_i. Eventually, the contribution of induced lacrimation to tear flow disappears, and the rate at which this occurs is governed by the rate constant k_l.

The essential feature of Eq. 5 concerns the dimension of the quantity:

$$\left[\frac{p_p + p_n}{V_A(t)} + \frac{Q_T(t)}{V_A(t)}\right]$$

which is that of a first-order rate constant. Nevertheless, it should not be considered as a constant since $V_A(t)$ and $Q_T(t)$ are functions of time. Thus, the quantity derived from the slope of a semilogarithmic plot of tear film drug concentration *versus* time is time averaged. The implication is that to convert the experimental first-order rate constant to the parameters p_p and p_n, knowledge of a time-averaged volume is needed. Of course, if a nonsampling technique that continuously monitors tear film drug concentration is available, $d[A]/dt$ will be known accurately as a function of time and a time-averaged volume is not needed. In this report, the first approach will be adopted, but $V_A(t)$ cannot be construed as a constant in subsequent computer simulations. The procedure for finding the time-averaged volume will be presented under *Discussion*.

For the case in which:

$$\frac{p_p}{V_A(t)}[B] \ll \left(\frac{p_p + p_n + Q_T(t)}{V_A(t)}\right)[A]$$

and $k_l = k_d$, Eq. 5 can be combined with Eqs. 4 and 7 to give an expression which, upon integration, yields a final expression describing the time dependence of drug concentration in the precorneal area following topical instillation:

$$[A] = [A]_i \left(\frac{V_i}{V_A(0)}\right)\left(\frac{V_A(0)}{V_A(t)}\right)^b \exp(-k''t) \qquad \text{(Eq. 8)}$$

where $[A]_i$ is the instilled drug concentration, V_i is the instilled volume, and:

$$b = \left(\frac{p_p + p_n + Q_0}{V_0} - \frac{Q_i}{V_i}\right)\frac{1}{k_d}$$

$$k'' = \frac{p_p + p_n + Q_0}{V_0}$$

$$V_A(0) = V_i + V_0$$

Several features of Eq. 8 are of interest. First, the parameters governing nonproductive loss, p_n, and pilocarpine transfer between the precorneal area and the corneal epithelium, p_p, contribute, along with the parameter governing normal tear flow, Q_0, to the exponent b. This result will not be obtained when one neglects the effect of volume on apparent first-order rate constants (17), in which case only Q_0 contributes. Second, the initial dilution of an instilled dose is accounted for by the term $V_i/V_A(0)$. Third, the equation accounts for the peculiar influence of drainage on drug concentration decline, which is one of deceleration. The key to the preceding statement lies in the terms $[V_A(0)/V_A(t)]^b$ and $\exp(-k''t)$ in Eq. 8. Whereas the term $\exp(-k''t)$ decreases continuously with time, the term $[V_A(0)/V_A(t)]^b$ increases with time [until $V_A(t)$ = normal resident tear volume], thereby counteracting the diminishing effect of the former. Fourth, the term $[V_A(0)/V_A(t)]^b$ yields the expected curvature observed in a semilogarithmic plot of tear film drug concentration *versus* time.

Equation 8 will be employed, as shown in the *Appendix*, to establish an expression relating the coefficient p and the apparent first-order rate constant k_a for a given parameter associated with the decline of the pilocarpine concentration in the precorneal area. Whether it is a good approximate equation to use depends largely on the relative magnitude of b and k''; the approximation improves as b exceeds k'' by a factor of at least 5. For the range of parameter values encountered in this study, the error due to the approximation does not exceed 7%, a tolerable error for these purposes[2].

Finally, mass balance equations similar to Eq. 5 can be written for the other compartments in Scheme III. They are:

$$V_B \frac{d[B]}{dt} = p_p([A] - [B]) - p_m[B] - p_a([B] - [C]) \qquad \text{(Eq. 9)}$$

$$V_C \frac{d[C]}{dt} = p_a([B] - [C]) - p_r([C] - [D]) - p_{ao}[C] \qquad \text{(Eq. 10)}$$

$$V_D \frac{d[D]}{dt} = p_r([C] - [D]) - p_{ro}[D] \qquad \text{(Eq. 11)}$$

In Eqs. 9–11, A represents the precorneal area, B represents the corneal epithelium, C is the corneal stroma–endothelium–aqueous chamber, and D is the reservoir; the V's refer to the volumes of the compartments indicated by the subscripts. Analytical expressions that might be obtained from these equations are too complicated to have practical value. Fortunately, much can be learned about the behavior of pilocarpine in the eye by solving the system of first-order linear differential equations, *i.e.*, Eqs. 3, 5, 6, and 9–11, using numerical methods. This approach was used in the simulation studies.

Simulation studies with the precorneal drug loss parameter values listed in Table II failed to duplicate tear film drug loss, suggesting the need for additional loss routes to complete the profile. Establishing these loss routes and quantifying the influence of all precorneal factors on ocular drug bioavailability are the purposes of this report.

EXPERIMENTAL

Materials—Tritiated pilocarpine alkaloid (specific activity of 4.2 Ci/mmole) in ethanol was obtained commercially[3] and purified by vacuum evaporation immediately prior to each run, as previously described (8). [^{14}C]-Glycerol[3] sterile aqueous solution (specific activity of 96.0 μCi/mg) was used as received. All other chemicals were either reagent or analytical grade and were used as received.

Male albino rabbits[4], 1.8–2.4 kg, were used throughout the studies. They were fed a regular diet with no restrictions on food or water consumed. Lighting and auditory stimuli were maintained on a 24-hr basis in the caging facility.

Solution Preparation—*0.01* M *Pilocarpine Alkaloid Solution*—A 0.01 *M* pilocarpine alkaloid solution, prepared in Sorensen phosphate buffer at the appropriate pH (6.24 or 7.38), was added to the labeled material prior to each experiment. Solutions were made isoosmotic with tears using sodium chloride. Each microliter of the resulting solution had an activity of ~75,000 cpm.

0.14 M *Glycerin Solution*—An aqueous aliquot of the isotopic species was added, prior to each experiment, to a 0.14 *M* glycerin solution prepared in pH 6.24 Sorensen phosphate buffer. The resulting solution was isoosmotic with tear fluid, and each microliter of solution had an activity of ~10,000 cpm.

0.14 M *Glycerin Solution Containing 0.01* M *Pilocarpine Alkaloid*—Equal portions of a 0.28 *M* glycerin solution and a 0.02 *M* pilocarpine alkaloid solution, both in pH 6.24 Sorensen phosphate buffer, were mixed immediately prior to each experiment. An aqueous aliquot of [^{14}C]-glycerin was added to the mixture, and the resulting solution was made isoosmotic with tears using sodium chloride. Each microliter of the final solution had an activity of ~10,000 cpm.

The small amount of labeled substances added to these three solutions did not appreciably alter the molarity of the final solutions. The final counts per minute for each solution was selected to achieve optimal counting efficiency.

0.1% Epinephrine Bitartrate and 0.25% Histamine Dihydrochloride Solutions—A 0.1% epinephrine bitartrate solution was prepared by diluting a commercial solution[5] with isoosmotic pH 7.38 Sorensen phosphate buffer. A 0.25% histamine dihydrochloride solution was prepared by dissolving the salt[6] with the same buffer. To minimize any direct irritating effect from these solutions on precorneal pilocarpine disposition, pilocarpine solutions were not instilled onto the eyes of experimental

[2] Unpublished calculations.
[3] New England Nuclear, Boston, Mass.
[4] Klubertanz, Edgerton, Wis.
[5] Epitrate (2%), Ayerst Laboratories, New York, N.Y.
[6] Sigma Chemical Co., St. Louis, Mo.

animals until 15 min had elapsed following application of the histamine or epinephrine solutions.

Epinephrine bitartrate was employed for its vasoconstrictive effect on blood vessels perfusing the nictitating membrane and the conjunctiva, whereas histamine dihydrochloride was employed for its vasodilating effect. The concentration of each agent was selected based on conventional usage and appeared, by visual inspection, to exert the desired effect over the experiments.

Anesthesia—When necessary, anesthesia was induced and maintained by phenobarbital sodium (50 mg/ml iv) administered *via* the marginal ear vein. The rabbits were anesthetized ~15 min prior to instillation of the pilocarpine solution.

Tear Film Pilocarpine Concentration–Time Profile—Disappearance of pilocarpine from the tear film was monitored under the following experimental conditions: (*a*) normal unanesthetized rabbits, (*b*) normal anesthetized rabbits, (*c*) unanesthetized rabbits whose ocular surfaces were treated with 25 μl of a 0.1% epinephrine bitartrate solution 15 min prior to instillation of the pilocarpine solution, (*d*) anesthetized rabbits whose ocular surfaces were treated as in (*c*), (*e*) unanesthetized rabbits whose ocular surfaces were dosed with 25 μl of a 0.01 *M* nonradioactive pilocarpine solution 15 min prior to instillation of the dosing solution, (*f*) unanesthetized rabbits whose ocular surfaces were dosed with 25 μl of a 0.25% histamine dihydrochloride solution 15 min prior to instillation of the pilocarpine solution, and (*h*) unanesthetized rabbits receiving 25-μl doses of a 0.01 *M* pilocarpine solution at pH 7.38.

During the experiments, all test animals were kept in restraining boxes in a normal upright posture. Both eyes of the rabbit were employed. Pilocarpine solution, 25 μl, was instilled directly onto the cornea of the test animal, collecting in the cul-de-sac. During instillation, the upper lid was slightly raised and the lower lid was pulled slightly away from the globe. The lids were immediately returned to their normal position after instillation.

One-microliter tear samples were removed at 0.25, 1, 2, 3, 4, and 5 min postinstillation using 1-μl disposable glass capillary pipets[7]. The earliest sample was taken 15 sec after instillation to allow for mixing of the instilled solution with the tear fluid. During the early time period following drug instillation, sample removal has a small influence on the overall rate constant governing the decline in pilocarpine concentration since the precorneal tear volume is substantial and the number of samples taken is small. Moreover, removal of a small volume of fluid from the tear film–drug pool has only an indirect influence on drug concentration in subsequent samples.

During sampling, extreme care was exercised to avoid eye irritation. Thus, the capillary pipet tip was placed into the tear pool in contact with the cornea along the lower lid margin without touching any eye tissue. For consistency, all samples were withdrawn from the center of the marginal tear strip. On those rare occasions when the entire pipet was not filled with tear fluid, the volume of sample withdrawn was estimated from the depth to which the capillary pipet was filled. Pipets containing tear samples were transferred to vials[8] containing 5 ml of prerefrigerated scintillation cocktail[9] and counted in a liquid scintillation spectrometer[10] after 24 hr of storage in the dark. The presence of glass capillaries in the scintillation cocktail did not alter the counting efficiency or affect the results in any way, in agreement with earlier findings (18).

After suitable corrections, the data in number of counts per minute per microliter of tear fluid were plotted semilogarithmically as a function of time and subjected to linear regression analysis. First-order rate constants were obtained for the decline of the pilocarpine concentration in the precorneal area of each rabbit.

Tear Film Glycerin Concentration–Time Profile—Only anesthetized rabbits were employed in this portion of the study. The dosing solutions were: (*a*) glycerin solution containing no pilocarpine and (*b*) glycerin solution with 0.01 *M* pilocarpine. Positioning of the test animals, dosing and sampling procedures, and data analysis were as described in the preceding sections.

Influence of Instilled Dose Volume on Pilocarpine Disappearance from Tear Film—Ten microliters of a 0.01 *M* pilocarpine alkaloid solution was instilled onto the cornea of anesthetized rabbits as described. Sampling procedure and data analysis were as described.

Computer Simulation Studies—The computer program to simulate pilocarpine disposition in the albino rabbit eye consists of two major subroutines, DERIVS and INTGRT. The system of differential equations is presented in the subroutine DERIVS, which is introduced into the main program *via* the differential equation routine DEPC[11]. DEPC employs the predictor–corrector method outlined previously (19) to solve numerically initial value problems of ordinary first-order differential equations.

The subroutine INTGRT performs integration by Simpson's rule on the results produced by the differential equation routine. In addition to simulating single-dose situations, the program is capable of treating: (*a*) multiple-dose problems with variable dose size and volume given at intervals that need not be equal and (*b*) situations where the dose is removed before absorption is complete.

All computer calculations were performed on a digital computer[12], and the specific simulations carried out are indicated under *Results* and *Discussion*.

Table III—Compilation of First-Order Rate Constants Governing Initial Decline in Drug (Pilocarpine and Glycerin) Concentration under Various Experimental Conditions

Experimental Condition	k_a, min^{-1}
Pilocarpine (pH 6.24, 25-μl dose)	
Unanesthetized	0.63 (0.06)[a] [10][b]
Pretreated with epinephrine	0.40 (0.050) [7]
Pretreated with pilocarpine	0.68 (0.11) [4]
Pretreated with histamine	1.03 (0.14) [6]
Anesthetized	0.30 (0.05) [5]
Pretreated with epinephrine	0.072 (0.012) [6]
Pilocarpine (pH 6.24, 10-μl dose)	
Anesthetized	0.46 (0.009) [7]
Pilocarpine (pH 7.38, 25-μl dose)	
Unanesthetized	0.43 (0.042) [6]
Glycerin (pH 6.24, 25-μl dose)	
Anesthetized	0.13 (0.014) [8]
Dosing solution incorporated with pilocarpine	0.39 (0.043) [5]

[a] Number in parentheses represents standard error of the mean. [b] Number in brackets represents number of determinations.

RESULTS

Disposition of Pilocarpine and Glycerin in Precorneal Area—With the possible exception of histamine dihydrochloride, the compounds instilled into the cul-de-sac of the rabbits at the concentrations employed do not damage the corneal epithelium (20, 21). Consequently, alteration in apparent rate constants could not be due to enhanced absorption of pilocarpine as a result of corneal damage from these compounds.

In fitting the data to first-order decline by linear regression analysis, no attempt was made to force the best fit line through the theoretical counts per minute per microliter of tear fluid at time zero, due mainly to uncertainties in the mixing efficiency of instilled dose with the resident tear fluid. On the average, the deviations of the time zero values given by these best fit lines from the corresponding values calculated by assuming instantaneous mixing were less than 5%. In most cases, the coefficient of determination for the regression exceeded 0.95.

Following topical instillation of a given volume of pilocarpine solution in the eye, the tear film drug concentration declined rapidly. Figure 1 is an example of a semilogarithmic plot of counts per minute per microliter of tear fluid against time. Table III summarizes the apparent first-order rate constants governing tear film drug concentration decline under various experimental conditions. In normal unanesthetized rabbits, the decline in pilocarpine concentration had an associated rate constant of 0.63 min^{-1}. Changing the instilled solution pH from 6.24 to 7.38 produced a 1.5-fold reduction in the associated rate constant. In contrast, in the presence of anesthesia, the rate constant was reduced by ~50%. Predosing the eye with vasoactive agents also modified the first-order rate constant. Specifically, histamine dihydrochloride, a vasodilator, increased the rate constant by a factor of 1.5; epinephrine bitartrate, a vasoconstrictor, reduced it by the same factor. The reduction in the rate constant due to epinephrine was more prominent when anesthesia was present (Table III).

Table III also shows that, in the presence of anesthesia, glycerin disappeared from the precorneal area with an associated rate constant ~50% less than that for pilocarpine. However, when administered in combination with pilocarpine, the associated rate constant increased and was

[7] Curtis Matheson Scientific, Houston, Tex.
[8] Mini Vial, Research Products International Corp., Elk Grove Village, Ill.
[9] Aquasol, New England Nuclear, Boston, Mass.
[10] Model 2002, Packard Instrument Corp., Downers Grove, Ill.
[11] Differential Equations Reference Manual for the 1108, Madison Academic Computing Center, Madison, Wis.
[12] UNIVAC 1110, Madison Academic Computing Center, Madison, Wis.

Table IV—Significance Testing [a] for Selected Cases Listed in Table III

Experimental Condition	p
Unanesthetized[b] *versus* (unanesthetized + pilocarpine)	<0.3366
Unanesthetized *versus* (unanesthetized + epinephrine)	<0.0036
Unanesthetized *versus* (unanesthetized + histamine)	<0.0106
Anesthetized[b] *versus* (anesthetized + epinephrine)	<0.0009
Anesthetized (25 μl) *versus* anesthetized (10 μl)	<0.0056
Anesthetized (glycerin) *versus* anesthetized (pilocarpine)	<0.0041
[Anesthetized (glycerin) + pilocarpine] *versus* anesthetized (pilocarpine)	<0.1004
Unanesthetized *versus* anesthetized (10 μl)	<0.0047
Unanesthetized (pH 6.24) *versus* unanesthetized (pH 7.38)	<0.0065
Anesthetized (pH 6.24) *versus* unanesthetized (pH 7.38)	<0.0366

[a] Student t-test. [b] Unless otherwise indicated, these cases referred to those in which pilocarpine was the drug employed.

statistically indistinguishable from that obtained for pilocarpine at the 95% confidence level (Table IV).

Finally, the first-order rate constant of decline increased by a factor of 1.5 when the instilled volume was reduced from 25 to 10 μl. At the 95% confidence level, this difference was statistically significant (Table IV).

Computer Simulation of Precorneal and Intraocular Pilocarpine Disposition—Table V lists the literature-adapted parameter values used in the initial simulation (1, 7, 11, 17, 22). Each parameter, p, associated with drug transfer between compartments, *e.g.*, aqueous humor → reservoir, was obtained by multiplying the corresponding first-order rate constant by the donor compartment volume. Since the corneal epithelium and stroma volumes were not determined, only their estimates are included. In regard to precorneal factors, the associated first-order rate constants (Table II) were employed. The exception was nonproductive loss, for which a value of 0.258 min^{-1} was shown (17) to yield better agreement with the tear film pilocarpine concentration–time profile established in the present study. For estimating p_p and p_n, a time-averaged precorneal fluid volume was employed; its rationale was discussed earlier. The final version of the precorneal parameter values is presented in Table VI. The initial estimates shown in Table V were modified only slightly.

Table V—Initial Estimates of the Parameter Values Associated with Ocular Distribution of Pilocarpine in the Albino Rabbit

Parameter	Value
Precorneal factors	
p_p	0.13 μl/min
k_d	0.54 min^{-1}
p_n	8.38 μl/min
Q_0	0.66 μl/min
Q_i	2.48 μl/min
k_l	0.54 min^{-1}
Intraocular factors	
p_a	0.6 μl/min
p_{ao}	4.04 μl/min
p_r	2.02 μl/min
p_{ro}	0 μl/min
Volumes	
V_0	7.5 μl
V_B	6 μl
V_C	250 μl[a,b]
V_D	325 μl[b]

[a] Volume of stroma is assumed to be 32 μl. [b] V_C and V_D add up to the volume of distribution value of 575 μl reported by Conrad and Robinson (22).

Figure 2 displays the simulated tear film drug concentration profile in unanesthetized rabbits for the first 5 min postinstillation. The slight curvature evident was due to drainage. Unless the experimental data are extremely precise, this curvature usually will not be detected. Consequently, the initial decline in drug concentration can often be approximated by a first-order process. The slope of the best-fit line yielded a first-order rate constant of 0.68 min^{-1}, which resided within the 95% confidence interval of the experimental value. This observation, in conjunction with the values reported for previously identified precorneal disposition factors, supported the notion that additional factors are critical to precorneal loss of pilocarpine.

The corresponding plot for the amount of drug in the tear film is depicted in Fig. 3. Here, the slope was approximately 1.5 times larger than that governing the decline in drug concentration. The important point is that the apparent first-order rate constant controlling the decline in drug concentration is not necessarily that controlling the decline in the amount of drug.

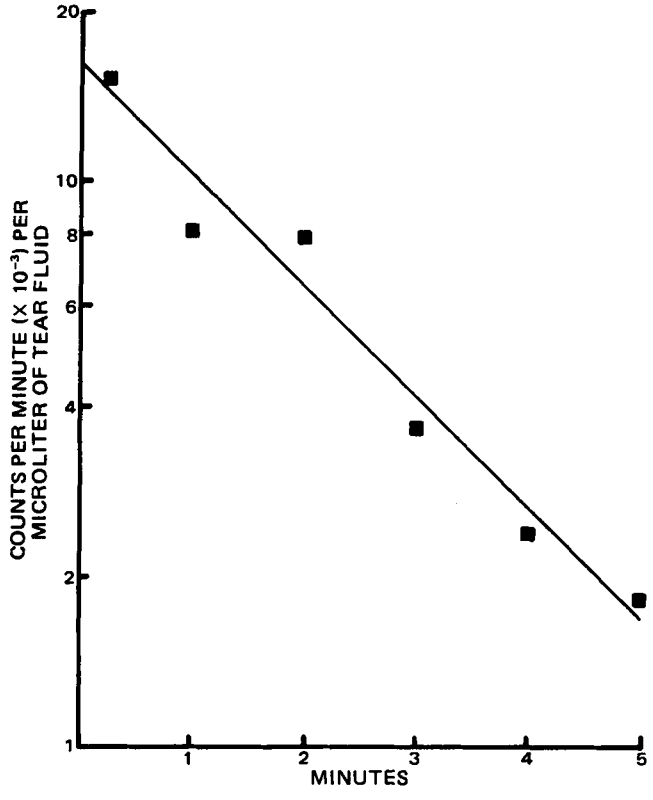


Figure 1—*Decline in the pilocarpine concentration (expressed as counts per minute per microliter of tear fluid) in the tear film following instillation of 10 μl of a 0.01* M *pilocarpine solution in an anesthetized rabbit.*

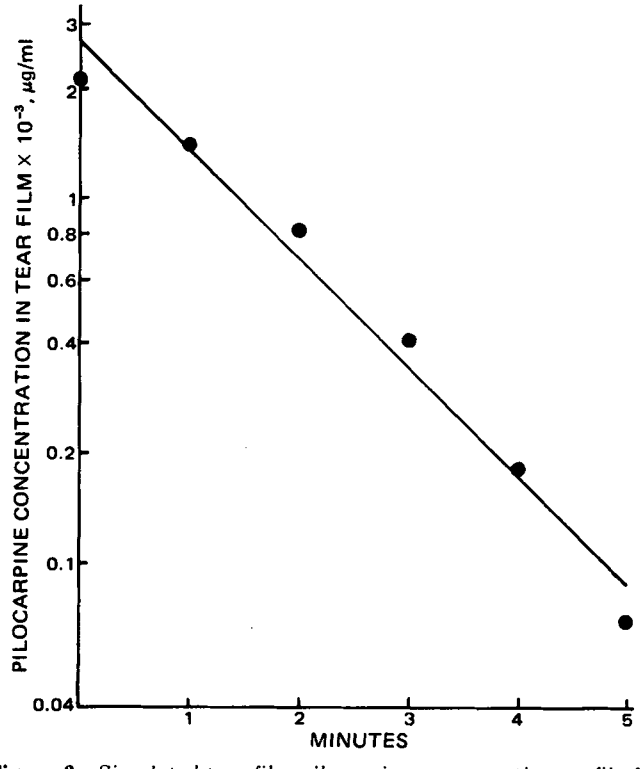


Figure 2—*Simulated tear film pilocarpine concentration profile for the first 5 min postinstillation. The solid circles represent the concentration of drug in the tear film calculated for a given time from the parameter values listed in Table V. The solid line represents the best-fit line, with* $R^2 = 0.98$ *and slope* $= -0.68\ min^{-1}$.

Table VI—Relationship between the Volume-Independent Value and First-Order Rate Constant of Precorneal Drug Loss Parameters

Parameter Associated with	p^a, μl/min	k^b, min^{-1} Unanesthetized	Anesthetized
Corneal absorption	0.13	0.006	0.005
Conjunctival absorption	0.71	0.033	0.025
Nonconjunctival loss	2.80	0.13	0.10
Vasodilation due to pilocarpine	4.76	0.22	0.17
Normal tear flow	0.66	0.031	0
Induced lacrimation	4.75	0.22	0
Decline in induced lacrimation	—	2	0
Drainage	—	0.54	0.18
Equation relating p and k		$k = 0.047p$	$k = 0.036p$

[a] Volume-independent value. [b] Apparent first-order rate constant.

Figure 4 is a simulated aqueous humor drug concentration profile, and the fit to experimental data is good. Based on the profiles displayed in Figs. 2 and 4, the proposed model is excellent in describing precorneal disposition of pilocarpine. A deficiency of the model concerns accounting for pilocarpine disposition within the cornea beyond 20-min postinstillation. In particular, the amount of pilocarpine in the cornea for this time interval, calculated according to the model, is approximately twice that obtained experimentally. As a result of systematically varying the magnitude of relevant parameter values, it becomes clear that a model that satisfactorily describes both precorneal and corneal disposition of pilocarpine must await additional studies on drug movement within the cornea.

DISCUSSION

Mechanisms Governing Precorneal Pilocarpine Disposition—The existence of efficient parallel loss processes acting in concert to remove drug from the precorneal area of the eye is unequivocal. This is attested to by the early peak time established in the corneal epithelium and aqueous humor following instillation of a pilocarpine solution (10, 11) and by the small fraction of an instilled dose that successfully gains access to the internal eye structures (8, 9). The large rate constant, 0.63 min^{-1}, associated with the decline in pilocarpine concentration in the tear film of unanesthetized rabbits provides a quantitative measure of the overall parallel loss effect. Obviously, from the standpoint of improving ocular drug bioavailability by formulating maximally efficacious ocular drug delivery systems, it would be helpful to identify all components of precorneal drug loss as well as the associated rate constants.

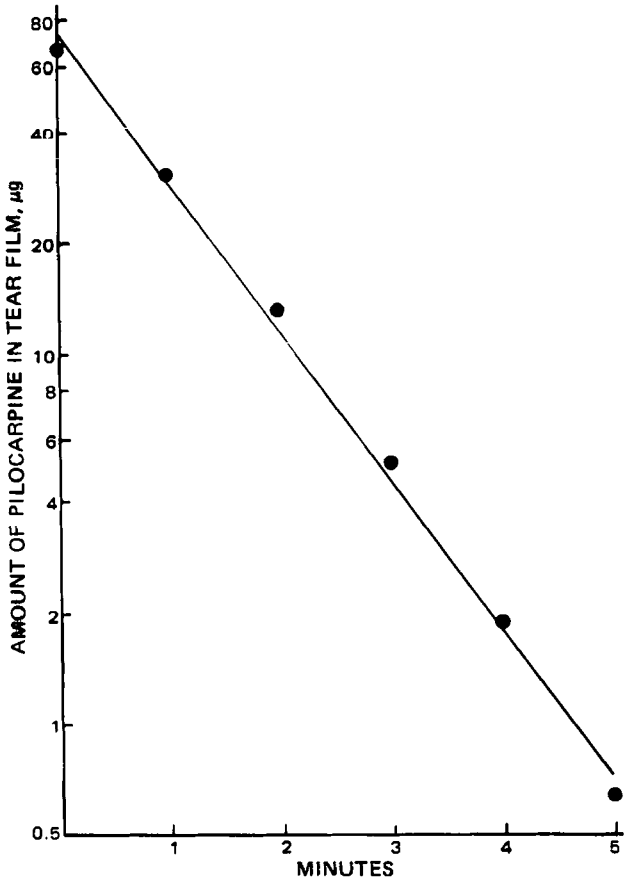


Figure 3—*Simulated profile for amount of pilocarpine in tear film for the first 5 min postinstillation. The solid circles represent the amount of drug in the tear film calculated for a given time from the parameter values listed in Table V. The solid line represents the best-fit line, with* $R^2 = 0.99$ *and slope* $= -0.93\ min^{-1}$.

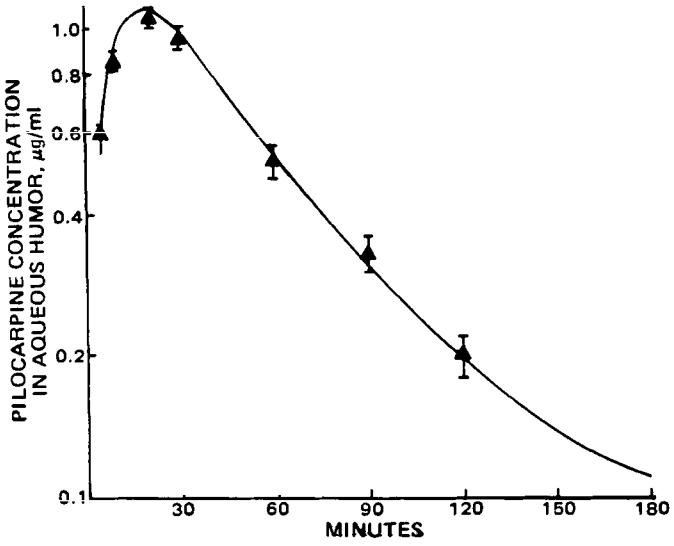


Figure 4—*Concentration of pilocarpine in aqueous humor of albino rabbits following topical instillation of a 0.01* M *solution. Key:* ▲, *data from Ref. 11; and —, simulated profile generated with parameter values listed in Table V.*

As mentioned earlier, the reported loss parameters of solution drainage (1, 2), drug protein binding (3–5), tear turnover (1, 6), and conjunctival absorption (7), by themselves, cannot account for the large first-order rate constant governing precorneal pilocarpine loss. To reconcile this discrepancy, several possibilities can be proposed, and each will be examined here in turn.

Incorrect Reported Rate Constants

1. Underestimation of Reported Rate Constants—The two reported loss routes of interest are corneal and conjunctival absorption. Although the rate constant for corneal pilocarpine absorption has not been determined, good estimates place it at least one or two orders of magnitude less than the rate constant governing conjunctival absorption and overall tear film drug loss. As a result, it is of minor consequence to the overall tear film drug loss rate constant.

The 0.0217 min^{-1} rate constant reported for nonproductive loss due to conjunctival pilocarpine absorption is in line with reported values for other substances, including the rate constant (23) for passive diffusion of sodium and chloride ions across the conjunctiva. Additional support comes from a consideration of the difference in surface area of the cornea and conjunctiva available for drug absorption (24) and of the data of a recent study (25) on conjunctival absorption. Therefore, it can be concluded that the reported conjunctival absorption rate constant for pilocarpine (7) is probably accurate.

2. Volume Effect on Apparent First-Order Rate Constants—It is of interest to evaluate the extent to which the discrepancy between 0.30 min^{-1}, observed in the anesthesia experiment[13], and 0.0217 min^{-1}, reported for nonproductive loss, can be attributed to a volume effect on apparent rate constants. In the study by Patton and Robinson (7), the experiment that yielded the nonproductive loss rate constant of 0.0217 min^{-1} was conducted in rabbits whose drainage apparatus were mechanically blocked. Under such circumstances, the precorneal fluid volume at all times would be approximately 32.5 μl, essentially the sum of instilled volume (25 μl) and resident tear fluid volume (7.5 μl). In contrast, the present study was performed with rabbits possessing functional drainage apparatus, meaning that the fluid volume in the precorneal area

[13] Ignoring the additional routes of precorneal drug loss for the moment leaves nonproductive loss as the only loss route available in the presence of anesthesia.

underwent an approximate fourfold change, from 32.5 μl at the moment of drop instillation to 7.5 μl shortly thereafter.

Teorell (14) showed that the first-order rate constant associated with drug movement from one compartment to the next is inversely related to the fluid volume in the donor compartment. In the present study, the effect of instilled volume on k_a was established in the experiment in which a 10-μl rather than a 25-μl dose was instilled into the cul-de-sacs of anesthetized rabbits. Table III shows that k_a underwent a 1.5-fold increase for a 2.5-fold reduction in instilled volume. On this basis, this volume effect by itself can *at most* contribute a factor of approximately 4.5, in which case a rate constant of 0.094 min^{-1} is obtained for nonproductive loss. Clearly, while volume influences the magnitude of the rate constant, it is not the principal explanation for the large rate constant associated with precorneal pilocarpine loss.

Different Mechanism for Corneal Pilocarpine Uptake—Some years ago, Kakemi *et al.* (26–29) reported that the amount of drug absorbed in the GI tract correlated well with its extent of binding to mucin lining the mucosa; they suggested that drug binding to mucin constituted the first step in absorption. Conceivably, the same phenomenon can occur in the mucin layer that coats the corneal surface (30). The anesthesia experiment in the present study, however, minimized the possibility of rapid uptake of pilocarpine by some component(s), including mucin, on the corneal surface as being primarily responsible for the large rate constant, k_a, associated with a decline in precorneal pilocarpine concentration. For this mechanism to be operative, k_a should register no appreciable change in its value under anesthesia.

Additional Loss Routes

1. Induced Lacrimation—If an increase in tear flow rate due to instilling a pilocarpine solution is a parallel loss route, the apparent rate constant, k_a, governing drug loss from the precorneal area must show a reduction in its value in the presence of anesthesia since lacrimation as well as normal tear flow is inhibited under this condition (1). The experimental value of 0.30 min^{-1} obtained in anesthetized rabbits, an appreciable reduction when compared with the value of 0.63 min^{-1}, indicates that induced lacrimation is indeed a pathway of drug loss. It is not the only route, however, because k_a does not converge to the value of 0.0217 min^{-1} determined (7) for nonproductive loss of pilocarpine to tissues surrounding the tear chamber.

The results of the experiment using a pH 7.38 instead of a pH 6.24 solution demonstrate that much of the lacrimation effect is due to vehicle formulation. Although this observation is consistent with other findings (16) on vehicle influence on drug bioavailability, it is inconsistent with those of Patton and Robinson (7). In particular, Patton and Robinson (7) can account fully for the difference in k_a between anesthetized and unanesthetized rabbits, both with blocked drainage apparatus, by merely including normal tear turnover. This apparent inhibition of lacrimation upon blocking the drainage apparatus has also been observed in humans (31). Aside from invoking a compensatory mechanism that resists an increase in tear volume beyond the large volume already present, no explanation is forthcoming at the present time. There is little doubt, however, that in normal unanesthetized rabbits, induced lacrimation is a component of the precorneal pilocarpine loss mechanism.

2. Nonconjunctival Loss of Pilocarpine (Uptake of Drug by Nictitating Membrane)—Examination of the anatomy of the rabbit's tear chamber reveals the nictitating membrane to be a structure that potentially can act in conjunction with the conjunctiva and the cornea to remove drug from the precorneal area. This discussion of nonconjunctival pilocarpine loss will focus on uptake by the nicitating membrane, even though additional components of nonconjunctival loss are possible.

Like the conjunctiva, the nictitating membrane is a vascularized tissue (32). It is not clear what gives it an apparent advantage over the conjunctiva in removing drug from the precorneal area. A direct determination of drug levels in the nictitating membrane as a function of time may not establish if it is the dominant factor in causing drug loss. So long as it is in contact with the fluid bathing the precorneal area, its concentration–time profile will probably show an early peak time of 5 min just like that for the corneal epithelium. On the other hand, surgical removal of the nictitating membrane may change the tear chamber volume and possibly the drainage process, just to name a few. Therefore, a simple approach of manipulating the vascularization of this tissue was adopted.

The role of blood flow on drug absorption was studied in the GI tract (33, 34) and was recently reviewed (35). In the eye, if the nictitating membrane contributes substantially more to precorneal drug loss than does the conjunctiva, in the presence of a vasoconstrictor the apparent rate constant of decline should register a corresponding reduction in value. Indeed, in the anesthetized rabbit, it is reduced by a factor of 4 upon the application of a 0.1% epinephrine bitartrate solution. The change is less dramatic in the unanesthetized rabbit because induced lacrimation due to vehicle formulation, which is not expected to be affected by this procedure, is still present. The difference in the magnitude of the rate constant obtained in unanesthetized and anesthetized rabbits was the same regardless of epinephrine pretreatment (Table III). This difference amounted to 0.33 min^{-1} and may be ascribed to tear flow, of which induced lacrimation is a part.

Just as vasoconstriction is accompanied by a substantial reduction in the apparent rate constant of parallel loss, it is reasonable to expect opposite changes in the aforementioned rate constant when vasodilation or inflammation is present. Indeed, histamine dihydrochloride, a potent vasodilator (36), at an instilled concentration of 0.25% caused an upward shift of the rate constant from 0.63 to 1.03 min^{-1}. This result may not be a pure vasodilation effect, however, because histamine at a concentration as low as 5×10^{-6} *M* loosened the superficial epithelial cells of excised beef corneas after several hours of incubation (37), an effect that could enhance corneal pilocarpine absorption. However, the contact time in the present experiment was short enough to preclude a surface effect.

3. Vasodilation due to Pilocarpine—Since pilocarpine itself is a vasodilator (36), this pharmacological effect is expected to manifest itself in the rate constant governing disappearance of the drug from the precorneal area. The vasodilation effect appears to be immediate since predosing the eye with an equivalent amount of pilocarpine yielded a rate constant that was statistically indistinguishable from that obtained under normal circumstances (Table IV). In contrast, when glycerin, which is devoid of vasodilation properties, was administered in combination with pilocarpine, the rate constant associated with the glycerin concentration decline increased by a factor of 3. Moreover, glycerin then disappeared from the precorneal area with a rate constant similar to that governing pilocarpine decline. Induced lacrimation was not a concern here since anesthetized rabbits were employed.

By taking the difference in rate constants obtained for pilocarpine and glycerin, both in anesthetized rabbits, a value of 0.17 min^{-1} was obtained. This value may be denoted as the rate constant due to vasodilation induced by pilocarpine in *anesthetized* rabbits. Suitable corrections for the influence of anesthesia on the drainage rate constant (1) must be applied in calculating the corresponding value for unanesthetized animals.

An implication associated with vasodilation induced by pilocarpine is its expected variation with instilled concentration. This, if operative, will be reflected in the pilocarpine levels obtained in a given tissue, *e.g.*, aqueous humor, and, obviously, the magnitude of the rate constant governing precorneal drug loss. The linearity of the dose–aqueous humor concentration (at 20 min postinstillation) profile over six orders of magnitude in dose (10^{-6}–10^{-1} *M*) (8) suggests that either the rate constant due to pilocarpine-induced vasodilation is a weak function of instilled concentration or, less likely, maximum vasodilation is attained at the lowest instilled concentration of 10^{-6} *M*. The dose–aqueous humor concentration plot is a log–log plot so that small differences will not be discerned.

Not only does induced lacrimation appear to be suppressed when the drainage apparatus is blocked, but the same is true for pilocarpine-induced vasodilation. This effect can be seen by comparing the magnitude of the rate constants involved: 0.17 min^{-1} for the vasodilation determined in this study *versus* 0.0217 min^{-1} for nonproductive loss reported elsewhere (7). Undoubtedly, a volume effect is making a contribution. Thus, in anesthetized rabbits with blocked drainage apparatus, the rate constant ascribed to vasodilation assumes a theoretical value of 0.11 min^{-1}, which is five times larger than the reported 0.0217-min^{-1} value. The difference can be reconciled, in part, by attributing the preponderance of vasodilator effect to the nictitating membrane, a consideration consistent with the view that the nictitating membrane, in some unknown fashion, does not participate in precorneal drug disposition as soon as the drainage apparatus is blocked. Whether this phenomenon is peculiar to pilocarpine can possibly be disclosed in an experiment involving other drugs such as glycerin.

Thus far, the volume effect on apparent first-order rate constants has been identified, the notion of rapid pilocarpine uptake by some components on the corneal surface has been mentioned, and induced lacrimation due to vehicle formulation, nonconjunctival pilocarpine loss, and vasodilation due to the drug have been established as additional aspects of precorneal pilocarpine disposition. It is still unclear why these additional, and yet critical, parameters controlling precorneal disposition are absent when the drainage apparatus is blocked. Similarly, there is the observation by DeSantis and Schoenwald (38) that disputes the importance of the nictitating membrane as a pathway contributing to pilocarpine loss

Table VII—Influence of Individual Parallel Loss Factors on Bioavailability

Parallel Loss Factor Present	Ratio A[a]	Ratio B[b]
Drainage	0.24	30.4
Normal tear flow	0.18	22.1
Induced lacrimation	0.13	16.4
Conjunctival absorption	0.16	20.9
Vasodilation due to pilocarpine	0.03	3.5
Nonconjunctival loss	0.05	5.9
All factors present	0.007	1.0

[a] Ratio A is referenced to the case when no parallel loss factors are present (*AUC* in aqueous humor is 9716 μg/ml min). [b] Ratio B is referenced to the case when all parallel loss factors are present (*AUC* in aqueous humor is 77.0 μg/ml min).

from the precorneal area. They monitored changes in pupil diameters as a result of surgical removal of the nictitating membrane. However, pupillary diameter measurements, as compared with aqueous humor drug level determinations (8), are not a sensitive indicator for pilocarpine bioavailability.

What remains to be done is to integrate these additional pathways responsible for precorneal pilocarpine loss with the previously identified pathways by assessing the relative importance of each to ocular drug bioavailability. But first it is necessary to derive from the experimental data the coefficients, p, associated with each pathway of loss shown in Scheme III because the experimental results, presented as first-order rate constants, were obtained in both anesthetized and unanesthetized rabbits and the rate of change in precorneal fluid volume differs under these conditions. In other words, the presence of drainage conceals the actual contribution of each pathway to total drug loss. With this background, one can proceed, as outlined in the *Appendix*, to calculate the volume-independent coefficients, p, associated with various routes of precorneal drug loss.

Table VI summarizes the values computed for various parameters associated with precorneal pilocarpine disposition. The corresponding k values for anesthetized and unanesthetized rabbits are obtained by applying Eqs. A1 and A3, respectively (see *Appendix*). From the volume-independent values, one can see that, in terms of effectiveness of removing drug from the precorneal area, the rank order of various precorneal disposition factors appears to be: vasodilation due to pilocarpine > nonconjunctival loss > conjunctival absorption > normal tear turnover > corneal absorption. How induced lacrimation fits into this sequence is not as straightforward because of its time dependence. The same is true of drainage, due to lack of knowledge of an effective volume to properly weight this parameter. Computer simulations will be employed to examine the relative importance of these precorneal factors in effecting drug loss.

Relative Importance of Precorneal Drug Disposition Factors on Extent of Drug Absorption—Tables VII and VIII list the various cases to be considered in an effort to understand the relative importance of precorneal drug disposition factors, alone or in combination, in influencing the extent to which pilocarpine is absorbed across the corneal epithelium. The area under the curve (*AUC*) from 0 to 120 min in the aqueous humor will serve as an indicator for the amount of drug absorbed. Ideally, the entire, rather than the truncated, *AUC* should be used. However, since only relative values are of interest, the *AUC* from 0 to 120 min is used.

Each case listed in Tables VII and VIII is referenced to two special cases. Ratio A is referenced to the ideal case in which no parallel loss factors are present and is, therefore, roughly equivalent to the fraction of dose absorbed in the presence of certain loss factors. Ratio B is referenced to the case corresponding to normal physiological conditions when all parallel loss factors are present and, therefore, reflects the improvement in bioavailability when one or more parallel loss factors are suppressed. As an illustration, Table VII shows that when all parallel loss factors except normal tear flow are suppressed: (*a*) the apparent fraction of dose absorbed (Ratio A) is 0.18, and (*b*) relative to normal physiological conditions, there should be a 22-fold increase in the amount of pilocarpine recovered in aqueous humor (Ratio B).

Consider Ratio A in greater detail. According to Table VII, when only a single parallel loss factor is present, the amount of dose absorbed is reduced by at least 75%. Under the usual circumstances of more than one parallel loss factor being operative, it is reduced by an additional 80% (Table VIII). From these two tables, one can establish, in principle, a single sequence ranking the various parallel loss factors in terms of their effectiveness in removing pilocarpine from the precorneal area. To this end, the most efficient parallel loss factor can be identified as the one possessing a Ratio A that has the smallest value in Table VII but the largest in Table VIII. Quite unexpectedly, two sequences differing only in the ranking of drainage are obtained. Whereas Table VII indicates that drainage is the *least* efficient parallel loss factor, Table VIII indicates that it is the *most* efficient one.

Table VIII—Influence of Combinations of Parallel Loss Factors on Bioavailability

Parallel Loss Factor Absent	Ratio A[a]	Ratio B[b]
Drainage	0.014	1.78
Induced lacrimation	0.0085	1.07
Conjunctival absorption	0.0084	1.06
Vasodilation due to pilocarpine	0.013	1.67
Nonconjunctival loss	0.010	1.29
All factors present	0.007	1.0

[a,b] See footnotes to Table VII for details.

These conflicting results can be explained by noting that the proposed model allows drug diffusion from the epithelium into the tear film, a realistic situation. Within this framework, the portion of drug that has diffused into the tear film is completely available for reabsorption as soon as normal resident tear volume is attained, so long as drainage is the only loss factor present. When factors in addition to drainage are present, the same portion of drug is subjected to continuous removal by routes in addition to corneal absorption. Of relevance here is the first case listed in Table VIII. It can be seen that eliminating drainage brings about the most improvement in bioavailability, equivalent to saying that drainage is the most efficient parallel loss factor.

Simply stated, when parallel loss factors are evaluated in the manner prescribed by Table VII, the conclusion of drainage being the least efficient parallel loss factor is an artifact of the system. Thus, the relative effectiveness of precorneal disposition factors in removing drug should be: drainage ≃ vasodilation > nonconjunctival loss > induced lacrimation ≃ conjunctival absorption > normal tear turnover.

Implications of Precorneal Drug Loss in Ocular Drug Delivery System Design—Having established the contributions of various precorneal drug disposition factors to precorneal loss of pilocarpine, their implications in designing ocular drug delivery systems that optimize ocular pilocarpine bioavailability can be examined. The aforementioned precorneal disposition factors can first be grouped into vehicle- and drug-related categories. Drainage and induced lacrimation belong to the category of vehicle-related factors, whereas conjunctival absorption, nonconjunctival loss, vasodilation, and corneal absorption are drug-related factors. Based on the magnitude associated with each parameter, impairing a single route of loss will not bring about more than a twofold improvement, on the average, in the fraction of dose absorbed (Ratio B, Table VIII).

By far, the route that has received the most attention is drainage. Previous studies (39–41) showed that delaying drainage by a factor of 10 through use of polymer solutions as vehicles results in only a factor of 2 improvement in bioavailability. As an alternative to polymer solutions, one can employ solid or semisolid dosage forms such as ointment (42), soft contact lenses (43–46), and controlled-release devices including the Ocusert (47, 48). With the possible exception of the Ocusert, this approach has thus far not met with satisfactory results.

Induced lacrimation, another vehicle-related loss factor, can be suppressed by formulating the solution at physiological pH and tonicity so long as they are compatible with drug stability. According to the proposed model, this approach alone causes no dramatic improvement in the amount of drug absorbed either. There is a 1.6-fold improvement in aqueous humor pilocarpine levels as the pH is varied (42).

The remaining factors, vasodilation, nonconjunctival loss (of which drug uptake by the nictitating membrane is a component), and conjunctival absorption, are more drug than vehicle related. Unfortunately, they include those factors that play dominant roles in causing drug loss. This finding suggests prodrug derivative formation to minimize vasodilation, thereby improving bioavailability for pilocarpine. Alternatively, administration of vasoconstrictors prior to instillation of pilocarpine solution shows promise for improving its bioavailability. The proposed model, in conjunction with the experimental data obtained in the present study, predicts a 2.5-fold improvement when a 0.1% epinephrine bitartrate solution is administered 15 min prior to instilling the dose. Chrai *et al.* (2) reported only a 1.16-fold increase, but these investigators administered both drugs in combination.

Since the nictitating membrane is absent in humans, drug uptake by this structure is not a concern in designing ocular drug delivery systems for humans.

From this discussion, it is clear that a combination of physical and

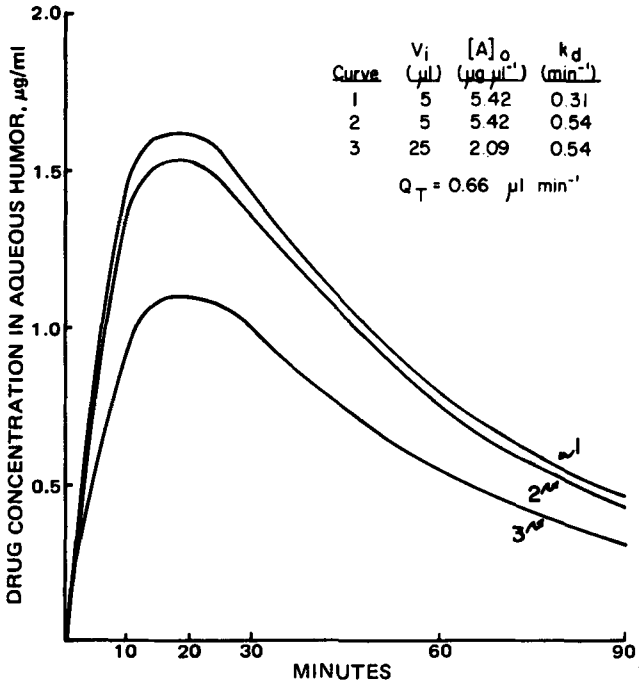


Figure 5—*Effect on aqueous humor drug levels upon varying instilled concentration and volume while holding amount of drug administered constant. Tear flow is present. Key:* V_i, *instilled volume;* $[A]_0$, *initial concentration of drug in tear film; and* k_d, *drainage rate constant. These profiles were generated for an instilled concentration of 13.56 μg/μl contained in a volume of 5 μl and for 2.71 μg/μl in 25 μl.*

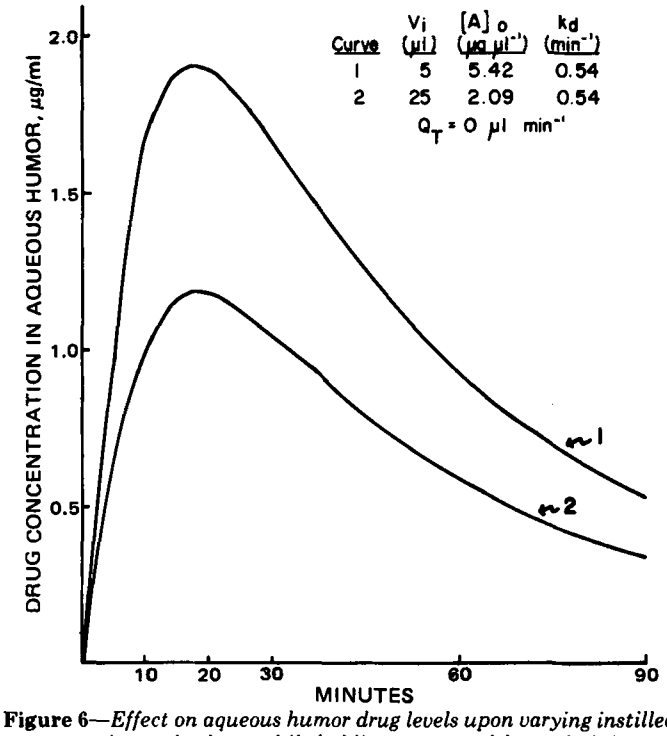


Figure 6—*Effect on aqueous humor drug levels upon varying instilled concentration and volume while holding amount of drug administered constant. Tear flow is absent. Key:* V_i, *instilled volume;* $[A]_0$, *initial concentration of drug in tear film; and* k_d, *drainage rate constant. These profiles were generated for an instilled concentration of 13.56 μg/μl contained in a volume of 5 μl and for 2.71 μg/μl in 25 μl.*

chemical approaches is needed to achieve maximum improvement in bioavailability of topically applied pilocarpine. Assuming that the enzymes necessary for bioconversion are available, one can design a prodrug derivative possessing the desirable attributes of favorable uptake by the corneal epithelium (a highly lipophilic structure), minimal vasodilating properties, and, ideally, minimal uptake by the conjunctiva. Such a derivative can then be placed into a suitable controlled-release device, which must satisfy the anatomical and physiological constraints imposed by the precorneal area of the eye. One may argue that administering larger amounts of drug would give the desired drug levels in ocular tissues, but there exists the concern of systemic toxicity since most of the applied drug is then available for systemic absorption (49).

Important Aspects Relating to Drainage—With the spectrum of of precorneal pilocarpine disposition factors placed in some perspective, the remaining sections of this report are devoted to some important aspects relating to drainage, the factor deemed to be crucial in precorneal drug loss.

Role of Instilled Dose Parameters in Ocular Drug Bioavailability—Given the strategic role of drainage in the parallel loss process and its sensitivity to the volume of dose instilled, it is instructive to compare the concentration–time profiles in a given intraocular tissue when a fixed amount of drug is delivered in various combinations of volume and concentration. Compartment pharmacokinetics will predict identical profiles under these conditions; this result can be seen in Eq. 12, which expresses the concentration, C, of drug in the central compartment as a function of time for a drug that obeys one-compartment model kinetics:

$$C = \frac{FD}{V_d}\left(\frac{k_a}{k_a - k_e}\right)(e^{-k_e t} - e^{-k_a t}) \qquad \text{(Eq. 12)}$$

where k_a is the absorption rate constant, k_e is the elimination rate constant, D is the amount of drug administered, F is the fraction of dose absorbed, and V_d is the apparent volume of distribution. In contrast, our model, like a previous one (12), predicts characteristic intraocular tissue concentration–time profiles for each combination of instilled concentration and volume. Figure 5 illustrates this point. In this figure, two curves (1 and 2) are included for the 5-μl case. Curve 1 represents the case where the drainage rate constant, k_d, possesses a value characteristic of this volume, *i.e.*, $k_d = 0.31$ min^{-1}; curve 2 represents the case where k_d is the same as that for a 25-μl dose, *i.e.*, $k_d = 0.54$ min^{-1}. In both cases, the concentration at any time is higher than that for a 25-μl dose (curve 3). This finding indicates that the differences that arise from the dose volume instilled are not due to a volume-dependent drainage effect alone; the initial concentration established in the tear film also plays a role. Moreover, since the volume instilled indirectly influences the contribution of tear flow, $Q_T(t)$, to parallel loss, it is necessary to see if the profiles would continue to be different even when $Q_T(t)$ is set equal to zero.

The answer is provided by the profiles displayed in Fig. 6. Again, the 5-μl dose gives a higher concentration than the 25-μl dose, indicating that the increase in extent of drug absorption from a smaller dose volume cannot be due largely to the indirect influence exerted by drainage on tear flow. Drainage is present in all these instances. Hence, when it ceases to be a parallel loss factor, the results given by the present model should coincide with those given by the pharmacokinetic treatment described earlier. In essence, the dose volume administered influences the tissue concentration–time profiles only if the fluid volume at the absorption site undergoes a time-dependent change in response to the volume administered. Therefore, this volume effect should be observed in regions of the body, *e.g.*, precorneal area, rectum, and skeletal muscles (50–53), which have a limited capacity to accommodate volume and/or to possess a small resident volume of fluid to begin with. The influence that fluid volumes may exert on the absorption of orally dosed drugs begins to be recognized. This influence is complex, as illustrated by studies on aspirin (54), amoxicillin and ampicillin (55), and erythromycin stearate (56).

For completeness, the role of the initial concentration as it relates to drug bioavailability from topical instillation should be explored. According to Table IX, the *AUC* in aqueous humor, and hence the amount of drug absorbed, is greater for a 25-μl dose than for a 5-μl dose, despite the negative influence of drainage experienced by the larger volume of dose. The increase is due to a larger amount of drug administered with the larger volume of dose. Also of interest in the same table is the higher instilled concentration associated with the 5-μl dose, yet the extent of drug absorbed still favors the 25-μl dose.

Thus, the relationship between instilled concentration and amount relative to drug bioavailability is an intricate one. On an absolute basis, for a given amount of drug, a small volume allows more drug to be absorbed. For a given initial or instilled concentration, the opposite is the case. However, in terms of the fraction of dose absorbed, as reflected by the value obtained on dividing the *AUC* by the instilled amount, a small volume of dose is still more efficient than a large one (Table IX). Such a volume effect was implied by previous studies in this laboratory (1, 2) and, more recently, was confirmed experimentally in rabbits (49, 57) and humans (58).

Table IX—Effect of Initial Concentration in Tear Film on Area under the Curve (*AUC*) in Aqueous Humor

Volume Instilled, μl	Instilled Concentration, μg/μl	Instilled Amount, μg	Initial Concentration, μg/μl	*AUC* in Aqueous Humor[a], μg/ml min	*AUC* ÷ Instilled Amount, μg/ml min/μg
5	5.22	26.08	2.09	43.6	1.67
25	2.71	67.82	2.09	77.0	1.14

[a] From 0 to 120 min.

Role of Drainage in Determining Early Peak Time in Corneal Epithelial Drug Concentration–Time Profile—Not only does drainage exercise a definite role in controlling the fraction of instilled dose available for absorption, it also complicates the interpretation of the early peak time observed in the corneal epithelium. Despite the magnitude of the drainage rate constant, the peak time obtained when it is the only parallel loss factor is about three times *longer* than that obtained when it is the only one absent (Table X). This observation says that, by itself, drainage is less effective than the other parallel loss factors combined in causing an early peak time in the epithelium concentration–time profile.

The picture changes when drainage is allowed to act in conjunction with at least one other parallel loss factor. As an example, in the presence of tear flow, drainage gives a peak time of 10 min, as compared with the 20 min obtained otherwise. This example illustrates an additional important point; so long as drainage is an integral component of the precorneal drug loss mechanism, the peak time derived from a corneal epithelial drug concentration–time profile is an insensitive indicator for the existence of such equally efficient parallel loss pathways as vasodilation and uptake of drug into the nictitating membrane. The present study demonstrates that only by directly monitoring tear film drug levels, under a variety of experimental conditions, can a complete picture of precorneal drug disposition emerge.

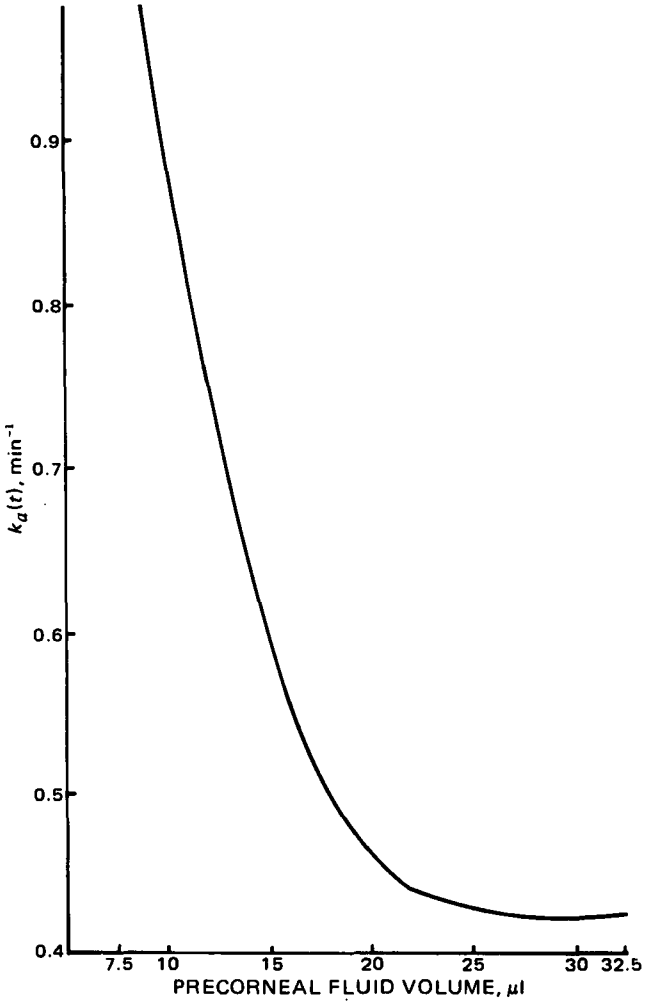


Figure 7—*Plot of* $k_a(t)$ *versus volume,* $V_A(t)$, *of precorneal fluid at a given time. See text for meaning of* $k_a(t)$.

Role of Precorneal Fluid Volume in Influencing Magnitude of Apparent First-Order Rate Constants Associated with Decline in Tear Film Drug Levels—Treatment of data according to compartment pharmacokinetics carries with it the implicit assumption of a single rate constant being adequate to describe the change in both amount and concentration of drug with time. Table XI indicates that this assumption does not hold in the precorneal area of the eye since the volume of fluid there continuously changes with time in an effort to attain resident tear volume. As expected, the apparent rate constant is larger for amount than for concentration, the difference being due to drainage, which exerts a direct influence on amount as opposed to an indirect one on concentration.

Furthermore, this apparent rate constant is time dependent. This is evident in Eq. 13, which describes the change in tear film drug concentration for the first 5 min postinstillation when the drug contribution from the epithelium to tear film drug levels is negligible:

$$\frac{d[A]}{dt} = -\left(\frac{p_p + p_n + Q_T(t)}{V_A(t)}\right)[A] \qquad \text{(Eq. 13)}$$

where the term within parentheses can be identified with the apparent first-order rate constant, $k_a(t)$, governing the decline in drug concentration. Notice that $Q_T(t)$ and $V_A(t)$ are functions of time. Thus:

$$k_a(t) = \frac{p_p + p_n + Q_T(t)}{V_A(t)} \qquad \text{(Eq. 13}a\text{)}$$

The first-order rate constant derived from the slope of a semilogarithmic plot of drug concentration *versus* time is, therefore, a time-averaged quantity, as indicated earlier. It is denoted as $\bar{k}_a$ and, conforming to experimental conditions in the present study, is given by:

$$\bar{k}_a = \frac{1}{V_A(5) - V_A(0)} \int_{V_A(0)}^{V_A(5)} k_a(t)\, dV_A(t) \qquad \text{(Eq. 14)}$$

where $V_A(0)$ is the fluid volume in the precorneal area at time zero and $V_A(5)$ is that at 5 min. Evaluation of the integral in Eq. 14 is simplified if $Q_T(t)$ is constant or zero. The result of the integration by assuming $Q_T(t) = 0$ is:

$$\bar{k}_a = \frac{p_p + p_n}{V_A(5) - V_A(0)} \ln\left(\frac{V_A(5)}{V_A(0)}\right) \qquad \text{(Eq. 15)}$$

For a 25-μl dose:

$$\bar{k}_a = \frac{p_p + p_n}{29.0} \qquad \text{(Eq. 16)}$$

whereas for a 10-μl dose:

$$\bar{k}_a = \frac{p_p + p_n}{18.9} \qquad \text{(Eq. 17)}$$

The conditions leading to Eqs. 16 and 17 are resident tear volume V_0 = 12 μl and drainage rate constant k_d = 0.177 min^{-1}. Equation 16 is very similar to Eq. A1 obtained by the procedure detailed in the *Appendix*. The essence of Eqs. 16 and 17 is twofold. First, contrary to what one might anticipate, the effective volume contributing to $\bar{k}_a$ is neither the instilled volume nor the total volume at time zero. Second, $\bar{k}_a$ is larger for a smaller instilled volume. Dividing Eq. 17 by Eq. 16 gives a ratio of 1.5, in excellent agreement with experimental findings on anesthetized rabbits, thus lending credence to the interpretation of apparent first-order rate constants offered by Eq. 14.

When $Q_T(t)$ is not equal to zero, the integral in Eq. 14 can be evaluated graphically. With the parameter values listed in Table VI, a plot of $k_a(t)$ *versus* $V_A(t)$ can be generated (Fig. 7). From this plot, a $\bar{k}_a$ of 0.41 min^{-1} is derived, which is less than the value of 0.63 min^{-1} obtained from the slope of a semilogarithmic plot of tear film drug concentration *versus* time. The discrepancy can be partly ascribed to rounding-off errors incurred in graphical integration.

An expression analogous to Eq. 15 for the time-averaged rate constant, $\bar{k}'$, governing the decline in amount of drug in the tear film can now be obtained. Equation 2 is cast into its equivalent form:

$$\frac{dA_t}{dt} = -\left(\frac{p_p + p_n + Q_T(t) - k_d V_0}{V_A(t)}\right) A_t - k_d A_t \qquad \text{(Eq. 18)}$$

Table X—Influence of Selected Parallel Loss Factors on Peak Time in Corneal Epithelial Drug Concentration–Time Profile

Parameter	Peak Time, min
Tear flow	9
Drainage	20
Drainage and tear flow	10
All but drainage	6

where A_t is the amount of drug in the tear film. Equation 18 assumes that the contribution from the term $p_p[B]$ is negligible. It follows that the apparent first-order rate constant, $k'(t)$, is given by:

$$k'(t) = \frac{p_p + p_n + Q_T(t)}{V_A(t)} + k_d\left(1 - \frac{V_0}{V_A(t)}\right) \qquad \text{(Eq. 19)}$$

Then:

$$\bar{k}' = \frac{1}{V_A(5) - V_A(0)} \int_{V_A(0)}^{V_A(5)} k'(t)\, dV_A(t) \qquad \text{(Eq. 20)}$$

where the symbols are as previously defined. Again, for $Q_T(t) = 0$, Eq. 20 becomes:

$$\bar{k}' = \frac{p_p + p_n}{V_A(5) - V_A(0)} \ln\left(\frac{V_A(5)}{V_A(0)}\right) + k_d\left[1 - \frac{V_0}{V_A(5) - V_A(0)} \ln\left(\frac{V_A(5)}{V_A(0)}\right)\right] \qquad \text{(Eq. 21)}$$

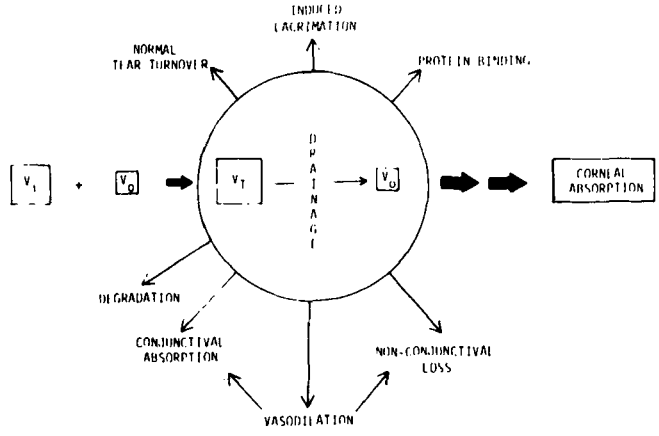
Equation 21 differs from Eq. 15 by the additional term containing k_d. For a 25-μl dose, $\bar{k}' = 0.036p + 0.10$ where $p = p_p + p_n$.

CONCLUSIONS

A picture concerning the mechanisms that control precorneal pilocarpine disposition following topical instillation (Scheme IV) has been presented. Through experimentation and computer simulation, it was found that competing with corneal pilocarpine absorption from the precorneal area are the processes of solution drainage, vasodilation due to the drug, nonconjunctival loss (of which uptake of drug by the nictitating membrane is a component), conjunctival absorption, induced lacrimation, and normal tear turnover, with some of these processes being more efficient than others. The enormous influence of drainage on ocular drug bioavailability was recognized.

Equally as important, the analysis showed that the burden of removing drug from the precorneal area was shared almost equally by drainage, vasodilation due to pilocarpine, and nonconjunctival loss. Consequently, suppressing a single factor only results in a modest improvement in pilocarpine bioavailability. The experience with manipulating drainage is an illuminating example. It follows that the strategy for optimizing absorption efficiency through controlled drug delivery should involve both physical and chemical approaches. This process should be enhanced since estimates on the magnitude of precorneal disposition parameters are now available.

An additional aspect of this study centers on defining the effect of instilled volume on the magnitude of the apparent first-order rate constant governing the decline in concentration and amount of drug in the precorneal area. According to the proposed model, the rate constant controlling drug loss is a time-dependent quantity. Strictly speaking, it should be denoted as a coefficient rather than as a rate constant. The time-average first-order rate constant is shown to be a function of, among other parameters, the initial volume of fluid in the precorneal area. In contrast to the situation where the volume of fluid at the absorption site remains essentially constant with time, the first-order drug loss rate constant viewed in the context of the proposed model varies depending on whether concentration or amount is being considered.



Scheme IV—Precorneal disposition of pilocarpine in the albino rabbit

Table XI—Apparent First-Order Rate Constants Governing Decline in Amount and Concentration of Pilocarpine in Tear Film following Instillation of 25 μl of 0.01 M Solution

Condition	k_a, min^{-1} [a]	
	Amount[b]	Concentration[c]
Unanesthetized	0.90	0.63
Anesthetized	0.40	0.30

[a] For the first 5 min postinstillation. [b] Calculated value. See text for details. [c] Experimental value.

Additionally, as a result of analyzing instilled dose parameters such as volume and concentration in terms of influence on the rate and extent of absorption, the contention that the volume of dose instilled affects ocular drug bioavailability through a combined influence on initial concentration established in the tear film and on the magnitude of drainage that results was put on a relatively definitive basis.

The proposed model is satisfactory from the standpoints of physical meaning as well as a quantitative description concerning precorneal disposition, of accounting for the role of instilled volume in ocular drug bioavailability, and of exposing the hidden aspects of time dependence as it pertains to first-order rate "constants" commonly employed to describe drug distribution.

APPENDIX

This section outlines the procedure for computing the values for various precorneal disposition parameters from experimental results listed in Table III. Of central importance in these calculations is Eq. 8. According to this equation, $[A]$ can be estimated if p_n (the coefficient associated with nonproductive loss) and Q_i (the coefficient associated with induced lacrimation) are known since values for the other parameters are available. Only one unknown remains if the condition of anesthesia is selected, in which case $Q_0 = Q_i = 0$. Thus, one can calculate $[A]$ for a given value of p_n for $t = 0, 1, 2, 3, 4$, and 5 min. These numbers are then plotted semilogarithmically against t. From the slope of this plot, an apparent first-order rate constant is derived. The same procedure is then repeated for other p_n values. On correlating the resulting slopes with the corresponding p_n values, an equation relating the volume-independent value (p, in microliters per minute) and the apparent first-order rate constant (k, in minute^{-1}) for a given parameter is obtained.

Consider now the experiment in which a 25-μl dose of pilocarpine solution was instilled onto the cornea of anesthetized rabbits. Under this condition, the apparent first-order rate constant has a value of 0.30 min^{-1} (Table III). Chrai *et al.* (1) reported that in the presence of anesthesia, the normal resident volume of tear fluid is 12 μl, the drainage rate constant has a value of 0.177 min^{-1}, and tear turnover is inhibited, *i.e.*, $Q_i = Q_0 = 0$ μl min^{-1}. With this information, the equation relating p and k takes the form:

$$k = \frac{p}{28.0} = 0.036p \qquad \text{(Eq. A1)}$$

Equation A1 holds for anesthetized rabbits only. For $k = 0.30$ min^{-1}, $p = 8.40$ μl/min. This, when applied to an instilled dose of 10 μl in anesthetized rabbits, yields $k = 0.46$ min^{-1}, in excellent agreement with the observed value (Table III).

The key elements involved in partitioning $p = 8.40$ μl/min just obtained into its components are as follows. Since $p = p_p + p_n$ and p_p has been estimated to be 0.13 μl/min (Table V), $p_n = 8.27$ μl/min. The parameter p_n is the sum of p_{nc}, p_{nm}, and p_{nd}, where p_{nc} refers to conjunctival absorption, p_{nm} refers to nonconjunctival loss, and p_{nd} refers to vasodilation induced by pilocarpine. Among these values, an estimate for p_{nc} is available. It is 0.71 μl/min, calculated from 0.0217 min^{-1}, the first-order rate constant associated with conjunctival absorption, and 32.5 μl, the volume of fluid under the prevailing experimental conditions (7). The first-order rate constant governing vasodilation is 0.17 min^{-1}. According to Eq. A1, this value corresponds to a p_{nd} of 4.76 μl/min. Now p_{nc} can be shown to take on a value of (8.27 − 0.71 − 4.76) or 2.80 μl/min. The only parameters remaining to be calculated are Q_i and k_l, which will

be discussed after the companion form of Eq. A1 for unanesthetized rabbits is obtained.

In this instance, the procedure is essentially that outlined for anesthetized rabbits, the exception being that the resident tear volume is 7.5 μl. Since Q_i is weighted with V_i instead of V_0 (Eq. 8), it is set equal to zero to simplify calculations. Accordingly, Eq. A2 is the appropriate form relating p and k in unanesthetized rabbits, provided that induced lacrimation is not a loss factor:

$$k = \frac{p}{14.1} = 0.071p \qquad \text{(Eq. A2)}$$

Applying Eq. A2 to the sum of p_p, p_n, and Q_0, *i.e.*, 9.06 μl/min, produces a value of 0.64 min^{-1}, implying that induced lacrimation is not needed to explain the apparent first-order rate constant obtained in normal unanesthetized rabbits. On the one hand, this finding is consistent with the assumption made on induced lacrimation in deriving Eq. A2. On the other hand, it appears to contradict the data on the influence of instilled solution pH on the apparent first-order rate constant. In essence, Eq. A2 is not obeyed by unanesthetized rabbits for whom pH-induced lacrimation constitutes a pathway of drug loss.

To take this factor into account, two assumptions are appropriate: (*a*) no induced lacrimation occurs with an instilled solution at pH 7.38 and (*b*) alterations in solution pH cause no significant changes in the remaining precorneal disposition parameters. By noting that the apparent first-order rate constant for the condition under which these assumptions hold is 0.43 min^{-1} (Table III), Eq. A3 can be obtained:

$$k = \frac{p}{21.1} = 0.047p \qquad \text{(Eq. A3)}$$

With this background, the parameters associated with induced lacrimation can be estimated. In the proposed model, they include the induced lacrimation rate constant, Q_i, and the rate constant, k_l, governing its decline. In the absence of additional information, the situation consists of solving one equation with two unknowns. Consequently, one has less confidence in these computed values as compared with others. In any event, from Eq. A3, one can obtain p = 13.29 μl/min for k = 0.63 min^{-1} (Table III). Subtracting p_p, p_{nm}, p_{nc}, and p_{nd} from p yields a value of 4.89 μl/min, which presumably can be attributed to induced lacrimation. Computer-simulation results suggest a value of 4.75 μl/min for the induced lacrimation rate constant, whose decline appears to be controlled by a rate constant with a value in the order of 2 min^{-1}. Applying these estimates to the case in which the ocular surfaces were pretreated with epinephrine (Table III) generates an apparent first-order rate constant of 0.2 min^{-1}, which is in only fair agreement with the observed value of 0.4 min^{-1}.

Table VI summarizes the values computed for the various parameters associated with precorneal disposition of pilocarpine.

REFERENCES

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

(2) S. S. Chrai, M. C. Makoid, S. P. Eriksen, and J. R. Robinson, *ibid.*, **63**, 333 (1974).

(3) T. J. Mikkelson, S. S. Chrai, and J. R. Robinson, *ibid.*, **62**, 1648 (1973).

(4) *Ibid.*, **62**, 1942 (1973).

(5) S. S. Chrai and J. R. Robinson, *J. Pharm. Sci.*, **65**, 437 (1976).

(6) T. F. Patton and J. R. Robinson, *ibid.*, **64**, 267 (1975).

(7) *Ibid.*, **65**, 1295 (1976).

(8) S. S. Chrai and J. R. Robinson, *Am. J. Ophthalmol.*, **77**, 735 (1974).

(9) R. Lazare and M. Horlington, *Exp. Eye Res.*, **21**, 281 (1975).

(10) M. C. Makoid and J. R. Robinson, *J. Pharm. Sci.*, **68**, 435 (1979).

(11) J. W. Sieg and J. R. Robinson, *ibid.*, **65**, 1816 (1976).

(12) K. J. Himmelstein, I. Guvenir, and T. F. Patton, *ibid.*, **67**, 603 (1978).

(13) K. B. Bischoff and R. L. Dedrick, *ibid.*, **57**, 1346 (1968).

(14) T. Teorell, *Arch Int. Pharmacodyn. Ther.*, **57**, 205 (1937).

(15) D. S. Riggs, "The Mathematical Approach to Physiological Problems," Williams & Wilkins, Baltimore, Md., 1963, p. 185.

(16) J. M. Conrad, W. A. Reay, R. E. Polcyn, and J. R. Robinson, *J. Parent. Drug Assoc.*, **32**, 149 (1978).

(17) V. H. L. Lee, M.S. thesis, University of Wisconsin, Madison, Wis., 1978.

(18) T. F. Patton, Ph.D. thesis, University of Wisconsin, Madison, Wis., 1975.

(19) R. W. Hamming, "Numerical Methods for Scientists and Engineers," 2nd ed., McGraw-Hill, New York, N.Y., 1973, pp. 393–411.

(20) H. Herrmann and F. H. Hickman, *Bull. Johns Hopkins Hosp.*, **82**, 182 (1948).

(21) R. R. Pfister and N. Burstein, *Invest. Ophthalmol.*, **15**, 246 (1976).

(22) J. M. Conrad and J. R. Robinson, *J. Pharm. Sci.*, **66**, 219 (1977).

(23) D. M. Maurice, *Exp. Eye Res.*, **15**, 527 (1973).

(24) N. Ehlers, *Acta Ophthalmol.*, **43**, 205 (1965).

(25) M. G. Doane, A. D. Jensen, and C. H. Dohlman, *Am. J. Ophthalmol.*, **85**, 383 (1978).

(26) K. Kakemi, T. Arita, R. Hori, R. Konishi, and K. Nishimura, *Chem. Pharm. Bull.*, **15**, 1883 (1967).

(27) *Ibid.*, **17**, 248 (1969).

(28) *Ibid.*, **17**, 255 (1969).

(29) K. Kakemi, H. Tezaki, S. Muranishi, and Y. Tsujimura, *Chem. Pharm. Bull.*, **17**, 1650 (1969).

(30) S. Mishima, *Arch. Ophthalmol.*, **73**, 233 (1965).

(31) M. S. Norn, *Acta Ophthalmol.*, **55**, 674 (1977).

(32) I. Eglitis, in "The Rabbit in Eye Research," J. H. Prince, Ed., Charles C Thomas, Springfield, Ill., 1964, p. 55.

(33) D. Winne and H. Ochsenfahrt, *J. Theor. Biol.*, **14**, 293 (1967).

(34) W. G. Crouthamel, L. Diamond, L. W. Dittert, and J. T. Doluisio, *J. Pharm. Sci.*, **64**, 664 (1975).

(35) D. Winne, *J. Pharmacokinet. Biopharm.*, **6**, 55 (1978).

(36) S. Gartner, *Arch. Ophthalmol.*, **32**, 464 (1944).

(37) H. Herrmann, *Bull. Johns Hopkins Hosp.*, **82**, 208 (1948).

(38) L. M. DeSantis and R. D. Schoenwald, *J. Pharm. Sci.*, **67**, 1189 (1978).

(39) S. S. Chrai and J. R. Robinson, *ibid.*, **63**, 1218 (1974).

(40) T. F. Patton and J. R. Robinson, *ibid.*, **64**, 1312 (1975).

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

(42) J. W. Sieg and J. R. Robinson, *J. Pharm. Sci.*, **66**, 1222 (1977).

(43) S. M. Podos, B. Becker, C. Asseff, and J. Hartstein, *Am. J. Ophthalmol.*, **73**, 336 (1972).

(44) R. M. Ramer and A. R. Gasset, *Ann. Ophthalmol.*, **6**, 1325 (1974).

(45) M. Ruben and R. Watkins, *Br. J. Ophthalmol.*, **59**, 455 (1975).

(46) J. S. Hillman, J. B. Marsters, and A. Broad, *Trans. Ophthalmol. Soc., U.K.*, **95**, 79 (1975).

(47) M. F. Armaly and K. R. Rao, *Invest. Ophthalmol.*, **12**, 491 (1973).

(48) H. A. Quigley, I. P. Pollack, and T. S. Harbin, *Arch. Ophthalmol.*, **93**, 771 (1975).

(49) T. F. Patton and M. Francoeur, *Am. J. Ophthalmol.*, **85**, 225 (1978).

(50) H. Schriftman and A. A. Kondritzer, *Am. J. Physiol.*, **191**, 591 (1957).

(51) R. B. Sund and J. Schou, *Acta Pharmacol. Toxicol.*, **21**, 313 (1964).

(52) T. Nishimura, H. Kobayashi, K. Okumura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.*, **22**, 1275 (1974).

(53) M. Hashida, Y. Takahashi, S. Muranishi, and H. Sezaki, *J. Pharmacokin. Biopharm.*, **5**, 241 (1977).

(54) B. K. Martin, *Adv. Pharm. Sci.*, **3**, 107 (1971).

(55) P. G. Welling, H. Huang, P. A. Koch, W. A. Craig, and P. O. Madsen, *J. Pharm. Sci.*, **66**, 549 (1977).

(56) P. G. Welling, H. Huang, P. F. Hewitt, and L. L. Lyons, *ibid.*, **67**, 764 (1978).

(57) T. F. Patton, *ibid.*, **66**, 1058 (1977).

(58) M. Sugaya and S. Nagataki, *Jpn. J. Ophthalmol.*, **22**, 127 (1978).

ACKNOWLEDGMENTS

Supported by Allergan Pharmaceuticals, Irvine, CA 92664, and Grant EY-01681 from the National Institutes of Health.

The authors thank Mr. Ho-Wah Hui for technical assistance.