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PRELIMINARY STUDIES ON DISPOSITION OF PILOCARPINE IN THE PIGMENTED RABBIT EYE AND A CRITICAL EVALUATION OF PRECORNEAL DISPOSITION FACTORS VIA COMPUTER SIMULATION TECHNIQUES

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(Under the supervision of Professor Joseph R. Robinson.)

Previous studies on the reduction of pharmacological response to a variety of autonomic drugs in the pigmented rabbit eye have centered on the single aspect of drug-pigment interaction without exploring their distribution patterns in the precorneal area as well as intraocular tissues. The inevitable result is that any interrelationships that may exist among these processes are unknown. This study was designed, using an isotopic technique, to gather some preliminary data on levels of pilocarpine in the cornea, aqueous humor and iris-ciliary body of pigmented rabbits. They were then compared with the data from previous work on albino rabbits so as to make a sketch of drug disposition in the pigmented rabbit eye. From the cornea concentration-time profile it was deduced that in the pigmented rabbit, an instilled dose of pilocarpine solution was subjected to essentially the same efficient parallel loss processes existing in the precorneal area of albino rabbits. In spite of this, an increase in uptake of drug

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by the pigmented rabbit eye was observed. Contrary to what one would anticipate from drug-pigment interaction, presumably occurring in the iris-ciliary body, the aqueous humor level of pilocarpine in the pigmented rabbit was statistically indistinguishable from that in the albino rabbit. The accumulation of radioactivity, tentatively as well as cautiously assigned to intact pilocarpine, in the iris and ciliary body was impressive. Its significance relative to the time course of miosis, hence dosage regimen, and to cornea and aqueous humor levels is discussed. Also, studies which are central to understanding the influence of drug-pigment binding on the overall distribution pattern of pilocarpine in the intraocular tissues of pigmented rabbits are suggested.

Unlike the pigmented rabbit, various aspects of disposition of pilocarpine from the moment of instillation of its solution in the cul-de-sac to its ultimate elimination from the aqueous humor have been investigated in considerable detail in the albino rabbit. From these combined data a preliminary model was constructed with the specific aim of isolating the relative importance of the already identified precorneal disposition factors in removing drug from its site of absorption. In contrast to existing models, the one presented in this report incorporates time-dependent change in precorneal fluid volume in response to instilled solution as an element. Although this is a more

realistic representation of the events occurring in the precorneal area, the very volume element leads to complicated integrated forms of rate equations. Consequently, a digital computer was employed to perform various simulations. Several important results emerged from such analyses. First, the impact of drainage on fraction of dose absorbed is not as large as its magnitude tends to suggest. Secondly, the value of non-productive loss rate constant determined experimentally is about ten times smaller than that inferred from computer simulation. Thirdly, 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 which results is now put on a more definitive basis. In addition to analyzing parallel loss factors, the role of instilled concentration versus amount in determining ocular drug bioavailability is examined. The study also raises questions with respect to uptake of drug by the corneal epithelium as well as its subsequent distribution within this tissue. Finally, with appropriate adjustments in parameter values, the model proposed for albino rabbits applies to pigmented rabbits as well.

APPROVED: Joseph R. Robinson

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BY

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I. INTRODUCTION

Considerable progress has been made in unraveling the mystery surrounding the disposition of a drug from the moment of instillation into the cul-de-sac to its ultimate elimination from a target tissue within the eye. Over the years research efforts in our laboratory have centered upon an examination of the total ocular disposition characteristics of the important miotic drug, pilocarpine. For instance, we have studied: (a) the various factors governing its extent of absorption from the precorneal area (1-6), (b) the mechanism of its transcorneal penetration (7), and (c) its overall disposition in various ocular tissues, including aqueous humor, iris, ciliary body, lens, and vitreous humor (8). From these combined data, it is possible to construct a preliminary model to account for the disposition of pilocarpine once it is delivered to the site of absorption and, equally as important, to enhance our understanding of the interrelationships among the various processes encountered by a pilocarpine molecule in its journey into and through ocular tissues.

Thus far, all of the above studies, as well as many of those conducted by other investigators, have employed albino rabbits as the experimental animal. An important physiological characteristic of the albino rabbit is the absence of pigment in its iris and ciliary body. It is

therefore important to recognize that although this strain of rabbit serves as a useful experimental model, relative to humans, in studying drug permeation, it may not provide an accurate picture of disposition for those drugs which possess varying degrees of affinity for pigment in the iris and ciliary body. Indeed, several drugs such as atropine, cocaine, ephedrine, phenylephrine and pilocarpine are known to produce differential effects in human pigmented and non-pigmented irides (10-19).

In light of these observations, the purpose of the present study is two-fold: (a) via computer simulation techniques to critically evaluate the precorneal factors influencing drug absorption as well as factors intimately related with its disposition once it penetrates the corneal epithelium and (b) to examine the disposition kinetics of pilocarpine in dark-eyed rabbits in an effort to understand how it differs from that of albinos.

Throughout this report we shall refer to rabbits with pigmented irides as dark-eyed or pigmented rabbits, and those with non-pigmented irides as albino or non-pigmented rabbits.

A. Background

Considering that the eye is an essential organ for day to day activities, it is not surprising to find that it is hostile to exogenous influences which tend to interfere

with its integrity and proper functioning. It thus presents an obstacle which must be surmounted by any drug before it can gain access to the internal structures of the eye. Many of the factors which reduce the amount of drug available for absorption have been detailed in previous reports (1-8). Among those studied are the binding of drugs to proteins in the tear fluid (2, 3, 20), drainage of the instilled solution down the nasolacrimal duct (1, 5), and the continual process of tear turnover (5, 21). As the major route of drug entry into the eye is the cornea (22), conjunctival absorption can potentially reduce the fraction of a given dose ultimately reaching a target tissue within the eye.

Sieg and Robinson (7) have shown that for pilocarpine, the epithelium apparently serves as both a barrier and a depot, while the stroma is kinetically indistinguishable from the aqueous humor. In contrast to other reports (23, 24), the endothelium does not appear to exercise much control over the movement of pilocarpine through the cornea.

Whereas it is sufficient to use data up to two hours to make statements on the corneal permeation process undergone by pilocarpine, this is not so when total disposition of the drug is to be considered. Makoid and Robinson (8) recently monitored pilocarpine aqueous humor levels beyond two hours only to find a fairly complex disposition pattern. A significant amount of pilocarpine was taken up by the

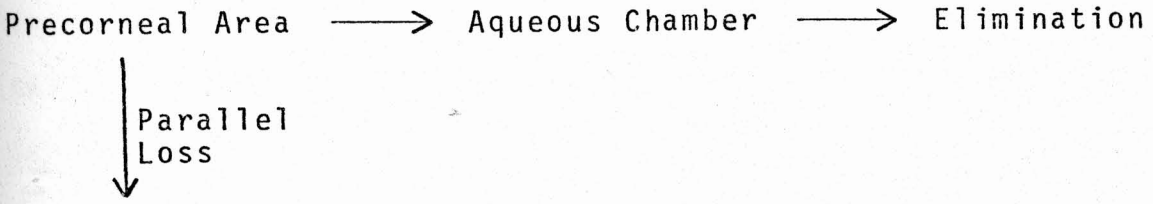
tissues surrounding the aqueous chamber and these included the iris, ciliary body, vitreous humor and lens.

Unlike albino rabbits, studies on the precorneal disposition of an instilled dose of drug as well as its permeation into and through the cornea of pigmented rabbits have not been reported in the literature. Almost all of the studies are concerned with the binding of drugs to pigments present in the iris and ciliary body or with some pharmacological response (9-19). The net result is that a complete picture of drug disposition in pigmented rabbits is not available. Nevertheless, it is reasonable to assume a similar precorneal disposition pattern of instilled dose in these two strains of rabbits. The present study was initiated in an attempt to obtain preliminary data on the movement of pilocarpine from the tear chamber, through the cornea and into the aqueous chamber, iris and ciliary body.

B. Development of the Model

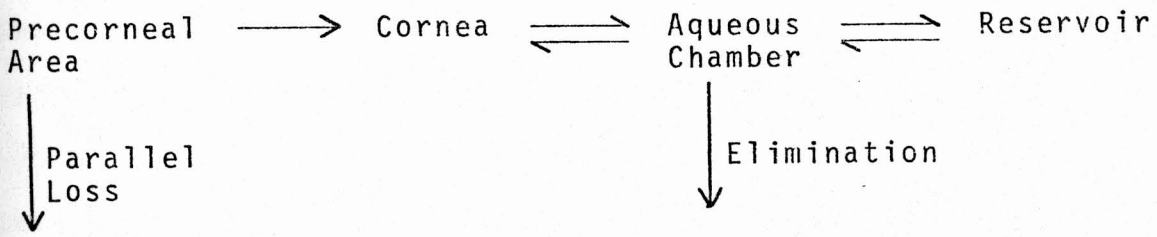
All of the previously described disposition factors must be incorporated into any model if it is to have predictive value in accounting for the fate of pilocarpine following topical instillation. Thus, models which are structured as in Scheme I are inadequate because they assume the cornea as a single membrane. The model proposed

Scheme I



by Makoid (Scheme II) (25) corrects this deficiency, but it does not distinguish the two components of the cornea, viz., epithelium and stroma, that play an important role in the movement of pilocarpine through this structure. Both

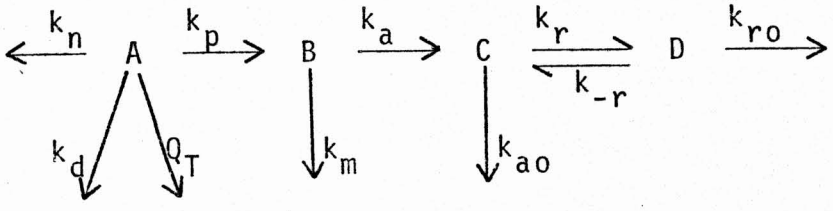
Scheme II



models suffer from the additional drawback that arises from their not considering the effects of instilled drop size explicitly. This important precorneal factor, however, forms a component of the model proposed by Himmelstein, Guvenir and Patton (26) which, unfortunately, treats the cornea as a single membrane, as in Scheme I. In short, for one reason or another, all these models are incomplete in so far as describing the fate of pilocarpine in the precorneal area and the various ocular tissues. The model to be

examined in this report, then, attempts to integrate all these approaches so that we can critically evaluate the various factors modulating the movement of pilocarpine in the tear chamber and the internal structures of the eye. Scheme III presents the key elements of the model. In

Scheme III



Scheme III, A represents the precorneal area; B, the epithelium; C, the stroma-aqueous chamber; and D, the reservoir. For simplicity we shall hereafter refer to the stroma-aqueous chamber as aqueous humor. The parameters of the model are detailed in Table I, and the major assumptions introduced while constructing it are listed below. They are:

(a) instantaneous mixing occurs in the tear chamber following the instillation of a drop,

(b) the rate of tear production is not significantly affected by the introduction of a drop,

(c) drug disposition is adequately described by first-order processes,

(d) with the exception of transfer between the

Table I. Parameters of the Model Described in Scheme III.

Parameter	Rate Constant Associated With:
k_p	Permeation into the epithelium
k_n	Non-productive loss including conjunctival absorption
k_d	Drainage
Q_T	Tear flow
k_a	Transfer of pilocarpine from the epithelium to the stroma-aqueous chamber
k_m	Loss of drug via metabolism or lateral diffusion from the epithelium
k_{ao}	Elimination of drug from the aqueous chamber
k_r	Transfer of pilocarpine from the stroma-aqueous chamber to the reservoir
k_{-r}	Transfer of pilocarpine from the reservoir to the stroma-aqueous chamber
k_{ro}	Elimination of pilocarpine from the reservoir

aqueous chamber and the reservoir, the other transfer processes depicted in Scheme III are unidirectional, i.e., irreversible flow operates,

(e) loss of drug via metabolism or lateral diffusion from the epithelium is negligible, i.e., $k_m \simeq 0$, and

(f) the iris, ciliary body, lens and vitreous humor constitute the reservoir.

Proceeding from these assumptions, appropriate differential equations can be easily written to account for the rate of change of mass of drug in each of the compartments shown in Scheme III. What distinguishes this model from Models I and II mentioned earlier is its treatment of disposition kinetics in the precorneal area, i.e., compartment A. The principles underlying this approach are modified from the treatment of Himmelstein, Guvenir and Patton (26) which, in turn, is based on the concept of flow-limited pharmacokinetics advanced by Bischoff and Dedrick (27). Accordingly,

Rate of change in mass in tear fluid = -Rate of loss by drainage - Rate of loss via tear flow - Rate of loss via corneal and non-productive absorption Eq. 1

When expressed in mathematical terms Equation 1 becomes:

$$\frac{dV_A[A]}{dt} = -Q_T[A] - k_d(V_A - V_o)[A] - (k_p + k_n)[A] \qquad \text{Eq. 2}$$

where: V_A is the volume of fluid in the tear chamber at a given time; V_o is the normal resident tear volume; the symbol [ ] denotes concentration which, in this instance, is that in the precorneal area; and the other symbols are as described previously. In Equation 2 the term $(V_A - V_o)$ represents the volume of fluid in the precorneal area in excess of the normal resident volume, and it is this excess which triggers the accelerated drainage of an instilled solution from the precorneal area in accordance with Equation 3 (1):

$$\frac{dV_A}{dt} = -k_d(V_A - V_o) \qquad \text{Eq. 3}$$

By taking the indicated derivatives in Equation 2 and substituting into it the value of dV_A/dt given by Equation 3, Equation 4 results.

$$\frac{d[A]}{dt} = -(k_p + k_n + \frac{Q_T}{V_A})[A] \qquad \text{Eq. 4}$$

In Equation 4 the term Q_T/V_A accounts for the influence of tear flow on instilled drug concentration. It is a time-

dependent quantity as V_A is. As one would expect, this factor becomes important only after the normal resident tear volume is once again attained. The time at which this occurs can be estimated from Equation 5.

$$V_A = V_o + V_i \exp(-k_d t) \qquad \text{Eq. 5}$$

where V_i is the volume instilled. Equation 5 can be combined with Equation 4 to give an expression which can be integrated to yield a final expression describing the time-dependence of drug concentration in the precorneal area following topical instillation, i.e., Equation 6.

$$[A] = [A]_i \left(\frac{V_i}{V_i + V_o}\right)\left(\frac{V_i + V_o}{V_o + V_i \exp(-k_d t)}\right)^{\frac{Q_T}{V_o k_d}} \exp\left\{-\left(k_p + k_n + \frac{Q_T}{V_o}\right)t\right\} \qquad \text{Eq. 6}$$

where $[A]_i$ is the instilled drug concentration. Similar mass balance equations can be written for compartments B, C and D. Specifically,

$$V_B \frac{d[B]}{dt} = k_p V_A [A] - (k_a + k_m) V_B [B] \qquad \text{Eq. 7}$$

$$V_C \frac{d[C]}{dt} = k_a V_B[B] + k_{-r} V_D[D] - (k_{ao} + k_r) V_C[C] \qquad \text{Eq. 8}$$

$$V_D \frac{d[D]}{dt} = k_r V_C[C] - (k_{-r} + k_{ro}) V_D[D] \qquad \text{Eq. 9}$$

In Equations 7, 8 and 9, the V's refer to the volumes of the compartments indicated by the subscripts. Analytical expressions can be obtained for these equations but they are far too complicated to be of 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., Equations 3, 4, 7, 8 and 9, using numerical methods, a process which is greatly expedited by the advent of computers. We shall adopt this approach in the simulation studies.

II. EXPERIMENTAL

A. Computer Simulation Studies

The computer program to simulate the disposition of pilocarpine in the eye consists of two major subroutines, DERIVS and INTGRT (see appendix). The system of differential equations (Equations 3, 4, 7, 8 and 9) is presented in the subroutine DERIVS, which is introduced into the main program via the differential equation routine DEPC[1]. DEPC employs the predictor-corrector method outlined by Hamming (28) to numerically solve initial-value problems of 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 in which the dose is removed before absorption is complete.

All computer calculations were performed on a digital computer at the Madison Academic Computing Center[2], and the specific simulations carried out are indicated in the "Results" section.

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[1] Differential Equations Reference Manual for the 1108, Madison Academic Computing Center, Madison, Wisconsin.

[2] UNIVAC 1110.

B. Pilocarpine Disposition Studies in Pigmented Rabbits

1. Materials. Tritiated pilocarpine alkaloid (specific activity 4.165 Ci mmole^{-1}) in ethanol was obtained commercially[3] and was purified by vacuum evaporation immediately prior to each run as previously described (29). All other chemicals used were either reagent or analytical grade and were used as received. Male, mixed breed, dark iride rabbits[4], weighing between 1.8 and 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 stimulation (radio) were maintained on a 24-hour basis in the caging facilities.

2. Methods

a. Preparation of 1 x 10^{-2} M pilocarpine solutions. A 1 x 10^{-2} M pilocarpine solution prepared in isotonic Sorensen's phosphate buffer at a pH of 6.24 was added to the labeled material prior to each experiment. It was determined that 0.25 mCi of the tritiated pilocarpine per milliliter of final solution was sufficient to insure good counting efficiency, i.e., 150,000-175,000 cpm μl^{-1} (22). The amount of labeled material added was small enough to have no effect on the molarity of the final solution.

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[3]New England Nuclear, Boston, Massachusetts.

[4]Klubertanz, Edgerton, Wisconsin.

b. Aqueous humor drug concentration versus time profile. The basic experimental techniques used for instilling solutions and for monitoring aqueous humor drug levels after topical dosing were described previously (30). Unanesthetized animals were used and a standard 25-µl dose was instilled.

c. Corneal drug concentration versus time profile. Immediately after the aqueous humor was aspirated from the anterior chamber, the eye was proptosed and secured with a hemostat behind the globe. A single incision was made with a scalpel at the corneal margin and the entire cornea was excised. Each cornea was carefully rinsed in saline to remove excess radioactivity and blotted on tissue. It was then placed into tared combustion cones[5] and the wet weight was determined using an analytical balance. A tissue oxidizer[6] was used to burn each cornea, and samples were counted in plastic vials[7] using a commercial liquid scintillation solution[8] and scintillation spectrometer[9]. After

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[5]Combusto-Cone, Packard Instrument Co., Downers Grove, Illinois.

[6]Model 306, Packard Instrument Co., Downers Grove, Illinois.

[7]The Vial, Research Products International Corp., Elk Grove Village, Illinois.

[8]Monophase-40, Packard Instrument Co., Downers Grove, Illinois.

[9]Model 2002, Packard Instrument Co., Downers Grove, Illinois.

suitable corrections, the final count for each sample was converted to micrograms of pilocarpine per gram of cornea.

d. Iris and ciliary body drug concentration versus time profile. Because of the difficulty of dissecting the iris free from the ciliary body in the rabbit both structures were used. Immediately after the cornea was excised, the entire iris and ciliary body were extracted intact and care was taken that they were free from choroidal adhesions. The tissues were then treated in a manner identical with the cornea. After suitable corrections, the final count for each sample was converted to micrograms of pilocarpine per gram of iris and ciliary body.

Throughout the studies the surgical procedures were performed as quickly as possible so that errors due to redistribution of drug during the time required to obtain samples were minimized.

e. Influence of instilled concentration on drug levels in the cornea, aqueous humor, and iris-ciliary body at 20 minutes post-instillation. Pilocarpine solutions of 0.5×10^{-2} M, 2×10^{-2} M and 4×10^{-2} M were prepared in the same manner as the 1×10^{-2} M solution. The exception was with the 0.5×10^{-2} M solution, for which a larger amount of tritiated pilocarpine was added to insure a final count of 600,000-700,000 cpm μl^{-1}. Again, unanesthetized

animals were used and a standard 25-µl dose was instilled. Cornea, aqueous humor and iris-ciliary body samples were obtained and analyzed as described earlier in this report.

III. RESULTS

A. Computer Simulation of Pilocarpine Disposition in Albino Rabbits

1. Choice of Parameter Values

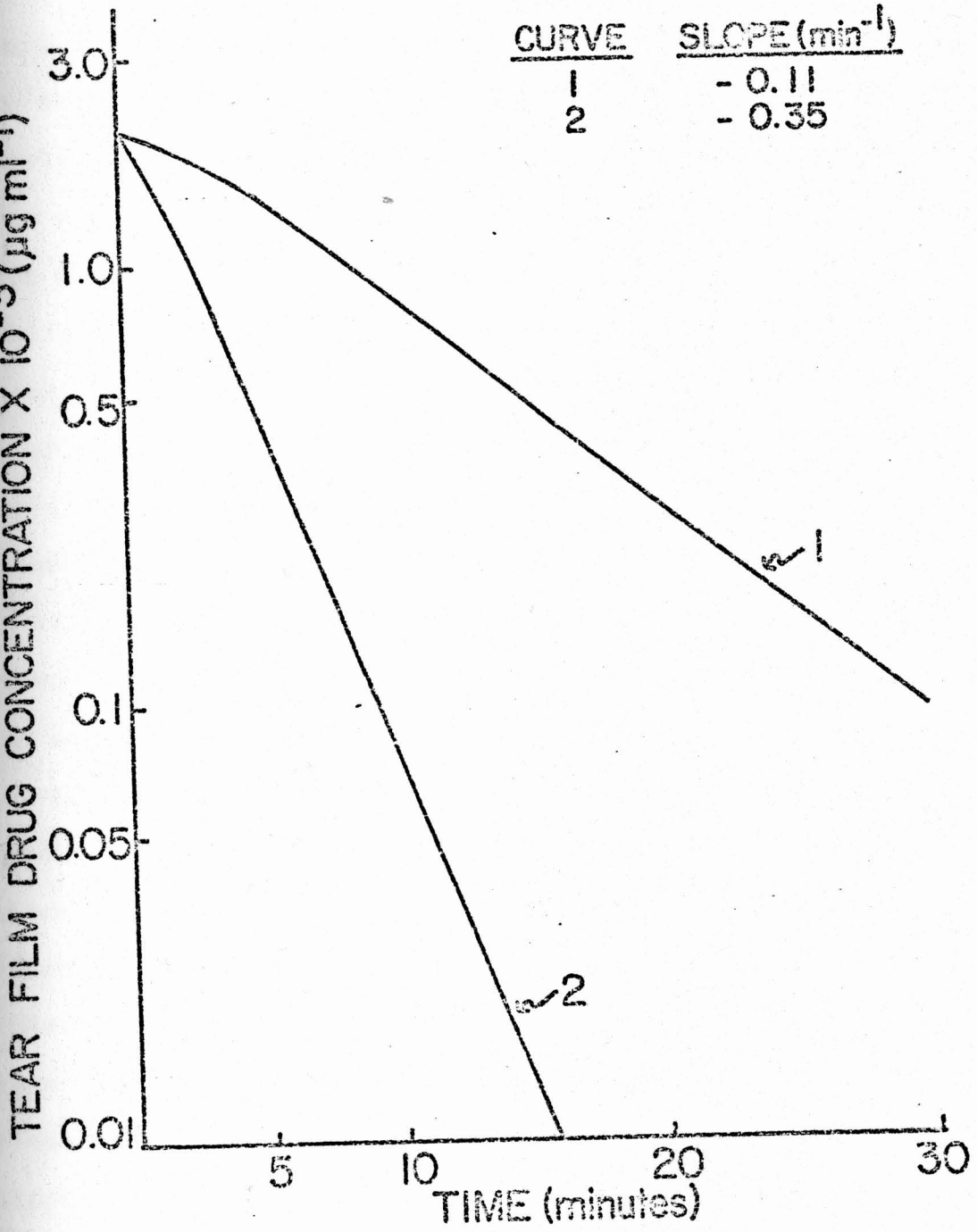
a. Precorneal factors. Table II lists the precorneal parameter values to be used in the initial simulation. It can be shown that those parameters associated with distribution of pilocarpine within the eye are of no relevance insofar as testing for the proper choice of precorneal factor values.

Curve 1 in Figure 1 describes the decline in drug concentration in the tear film as a function of time for the given set of parameter values. Linearity of this semilogarithmic plot is not observed until approximately ten minutes post-dosing. This behavior is predicted by Equation 6 and is primarily due to the influence of drainage during that time interval. In other words, immediately following the instillation of a drop, drainage predominates over the other factors of loss from the precorneal area. The curvature also reflects the presence of the other factors; otherwise, a slope of zero would be obtained since drainage by itself has no influence on concentration, only the amount. The slope of the linear portion of Curve 1 has a numerical value of 0.11 min^{-1}, corresponding to the sum of rate constants associated with corneal permeation,

Table II. Precorneal Parameter Values Used in the Initial Simulation.

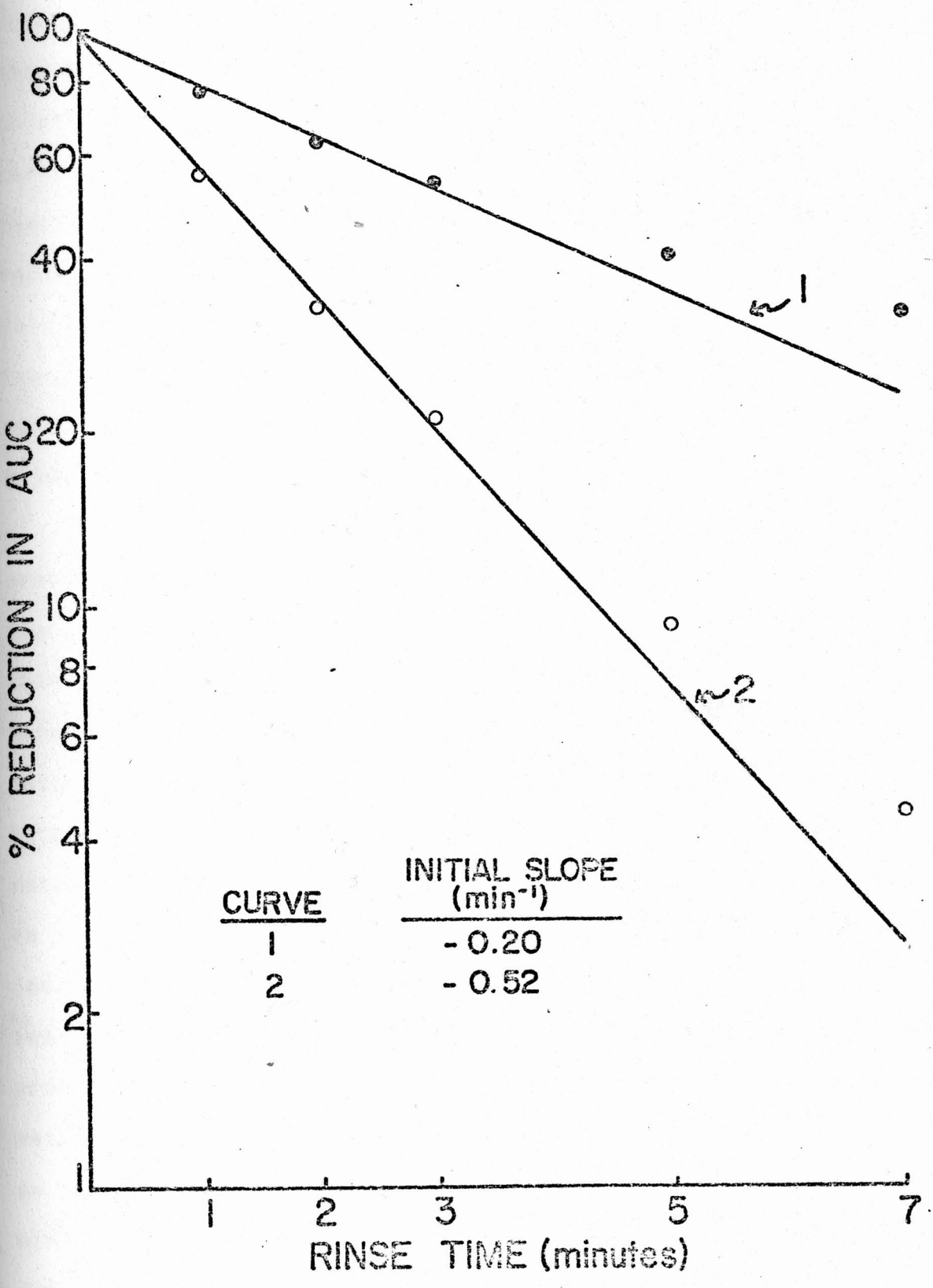
Parameter	Value	Reference
k_d	0.545 min^{-1}	1
Q_T	0.66 µl min^{-1}	1
V_o	7.5 µl	1
k_n	0.0217 min^{-1}	6
k_p	0.002 min^{-1}	7

Figure 1. Decline in drug concentration in the tear film. Both curves are generated with $k_p = 0.002\ min^{-1}$, $k_t = (Q_T/V_o) = 0.088\ min^{-1}$, and $k_d = 0.545\ min^{-1}$. For Curve 1, $k_n = 0.0217\ min^{-1}$; for Curve 2, $k_n = 0.258\ min^{-1}$. See text for details.


CURVE
SLOPE (min⁻¹)
1
- 0.11
2
- 0.35
3.0
1.0
0.5
0.1
0.05
0.01
TEAR FILM DRUG CONCENTRATION X 10⁻³ (μg ml⁻¹)
1
2
5
10
20
30
TIME (minutes)

non-productive absorption and tear turnover under basal conditions. Since this value is the upper limit of the slope in a tear film drug concentration versus time plot, the slope in the non-linear region (i.e., zero to approximately 10 minutes) of the curve will have a lesser value. This result, however, is in disagreement with the value of 0.36 min^{-1} determined by Sieg and Robinson (31) for the decline of glycerin concentration in the tear film for the first five minutes after instilling 25 µl of a pH = 8.0 solution. Based on the similar aqueous solubility characteristics expected of glycerin and pilocarpine, and on the assumption that the rate constant at pH 6.24 (which is the pH at which the pilocarpine permeation studies have been conducted) does not depart markedly from that at pH 8.0, it can be said that the initial estimate of 0.11 min^{-1} does not accurately describe the disappearance of pilocarpine from the tear chamber. Further support for this contention comes from the rinsing data of Sieg and Robinson (7). They observed approximately linear behavior when the percent reduction of area under the curve (AUC) of the aqueous humor profile as a result of rinsing was plotted against rinse time on semilogarithmic paper. Such a plot gave a slope of 0.57 min^{-1}. This is not the case when a similar plot is made from the parameter values given in Table II. Referring to Figure 2, if only the rinsing times of 0, 1, 2 and 3 minutes are considered, a slope of 0.20 min^{-1} is

Figure 2. Effect of rinse time on percent reduction in area under the curve (AUC) for pilocarpine in aqueous humor. Solid lines represent best fit lines through the first four points (0, 1, 2 and 3 minutes). Refer to legend of Figure 1 for parameter values used to generate Curves 1 and 2.


100
80
60
40
20
10
8
6
4
2
1
% REDUCTION IN AUC
1
2
3
5
7
RINSE TIME (minutes)
~1
~2
CURVE
INITIAL SLOPE (min⁻¹)
1
- 0.20
2
- 0.52

obtained; a slightly smaller value, 0.17 min^{-1} is obtained when the rinse time of five minutes is included. At this point, it is important to recognize that either value is larger than the value (0.11 min^{-1}) assigned to the rate constant of loss of pilocarpine from the tear chamber by routes other than that of drainage. This suggests that the value obtained from rinsing perhaps reflects the contribution from drainage. This additional contribution is, however, not additive; otherwise, the slope would be (0.11 + 0.54) min^{-1} or 0.65 min^{-1}.

Obviously, our immediate task is to decide on which precorneal factor (or factors) should be modified to bring the predicted tear film profile into agreement with that obtained experimentally. The approximately three-fold difference between observed and assigned overall disappearance rate constants of pilocarpine in the precorneal area cannot be accounted for solely by an underestimated permeation rate constant (0.002 min^{-1}). For now, the assumption made in the model that the instillation of a solution does not induce lacrimation rules out modifying the magnitude of tear flow rate as an alternative. This leaves the nonproductive absorption rate constant as the adjustable parameter, meaning that its magnitude has to be increased from an initial estimate of 0.0217 min^{-1} to one of, say, 0.258 min^{-1}. Curve 2 in Figure 1 illustrates the tear film profile produced by the set of revised parameter values. As

with Curve 1, its early portion shows some degree of curvature; in contrast with Curve 1, it has a steeper terminal slope. When the percent reduction in AUC of the aqueous humor profile due to rinsing was plotted against rinse time, an initial slope with a magnitude of 0.52 min^{-1} resulted. Clearly, better agreement with experimental result is obtained. One should note, however, that increasing the non-productive absorption rate constant (k_n) causes a reduction in the amount of pilocarpine absorbed unless the corneal permeation rate constant is increased accordingly. This means that information beyond that provided by the tear film profile is necessary. Also, the significance of this change in the magnitude of k_n must be evaluated.

b. Intraocular drug disposition factors. The proper choice of an estimated value for k_p as well as those associated with the distribution of pilocarpine within the eye rests on cornea and aqueous humor data, specifically the levels achieved and the peak times observed. Since the cornea and epithelium data of Sieg and Robinson (7) are reported in micrograms of pilocarpine per gram of tissue while the model treats concentration in units of micrograms of drug per volume of tissue, and also since the density of tissue is unknown it is necessary to compare the simulated and experimentally determined profiles on the basis of

amount of drug in the respective tissues. Table III lists the parameter values employed to generate the profiles in Figures 3, 4 and 5. Incidentally, unless otherwise specified, all subsequent simulations will adopt these values.

Whereas the k_a, k_{ao} and k_r values are modified from literature values (7, 25), the k_{-r} and k_{ro} values are assigned on a somewhat empirical basis. The rationale behind assigning the same numerical value to the rate constant of transfer from the aqueous chamber to the reservoir, k_r, and from the reservoir to the aqueous chamber, k_{-r}, derives from the finding of Makoid (25) that in the albino rabbit, the iris and ciliary body (which constitute portions of the reservoir) are as likely to receive as to retain pilocarpine since they are porous tissues (33-35). As they are also highly vascularized, it is not unreasonable to expect loss of pilocarpine into the circulation there. Considering that: (a) blood flows through the rabbit's eye at an approximate rate of 320 µl/min/g of tissue (36), (b) the volume of blood in the eye at any one time is approximately 211 µl (36), and (c) the weight of iris and ciliary body lies in the neighborhood of 40 mg (25), a first-order rate constant of 0.06 min^{-1} can be calculated and this is taken as k_{ro}, the rate constant of loss via the circulation supplying the iris and ciliary body.

Curves 1 in Figures 3, 4 and 5 represent the simulated epithelium, cornea and aqueous humor levels consistent with

Table III. Values for Parameters Associated With the Ocular Distribution of Pilocarpine.

Parameter	Value
k_p	0.004 min^{-1}
k_d	0.545 min^{-1}
k_n	0.258 min^{-1}
Q_T	0.66 µl min^{-1}
k_a	0.10 min^{-1}
k_{ao}	0.018 min^{-1}
k_r	0.008 min^{-1}
k_{-r}	0.008 min^{-1}
k_{ro}	0.06 min^{-1}
V_o	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 (32).

Figure 3. Amount of pilocarpine in epithelium of albino rabbits following topical instillation of its solution.

Key: ▲ Data from reference 7.

--- Simulated profile without feedback, Curve 1.

—— Simulated profile with feedback, Curve 2.

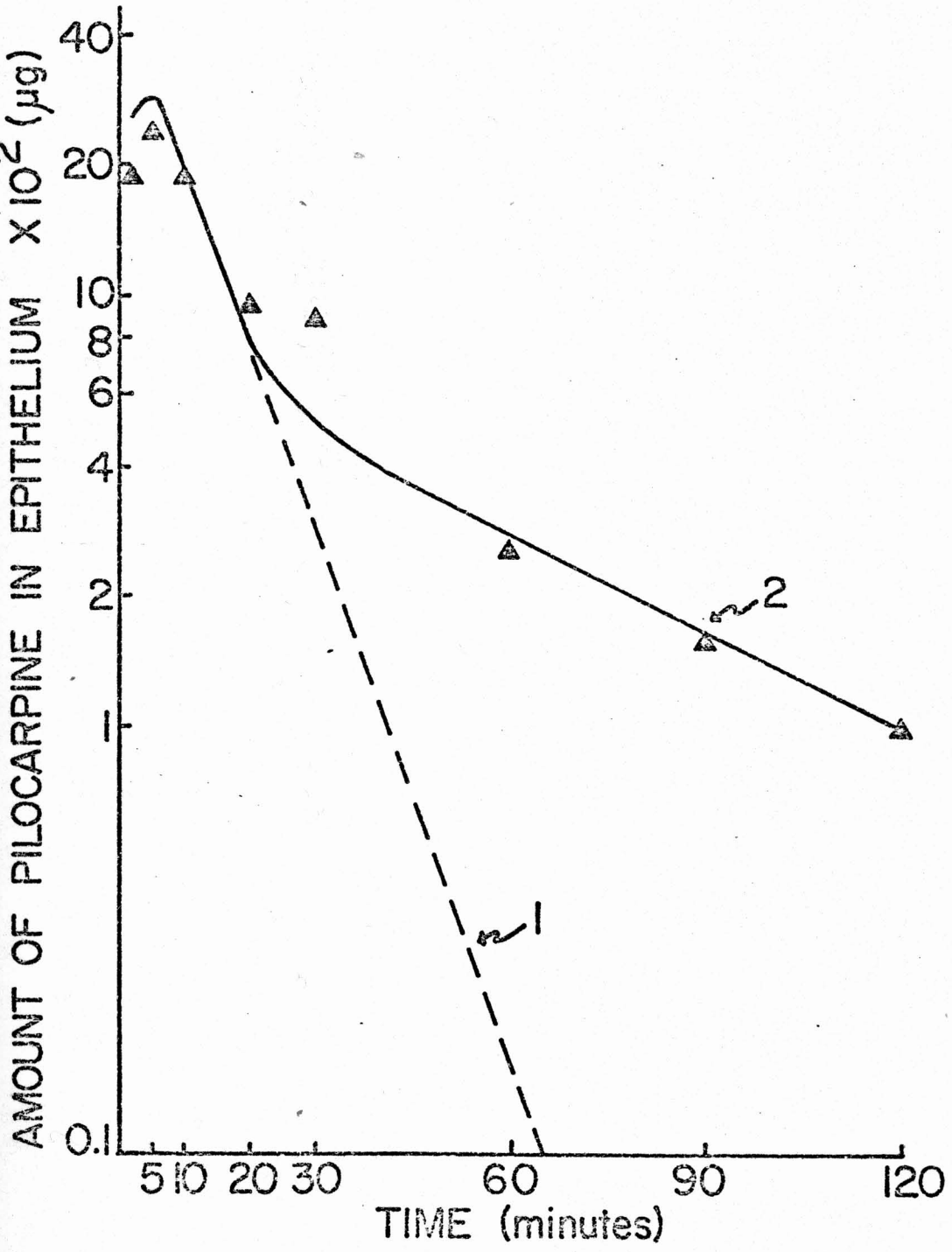

AMOUNT OF PILOCARPINE IN EPITHELIUM X 10² (µg)
40
20
10
8
6
4
2
1
0.1
5 10 20 30 60 90 120
TIME (minutes)
1
2

Figure 4. Amount of pilocarpine in cornea of albino rabbits following topical instillation of its solution.

Key: ▲ Data from reference 7.

--- Simulated profile without feedback, Curve 1.

—— Simulated profile with feedback, Curve 2.

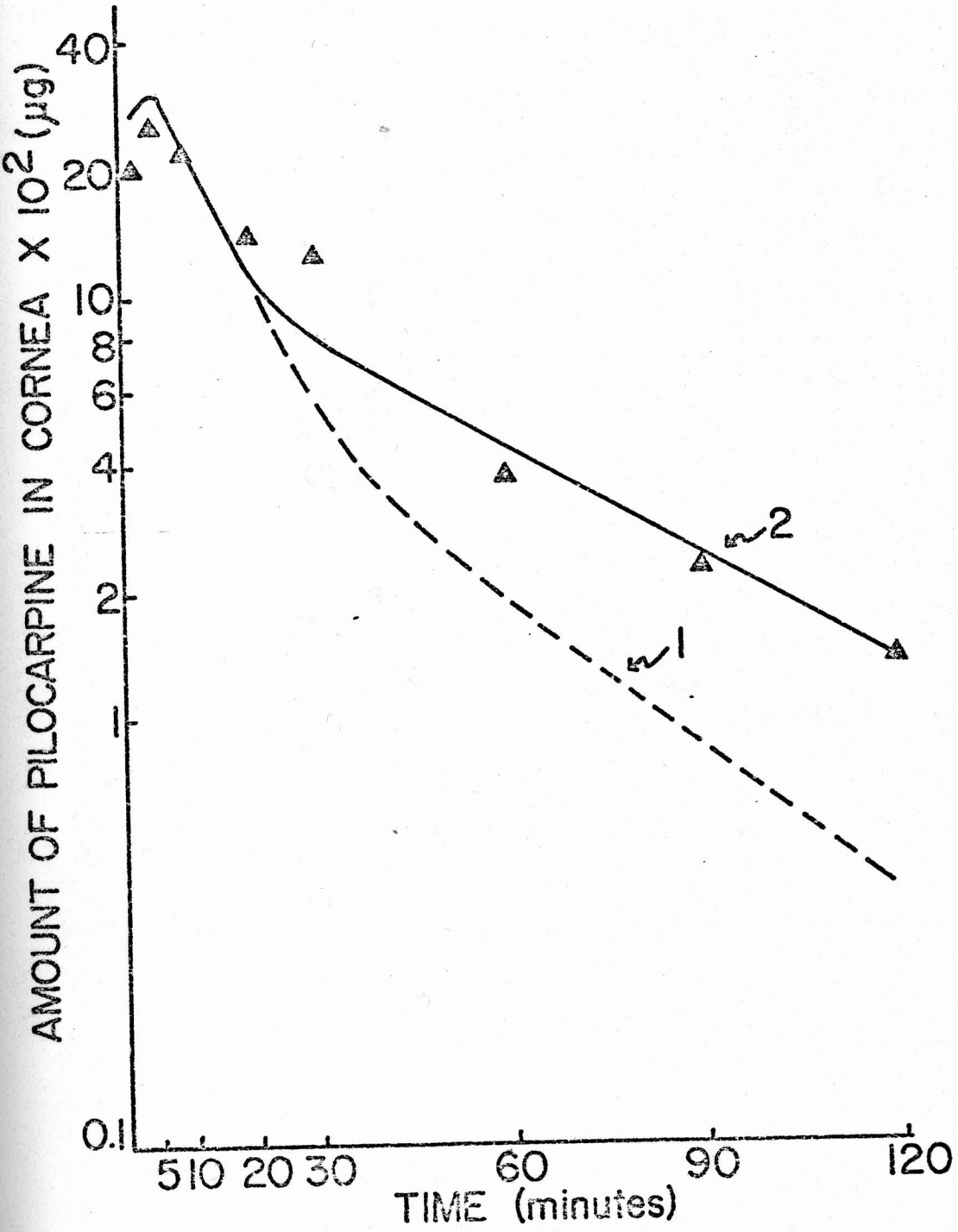
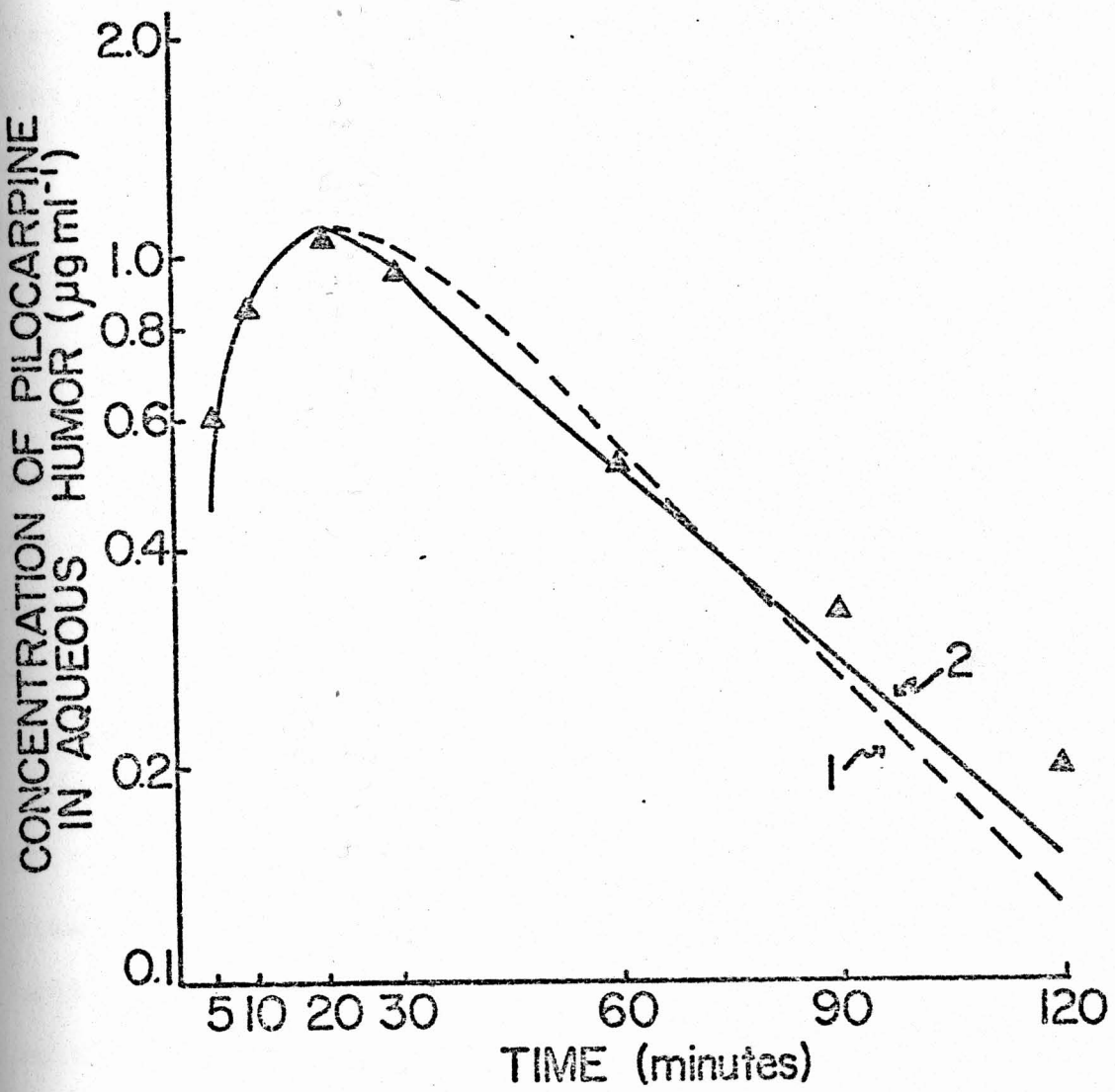

AMOUNT OF PILOCARPINE IN CORNEA X 10^2 (µg)
40
20
10
8
6
4
2
1
0.1
5 10 20 30 60 90 120
TIME (minutes)
2
1

Figure 5. Concentration of pilocarpine in aqueous humor of albino rabbits following topical instillation of its solution.

Key: ▲ Data from reference 7.

--- Simulated profile without feedback, Curve 1.

—— Simulated profile with feedback, Curve 2.


CONCENTRATION OF PILOCARPINE IN AQUEOUS HUMOR (μg ml⁻¹)
2.0
1.0
0.8
0.6
0.4
0.2
0.1
5 10 20 30 60 90 120
TIME (minutes)
1
2

the set of parameter values listed in Table III. The lack of biphasic decline in epithelium levels (Figure 3) is immediately apparent. While biphasic decline is present in the cornea plot (Figure 4), its terminal level is much lower than that observed experimentally and at the same time it declines with a perhaps insignificantly larger rate constant (0.019 vs. 0.017 min^{-1} experimentally). These results are not surprising in view of the fact that biphasic behavior in the epithelium is not inherent in the system of differential equations. Biphasic behavior is observed in the cornea because its levels receive contributions from both the epithelium and the stroma. Initially the levels in the epithelium are high so that the level in the cornea is primarily that of the epithelium. Eventually the epithelium is depleted of drug and the corneal drug profile is then controlled by the behavior in the stroma which, in this model, is necessarily that in the aqueous humor.

As a first step towards correcting this discrepancy between the predicted and simulated profiles, a "feedback" criterion is introduced into the system of differential equations. In its original form, the model allows drug to continue to accumulate in the aqueous humor in spite of an unfavorable concentration gradient governing the transfer of drug from the epithelium to the stroma-aqueous humor. However, the data of Sieg and Robinson (7) indicate that

beyond 30 minutes after dosing, pilocarpine levels in the epithelium appear to decline with a rate constant whose magnitude is identical with that of aqueous humor turnover. A comparison of epithelial and aqueous humor concentrations further reveals that during this period, the aqueous humor concentration is approximately 0.15 times that of the epithelium. Guided by these two pieces of information, a "feedback" criterion is established specifying that when the ratio of the concentration in the aqueous humor and the epithelium exceeds a certain value, say x, the rate constant governing the decline in epithelial levels shifts from its input value to the value assigned to the elimination rate constant from the aqueous humor, k_{ao}.

Letting x have a value of 0.12, Curves 2 in Figures 3, 4 and 5 result. Biphasic behavior is evident in both simulated epithelium and cornea profiles. The rate constant associated with the terminal portion of the epithelium profile has a value of 0.018 min^{-1} which, as it should be, is that governing the elimination of pilocarpine from the aqueous chamber. Its counterpart in the cornea has a slightly larger value, 0.020 min^{-1}, corresponding to the value of the terminal slope of the aqueous humor plot.

In addition to the biphasic behavior, Figures 3 and 4 show that the simulated pilocarpine levels in the epithelium and cornea at two and five minutes are somewhat higher than the experimental values. This can be rationalized by

the choice of precorneal parameter values. Although only the 30-minute value is available, it is apparent that these profiles do not satisfactorily simulate the behavior interpolated from experimental results in the region between 20 and 60 minutes. This resides in the very method of specifying the "feedback" criterion. Specifically, this approach tacitly assumes that the rate constant of transfer from the epithelium to the aqueous humor undergoes a discontinuous change when in fact it is not so. Consequently, the behavior in the epithelium and cornea in the region of concern here perhaps is better described by some function associating the rate constant of transfer to some indicator of concentration differential such as ratio of concentrations with the added constraint that the final value adopts the value of the rate constant of elimination from the aqueous chamber.

In contrast to the predicted levels in the epithelium and cornea, those in the aqueous humor agree with the experimental values rather well (Figure 5). The terminal portion of the profile is slightly different as a rate constant of 0.018 min^{-1} rather than 0.016 min^{-1} is used. The input value 0.018 min^{-1} is further modified by the myriad of distribution processes postulated to occur between the aqueous chamber and its surrounding tissues. The net result is that a rate constant of 0.020 min^{-1} is found to characterize this phase of the aqueous humor curve.

Whereas the "feedback" criterion causes dramatic changes in the epithelium and cornea profiles, it appears to have a minimal influence on the aqueous humor level within the time interval of simulation. This is demonstrated by the area under the curve (AUC) values listed in Table IV. It is expected that the differences between the two instances will be magnified at later times. The peak time, t_p, and peak concentration, C_p, values in Table IV indicate that the "feedback" criterion, $x = 0.12$, is not operative until at least the peak time in the aqueous chamber is reached. Not shown in the table, however, is the peak time in the reservoir, which shifts from 35 to 38 minutes, clearly a consequence of incorporating the "feedback" criterion.

2. Influence of Mode of Instilling a Solution on Aqueous Humor Drug Levels. A cursory examination of the simulated aqueous humor profile depicted in Figure 5 reveals that the five minute value is about 1.5 times lower than that obtained experimentally. During the course of this study, several attempts have been made to improve agreement between the two. One approach involved increasing the rate constant (k_a) of transfer from the epithelium to the aqueous humor from its value of 0.10 min^{-1} to 0.15 min^{-1}. This proved to be unsatisfactory since improvement in the aqueous humor profile came at the

Table IV. Influence of Feedback on the Area Under the Curve (AUC), Peak Time (t_p), and Peak Concentration or Amount (C_p) in the Epithelium, Cornea, and Aqueous Humor.

	AUC[a] (μg min)		t_p (min)		C_p[b]	
Tissue	Case A[c]	Case B[d]	Case A	Case B	Case A	Case B
Epithelium	6.86	4.54	3.5	3.5	0.30	0.30
Cornea	9.08	6.79	4.0	4.0	0.31	0.31
Aqueous Humor	15.42	15.53	20.75	20.75	1.11	1.11

[a] From zero to 120 minutes.

[b] For epithelium and cornea, the unit is μg. For aqueous humor, it is μg ml^{-1}.

[c] Feedback.

[d] No feedback.

expense of the epithelium profile. It was then thought that the discrepancy could be related to the very manner in which a given dose was instilled. Ideally, we would like to explore the hypothetical situation of instilling a given volume, say 25 µl, at a given rate over a period of one minute. Unfortunately, this cannot be treated by the computer program in its present form. Nevertheless, considerable information can be gained by considering the several cases shown in Table V. In all cases, the concentration of the instilled solution is the same. One dose is administered at time zero and the other at one minute so that the total amount of drug (67.82 µg) is the same in each case.

The resulting levels are summarized in Table VI. It is evident that the discrepancy between predicted and observed levels cannot be accounted for by this mode of administration alone. This observation, coupled with the scatter expected of experimental data, could make the following discussion appear as an academic exercise.

At any rate, Case E simulates a situation where the dose is mixed with only a small volume of fluid bathing the cornea thereby creating a high local concentration and at the same time resisting removal of the dose by drainage. In the other cases it is assumed that instantaneous mixing of the dose occurs with the entire volume of fluid in the tear chamber and that drainage operates to an extent depending on the volume of dose. Inspection of the AUC

Table V. Several Modes of Instilling a Solution into the Cul-de-Sac.

Case	Volume Instilled (µl)		Tear Film Concentration ($\mu g\ \mu l^{-1}$)		Total Volume at One Minute (µl)
	0 min.	1 min.	0 min.	1 min.	
A	25.0	0.0	2.09	1.57	22.1
B	5.0	20.0	1.09	2.02	31.2
C	20.0	5.0	1.97	1.72	24.9
D	12.5	12.5	1.70	1.90	28.4
E	5.0	20.0	2.09	2.40	26.8

Table VI. Influence of Mode of Instillation on Aqueous Humor Levels and its AUC.

Time (min)	Case A	B	C	D	E
5	0.46[a]	0.39	0.46	0.43	0.41
10	0.86	0.85	0.90	0.89	0.91
20	1.11	1.16	1.19	1.20	1.26
30	0.96	1.02	1.04	1.05	1.11
60	0.51	0.54	0.55	0.56	0.59
90	0.27	0.29	0.30	0.30	0.32
120	0.15	0.16	0.16	0.16	0.17
AUC[b]	69.2	72.4	74.3	74.8	78.8

[a] $\mu g\ ml^{-1}$.

[b] $\mu g\ ml^{-1}$ min.

values reveals that this "dropwise" mode of instillation yields slightly larger values than when the entire dose is deposited in the cul-de-sac. Again, with the possible exception of Case E, this difference is probably well within experimental error (Table VII) and hence not discernible experimentally. The same can be said of the peak times and peak concentrations or amounts. The results of Cases B and C imply that establishing a high initial concentration by giving a larger volume of a solution of a given concentration can more than offset the unfavorable influence of drainage. This is reasonable to expect since drainage of the initial dose operates for only one minute and it is the excess volume at one minute that governs the ultimate drainage observed. Indeed, Table V indicates that the total volume at one minute is larger for Case B than for Case C. That Case B still yields a slightly larger AUC than Case A is related to a delay, albeit short, in drainage. By similar reasoning, Case D must have attained the proper balance between achieving a sufficiently high initial concentration on the one hand and optimal drainage on the other.

3. Relative Importance of Parallel Loss Factors on Extent of Drug Absorption. Table VIII lists the various cases to be considered in an attempt to gain an understanding of the relative importance of parallel loss

Table VII. Percent Error Incurred by Administering Divided Doses Rather Than a Single Dose for a Fixed Amount of Drug.

Case[a]	% Error
A	0.0
B	4.2
C	6.9
D	8.1
E	13.7

[a]See Table V for meaning of the cases.

Table VIII. Various Cases Illustrating the Relative Importance of Parallel Loss Factors on Extent of Absorption.

Case		Parameter Values (min^{-1})			
		k_{nl}[a]	k_{cj}[a]	k_t[b]	k_d
I	Only tear turnover -- Situation A	0.0	0.0	0.0203[c]	0.0
	-- Situation B	0.0	0.0	0.088[d]	0.0
II	Only conjunctival absorption	0.0	0.0217	0.0	0.0
III	Only non-conjunctival loss	0.2363	0.0	0.0	0.0
IV	Only drainage	0.0	0.0	0.0	0.5365
V	No tear turnover	0.2363	0.0217	0.0	0.5365
VI	No conjunctival loss	0.2363	0.0	0.088	0.5365
VII	No non-conjunctival loss	0.0	0.0217	0.088	0.5365
VIII	No drainage	0.2363	0.0217	0.0203	0.0
IX	No volume instilled	0.2363	0.0217	0.088	0.0
X	No drainage and no non-conjunctival loss	0.0	0.0217	0.0203	0.0
XI	All parallel loss factors present	0.2363	0.0217	0.088	0.5365

[a] $k_{nl} + k_{cj} = k_n = 0.258\ min^{-1}$ unless otherwise specified.

[b] $k_t = Q_T/V_o$.

[c] $V_o = 32.5\ \mu l$.

[d] $V_o = 7.5\ \mu l$.

factors, alone or in combination, in influencing the extent of drug absorbed across the corneal epithelium. Here, the rate constant (k_n) characterizing non-productive loss of drug in the precorneal area is divided into two components: one due to conjunctival absorption, k_{cj}, and the other due to processes other than conjunctival absorption, k_{nl}. For simplicity, k_{nl} will be denoted as the rate constant of non-conjunctival loss. The rationale behind this procedure will be elaborated upon later in this report. The AUC from zero to 120 minutes in the epithelium for the hypothetical case of no parallel loss is chosen as the reference standard to which the AUC's of all other cases are referred. It is believed that among all the tissues in the eye, the level achieved in the epithelium is most sensitive to changes in the precorneal factors. Ideally, the entire rather than the truncated AUC should be used. However, so long as we are interested only in relative values, it is justified to use the AUC from zero to 120 minutes. The ratios of AUC (Ratio A) of the various cases relative to that of the reference standard are shown in Table IX.

Note that Table VIII lists two situations for the case of 'only tear turnover' (Case I). Case IA gives a deceptive measure of the influence of tear turnover in causing parallel loss because by setting the remaining parallel loss factors equal to zero in this particular simulation and for an instilled dose of 25 µl, the resident tear

Table IX. Ratios of the Area Under the Curve of Various Cases Relative to (a) the Standard (Ratio A) and (b) the Case Where all Parallel Loss Factors are Fully Functional (Ratio B)[a].

	Case	Ratio A	Ratio B
I	Only tear turnover		
	-- Situation A	0.61	29.82
	-- Situation B	0.14	6.81
II	Only conjunctival absorption	0.58	28.38
III	Only non-conjunctival loss	0.051	2.48
IV	Only drainage	0.24	11.81
V	No tear turnover	0.022	1.09
VI	No conjunctival loss	0.021	1.03
VII	No non-conjunctival loss	0.048	2.32
VIII	No drainage	0.043	2.12
IX	No volume instilled	0.032	1.70
X	No drainage and no non-conjunctival loss	0.31	15.13
XI	All parallel loss factors present	0.021	1.00

[a]AUC for the ideal case is 334.3 µg min. AUC for Case XI is 15.9 µg min.

volume in the precorneal area is now 32.5 µl instead of 7.5 µl. For the same tear turnover rate, 0.66 µl min^{-1}, the first-order rate constant, k_t, for the process becomes 0.020 min^{-1}, instead of the value of 0.088 min^{-1} observed under normal conditions. Case IA essentially demonstrates the influence of tear turnover in the presence of a plugged drainage apparatus, and it assumes the presence of a mechanism maintaining the resident tear volume at 32.5 µl in spite of continuous tear turnover. Therefore, to appreciate the true influence of this parallel loss factor, the hypothetical case of delivering the same dose in no volume should be employed, i.e., Case IB.

According to this model, the parallel loss factors cause at least a 40% reduction in the amount of dose absorbed when compared with the ideal case where they are absent. Among them conjunctival absorption causes the least reduction. This is followed by tear turnover and then drainage. Non-conjunctival loss evidently causes the largest reduction in the percent of dose absorbed. The influence of drainage is less than what its magnitude tends to suggest since it is operative for only a small segment of the entire time course of drug absorption. The impact of these parameters on the extent of drug absorption can best be appreciated by examining the influence of combinations of individual ones in this particular regard. Thus, when only one of the four parallel loss factors is

eliminated, the ratio is reduced by approximately 80% relative to those cases where a single factor is operative. A comparison of the case where only non-conjunctival loss is present (Case III) with that where non-conjunctival loss is absent (Case VII) indicates that the combined influence of the precorneal factors other than non-conjunctival loss is rather equivalent to the influence of the latter alone. The predominant role played by non-conjunctival loss in total drug loss is further exemplified by Case VIII. Finally, when all parallel loss factors are functional, the AUC obtained is only 2% that of the ideal case.

Instead of taking the ideal case where no parallel loss is present as the reference, we can proceed to the other end of the spectrum and choose as the new reference the case where all the parallel loss factors are functional. This is equivalent to physiological conditions and is represented by Case XI. Obviously, Ratio B, defined as the ratio of AUC of a given case to Case XI, yields the same information on the relative contribution of the various precorneal factors to the percent of dose absorbed. However, it is more convenient to use Ratio B than Ratio A if one is interested in the fractional change in AUC for a given set of conditions where one or more loss routes are impaired.

In addition to AUC, the various cases in Table VIII can be characterized by the peak time (Table X). Taking

Table X. Peak Time in the Epithelium in the Presence of Various Parallel Loss Parameters.

	Case	t_p (min.)
I	Only tear turnover (A)	18.5, 100.0
	(B)	10.5
II	Only conjunctival absorption	18.5, 96.5
III	Only non-conjunctival loss	6.5
IV	Only drainage	20.0
V	No tear turnover	4.0
VI	No conjunctival loss	4.0
VII	No non-conjunctival loss	6.0
VIII	No drainage	5.5
IX	No volume instilled	5.0
X	No drainage and no non-conjunctival loss	14.5, 67.5
XI	All parallel loss factors present	3.5
XII	Parallel loss absent	33.5

Case XI which represents physiological condition as the reference, the peak time undergoes a shift to larger values when one or more of the parallel loss factors are absent. The magnitude of change is seen to be larger for Cases I, II and IV than for Cases V through IX. As with the AUC comparisons, non-conjunctival loss exerts a major influence on the magnitude of the peak time. Of course, it cannot be disputed that the changes for several of the cases (Cases III and V through IX) are not sufficiently large to be detected experimentally. A feature in Table X that warrants additional discussion is the double peak observed in the case where parallel loss is due only to tear turnover (Case IA), only to conjunctival absorption (Case II), and to conjunctival loss and tear turnover (Case X). Its presence can be explained by noting that a considerable amount of drug still remains in the tear chamber at the time the "feedback" criterion operates. Therefore, drug absorption into the epithelium continues after this time, but now the rate constant associated with transfer of drug from the epithelium to the aqueous humor is reduced, in fact, by approximately five times. Consequently, a second peak results. In all these cases, the two peaks are separated by at least 50 minutes.

B. Pilocarpine Disposition Kinetics in Pigmented Rabbits

1. Aqueous Humor Pilocarpine Levels as a Function of Time Following Instillation of a 1×10^{-2} M Solution.

Aqueous humor drug levels at various times after instilling a 1×10^{-2} M pilocarpine solution are summarized in Table XI, and the profile produced is displayed in Figure 6. An apparent peak concentration is reached within 30 minutes. One-compartment pharmacokinetic analysis of the data yields an apparent transcorneal permeation rate constant of 0.10 min^{-1} and an apparent elimination rate constant of 0.02 min^{-1}. Together these apparent rate constants predict a peak time of 20 minutes and a peak concentration of 0.91 $\mu g\ ml^{-1}$. Not only is the calculated peak occuring sooner than the observed peak, its level is also lower than the experimental value. The significance of this is to be discussed in a later section after some computer simulation results are presented.

2. Pilocarpine Levels in the Cornea as a Function of Time Following Instillation of a 1×10^{-2} M Solution.

Table XII presents the pilocarpine levels achieved in the cornea in terms of (a) micrograms of drug per gram of tissue and (b) total amount of drug in the tissue. Implicit in the former presentation of corneal data is the assumption that the drug is uniformly distributed in the various layers of this tissue. An examination of the profiles

Table XI. Concentration of Pilocarpine in Aqueous Humor Following Instillation of 1×10^{-2} M Solution.

Minutes	Number of Eyes	Micrograms per Milliliter of Aqueous Humor
5	10	0.51 (0.05)[a]
10	8	0.68 (0.04)
20	9	0.91 (0.04)
30	10	1.16 (0.11)
60	6	0.52 (0.05)
90	6	0.28 (0.027)
120	7	0.16 (0.009)

[a]Numbers in parentheses represent standard error of the mean.

Figure 6. Concentration of pilocarpine in aqueous humor of albino rabbits (◯) and of pigmented rabbits (●) following topical instillation of its solution. Error bars represent standard error of the mean. Albino rabbit data are taken from reference 7.

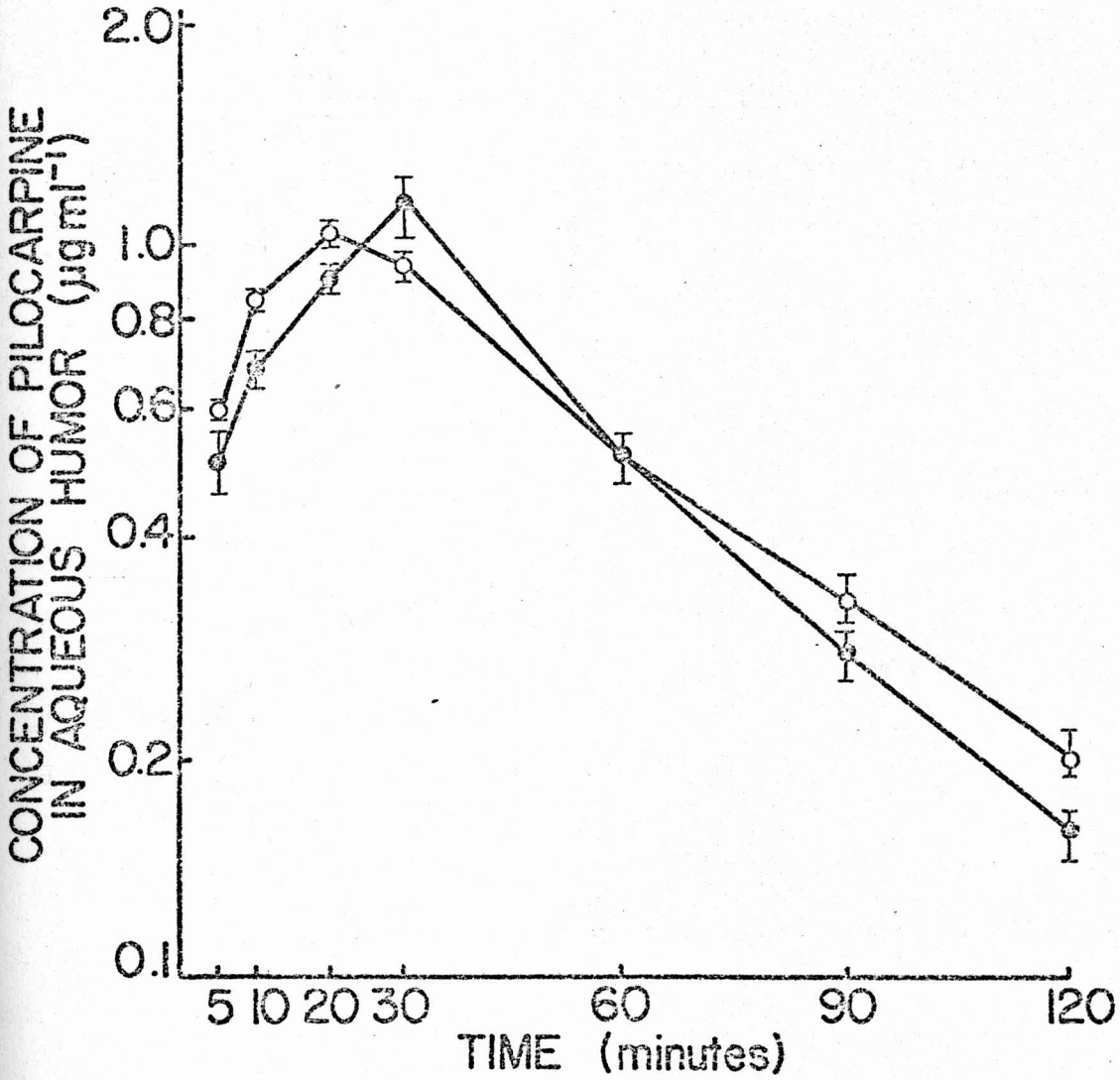

CONCENTRATION OF PILOCARPINE IN AQUEOUS HUMOR (μg ml⁻¹)
2.0
1.0
0.8
0.6
0.4
0.2
0.1
5 10 20 30 60 90 120
TIME (minutes)

Table XII. Concentration and Amount of Pilocarpine in the Cornea Following Instillation of a 1 x 10^{-2} M Solution.

Minutes	Number of Eyes	Microgram(s) per Gram of Cornea	Microgram[a]
5	5	5.44 (0.39)[b]	0.36 (0.04)
10	10	4.14 (0.44)	0.28 (0.02)
20	11	2.85 (0.19)	0.19 (0.01)
30	8	1.76 (0.18)	0.12 (0.008)
60	7	0.95 (0.06)	0.056 (0.002)
90	6	0.64 (0.06)	0.038 (0.003)
120	7	0.34 (0.03)	0.022 (0.002)

[a]Wet weight of corneas was 68.1 $\pm$ 1.2 mg (n = 67).

[b]Numbers in parentheses represent standard error of the mean.

plotted as a function of either concentration (Figure 7) or amount (Figure 8) reveals that peak levels are reached within five minutes of instillation of the dose. Beyond this time, biphasic decline of levels is evident. The first phase is associated with an apparent rate constant of 0.044 min^{-1}, in contrast to the 0.017 min^{-1} value describing the second phase. No attempt was made to estimate the apparent uptake rate constant as sufficient data points prior to the peak time were not available.

Since metabolism of drugs in ocular tissues is a possibility, and since we were measuring total radioactivity without discriminating intact from metabolized drug, the term "pilocarpine levels" as it appears in this report, strictly speaking, should be interpreted as "total drug". However, according to the data from incubation studies conducted by Makoid (25), the extent of pilocarpine degradation in the ocular tissues of albino rabbits would be minimal within the duration of a typical dosing study (two hours). Specifically, it varied from zero percent in the cornea after one hour of incubation to five percent in the aqueous humor, iris and ciliary body after two hours of incubation. It remains to be seen how well these findings will hold for the pigmented rabbit.

Figure 7. Concentration of pilocarpine in the cornea of albino rabbits (○) and of pigmented rabbits (●) following topical instillation of its solution. For the sake of clarity error bars are omitted. Albino rabbit data are taken from reference 7.

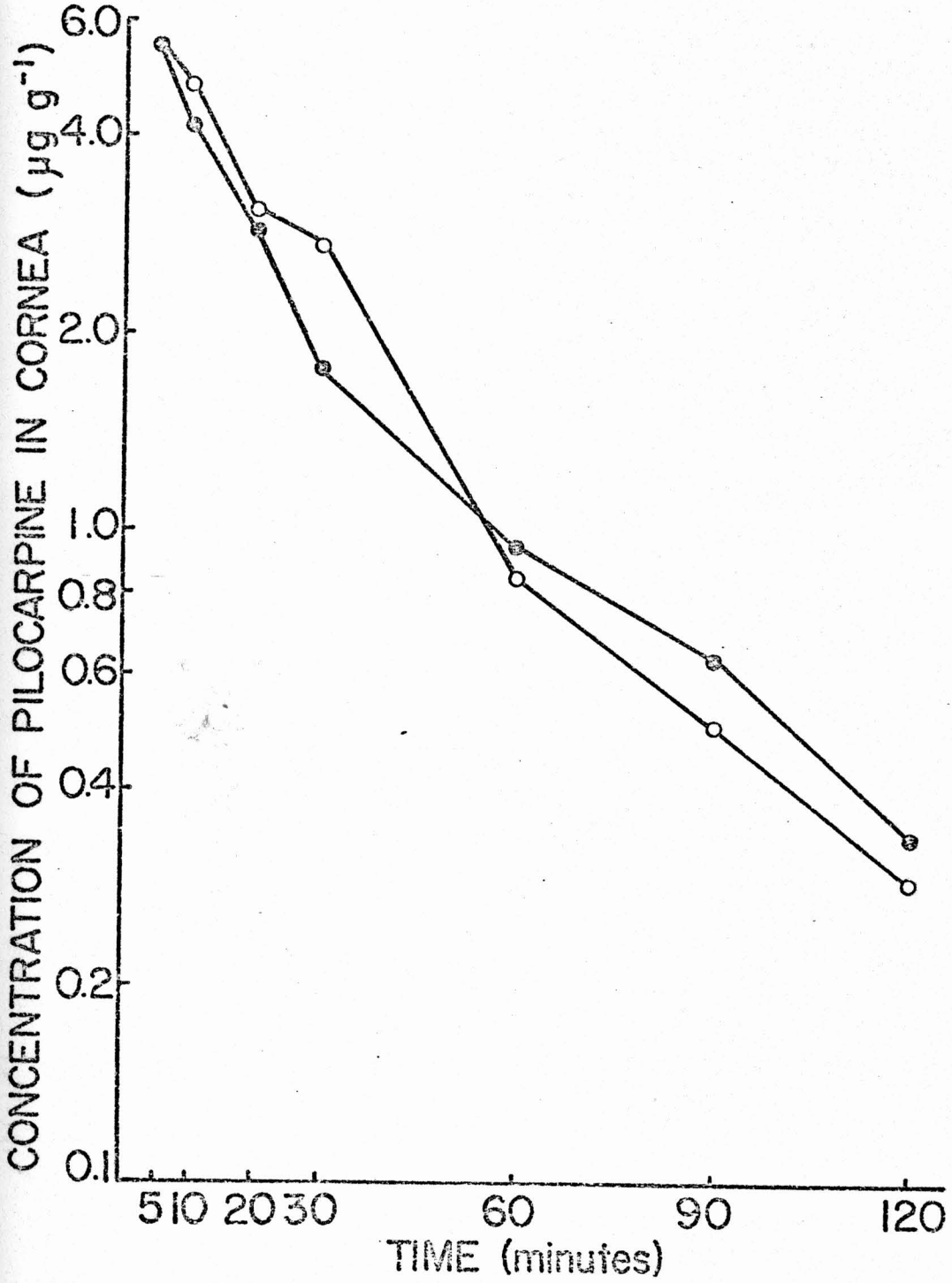
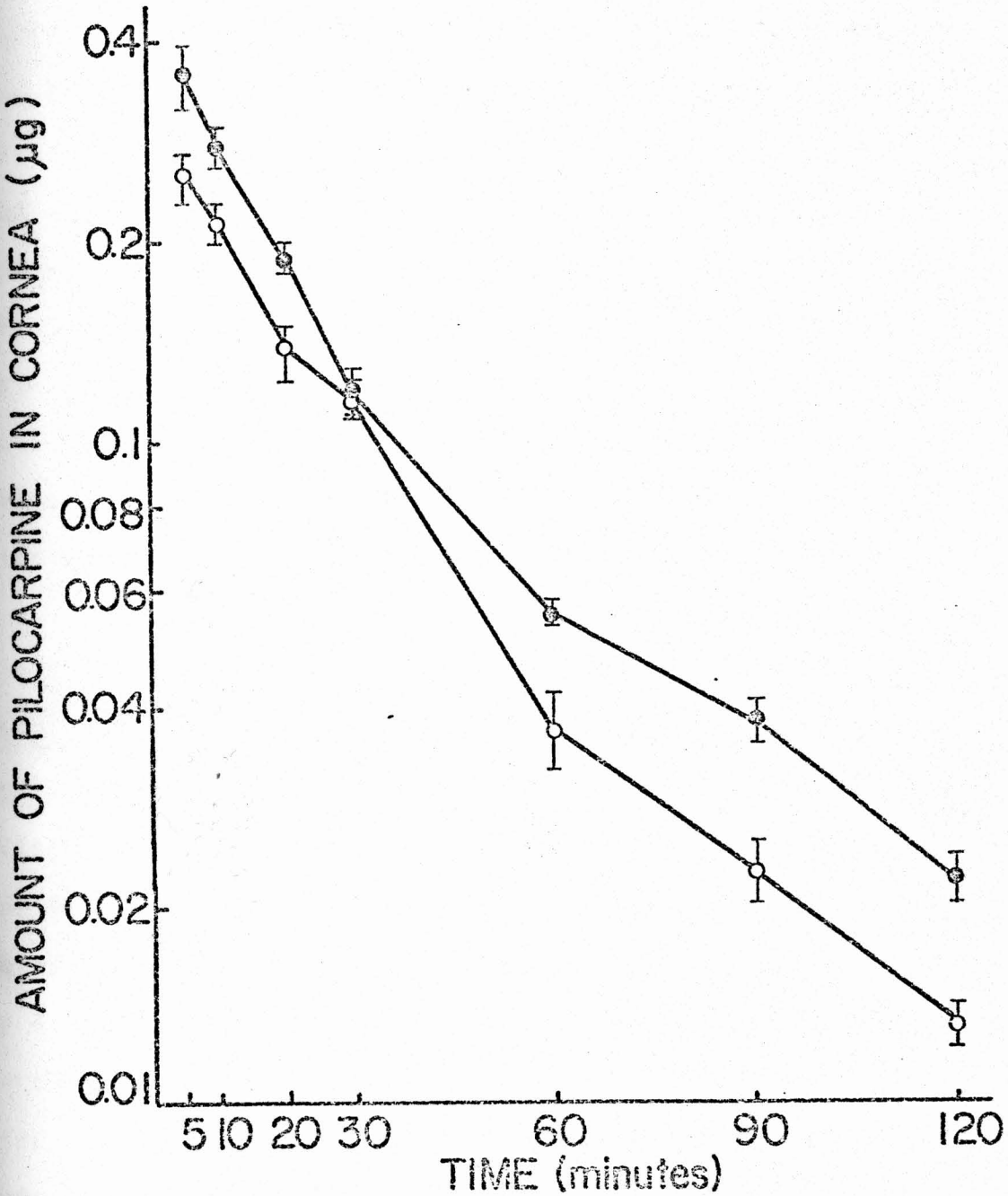

CONCENTRATION OF PILOCARPINE IN CORNEA (μg g⁻¹)
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
5 10 20 30
60
90
120
TIME (minutes)

Figure 8. Amount of pilocarpine in the cornea of albino rabbits (○) and of pigmented rabbits (●) following topical instillation of its solution. Error bars represent standard error of the mean. Albino rabbit data are calculated from reference 7.


AMOUNT OF PILOCARPINE IN CORNEA (µg)
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
5 10 20 30
60
90
120
TIME (minutes)

3. Pilocarpine Levels in the Iris and Ciliary Body as a Function of Time Following Instillation of a 1 x 10^{-2} M Solution. For the reasons indicated in the "Experimental" section, the iris and ciliary body are to be considered as one tissue. The data (Table XIII) indicate that the peak level lies in the neighborhood of 90 minutes (Figure 9). The apparent rate constants of uptake and elimination are not determined for two reasons. First, data beyond 120 minutes are not available. Secondly, the existing data points do not describe a smooth curve. This latter observation may be reflecting the likelihood that disposition of pilocarpine in the iris and ciliary body is not sufficiently similar for them to be treated as one tissue.

4. Influence of Instilled Concentration on Pilocarpine Levels in the Cornea, Aqueous Humor and Iris-Ciliary Body at 20 Minutes Post-Instillation. Table XIV summarizes the pilocarpine levels achieved in the cornea, aqueous humor, and iris-ciliary body at 20 minutes after instilling 25-µl doses of solutions with strengths ranging from 0.5 x 10^{-2} M to 4 x 10^{-2} M. These data are correlated with instilled concentration on a logarithmic basis and the results are displayed in Table XV. On the basis of the coefficient of determination, R^2, the regressions are less than satisfactory, especially so in the case of iris-ciliary body. However, as a first approximation it can be said that

Table XIII. Concentration and Amount of Pilocarpine in the Iris and Ciliary Body Following Instillation of a 1 x 10^{-2} M Solution.

Minutes	Number of Eyes	Microgram(s) per Gram of Iris-Ciliary Body	Microgram[a]
5	5	0.88 (0.05)[b]	0.08 (0.004)
10	10	1.72 (0.09)	0.12 (0.004)
20	11	2.04 (0.14)	0.14 (0.006)
30	8	3.26 (0.46)	0.23 (0.033)
60	9	3.97 (0.25)	0.24 (0.014)
90	4	4.61 (0.41)	0.28 (0.019)
120	6	2.73 (0.15)	0.18 (0.015)

[a]Wet weight of iris and ciliary body was 70.3 $\pm$ 1.6 mg (n = 67).

[b]Numbers in parentheses represent standard error of the mean.

Figure 9. Concentration of pilocarpine in the iris and ciliary body of albino rabbits (○) and of pigmented rabbits (●) following topical instillation of its solution. Error bars represent standard error of the mean. Albino rabbit data are taken from reference 25.

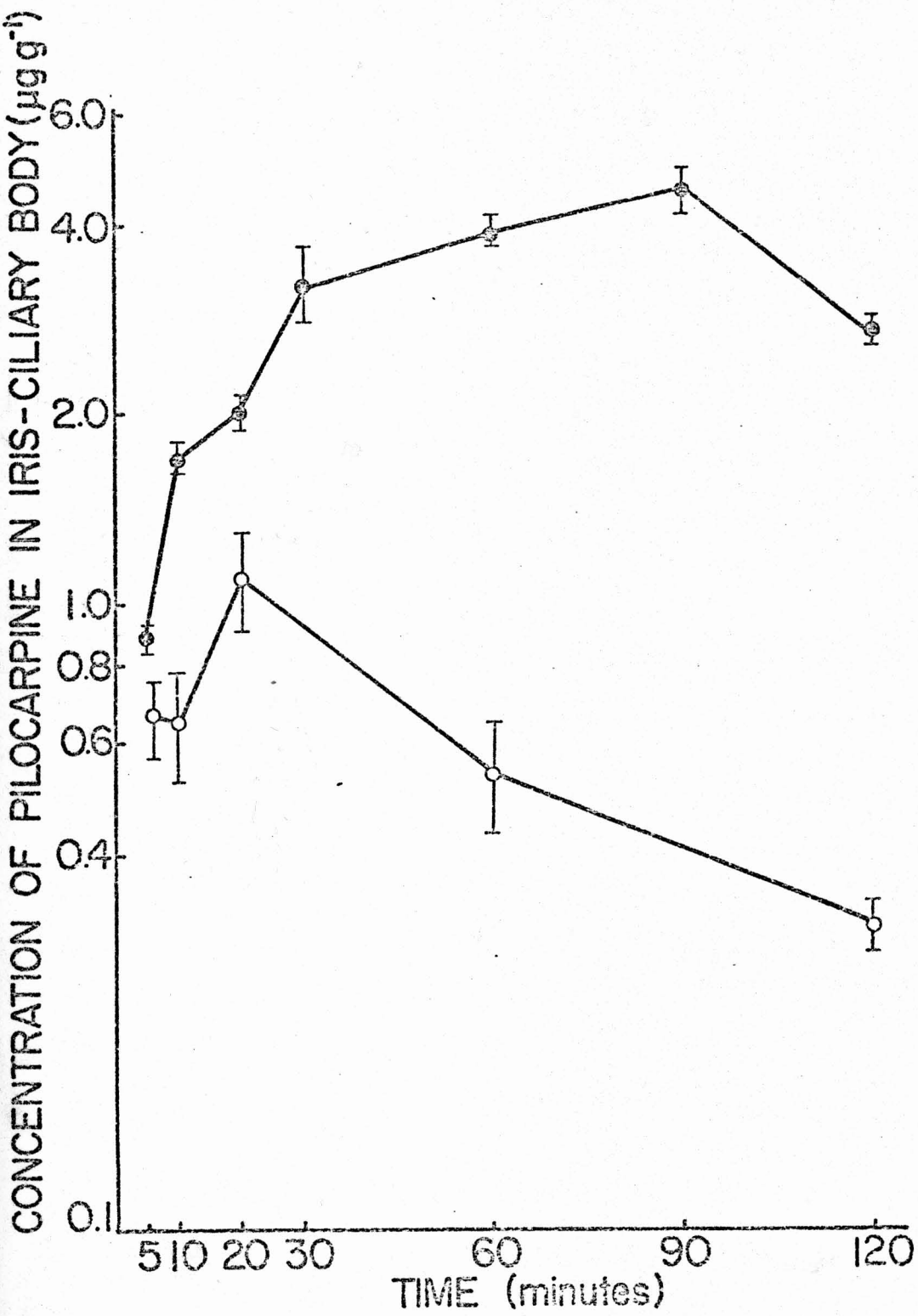

CONCENTRATION OF PILOCARPINE IN IRIS-CILIARY BODY (μg g⁻¹)
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.1
5 10 20 30 60 90 120
TIME (minutes)

Table XIV. Pilocarpine Levels in the Aqueous Humor, Cornea and Iris-Ciliary Body at 20 Minutes After Instillation of 25-µl Doses of Various Concentrations of Pilocarpine.

Tissues	Instilled Concentration ($\times 10^2$ M)			
	0.5	1	2	4
Aqueous Humor ($\mu g\ ml^{-1}$)	0.52 (0.04)[a,b]	0.91 (0.04)	3.02 (0.17)	4.92 (0.21)
Cornea ($\mu g\ g^{-1}$)	2.31 (0.11)	2.84 (0.19)	13.41 (0.44)	21.16 (0.87)
Iris-Ciliary Body ($\mu g\ g^{-1}$)	2.20 (0.08)	2.04 (0.14)	12.78 (0.60)	23.77 (0.70)

[a]Numbers in parentheses represent standard error of the mean.

[b]An average of eight eyes were used for each instilled concentration.

Table XV. Logarithmic Correlation of Ocular Tissue Levels of Pilocarpine at 20 Minutes Post-Dosing With Instilled Concentration.

Tissue	Equation	r	R^2
Aqueous Humor	$\log [AH]^a = (0.97 \pm 0.21)^c \log [S]^b - (3.36 \pm 0.40)$	0.956	0.914
Cornea	$\log [C]^a = (1.18 \pm 0.26) \log [S] - (2.31 \pm 0.48)$	0.956	0.915
Iris-Ciliary Body	$\log [I]^a = (1.30 \pm 0.36) \log [S] - (2.14 \pm 0.67)$	0.932	0.869

[a] [AH] represents concentration of pilocarpine in aqueous humor expressed in molar quantities; [C], cornea; and [I], iris-ciliary body. (A density of 1 g cm^{-3} was assumed for the cornea and iris-ciliary body.)

[b] [S] represents instilled pilocarpine concentration expressed in molar quantities.

[c] The figures within parentheses represent the 95% confidence interval on the regression coefficient.

instilled concentration and ocular tissue levels at 20 minutes are related logarithmically with a proportionality constant of about one. At least for the aqueous humor data, this result is in accord with that obtained in albino rabbits by Chrai and Robinson (29) when they varied the instilled concentration over six orders of magnitude. Such a relationship over a similar range of concentration is not expected to hold in the pigmented rabbit, for saturation of pigment binding sites in the iris-ciliary body is likely to occur at high dose levels causing a disproportionate increase in aqueous humor levels with instilled dose. In other words, the concentrations chosen for this study reside on the linear segment of the dose-response curve.

C. Computer Simulation of Pilocarpine Disposition in Pigmented Rabbits

Obviously, with only these preliminary data available it is necessary to make assumptions beyond those mentioned earlier when applying the model presented for albino rabbits to pigmented rabbits. The similarity in the early portions of the cornea and aqueous humor profiles in these two strains of rabbits (Figures 6-8) indicates a similar mechanism for the movement of pilocarpine from the tear chamber, through the cornea and then into the anterior chamber. The values to be used for the appropriate rate

constants are listed in Table XVI. For comparison purposes, those for the albino rabbits are also included. Here, it has to be stated without elaboration until a later section that the amount of pilocarpine recovered in the dark-eyed rabbit is larger than that recovered in the albino rabbit. Insofar as the precorneal factors are concerned, this result suggests that either the parallel loss factors are diminished in their magnitude or the permeation rate constant (k_p) value is increased, or both. Recall that the early peak time established in the cornea reflects the predominant influence of parallel loss factors (37). This, coupled with the less than dramatic increase in the amount of pilocarpine absorbed, lends credence to the assumption that the parallel loss factors share similar values in both kinds of rabbits. The only change made, then, is an increase in the value of k_p from 0.004 min^{-1} in the albino rabbit to 0.0045 min^{-1} in the dark-eyed rabbit.

The sharp distinction between the iris and ciliary body profiles in the albino and dark-eyed rabbits warrant some changes in the rate constants associated with transfer of drug between the aqueous chamber and the reservoir and with elimination of drug from the reservoir. By trial and error, it was found that the combination of 0.015 min^{-1} for k_r, 0.005 min^{-1} for k_{-r}, and 0.002 min^{-1} for k_{ro} reproduced the aqueous humor and corneal profiles rather well. This is equivalent to saying that relative to the albino rabbit,

Table XVI. Parameter Values for Simulation of Pilocarpine Disposition in the Albino and Dark-Eyed Rabbits.

Parameter	Value	
	Albino Rabbits	Dark-Eyed Rabbits
k_p	0.004 min^{-1}	0.0045 min^{-1}
k_d	0.545 min^{-1}	0.545 min^{-1}
k_n	0.258 min^{-1}	0.258 min^{-1}
Q_T	0.66 µl min^{-1}	0.66 µl min^{-1}
k_a	0.10 min^{-1}	0.08 min^{-1}
k_{ao}	0.018 min^{-1}	0.016 min^{-1}
k_r	0.008 min^{-1}	0.015 min^{-1}
k_{-r}	0.008 min^{-1}	0.005 min^{-1}
k_{ro}	0.06 min^{-1}	0.002 min^{-1}
V_o	7.5 µl	7.5 µl
V_B	6 µl	6 µl
V_C	250 µl	285 µl[a,b]
V_D	325 µl	475 µl[b]

[a]Volume of stroma is assumed to be 64 µl.

[b]V_C and V_D add up to the volume of distribution value of 760 µl reported for dark-eyed rabbits by Conrad and Robinson (32).

pilocarpine is transferred with a larger rate constant from the aqueous chamber into the reservoir, and a smaller rate constant for the reverse process as well as for removal of pilocarpine from the reservoir.

In addition to k_p, k_{-r} and k_{ro}, the volumes of several compartments have their values adjusted. Based on the approximately two-fold increase in weight of the cornea in the dark-eyed rabbit when compared to the albino rabbit, the volume of the stroma is assigned a value of 64 µl, giving a volume of 70 µl for the cornea. The volume of the aqueous chamber is assumed to be constant. However, the volume of the reservoir, V_D, is assigned a value of 475 µl which, when added to the volume of the stroma and of the aqueous chamber, results in a value of 760 µl, the volume of distribution for pilocarpine in dark-eyed rabbits (32).

Figures 10 and 11 respectively compare the observed and predicted profiles in the aqueous humor and in the cornea. As in the case of albino rabbits, the predicted value for the five-minute point in the aqueous humor is lower than the one observed. More importantly, the simulation yields a peak concentration of 0.98 µg ml^{-1} achieved at approximately 22 minutes post-instillation, in contrast with the experimentally determined peak concentration of 1.16 µg ml^{-1} achieved within 30 minutes. This result is rather similar to that obtained from one-compartment pharmacokinetic analysis mentioned previously. Obviously,

Figure 10. Concentration of pilocarpine in aqueous humor of pigmented rabbits following topical instillation of its solution.

Key: ● Experimental data

—— Simulated profile

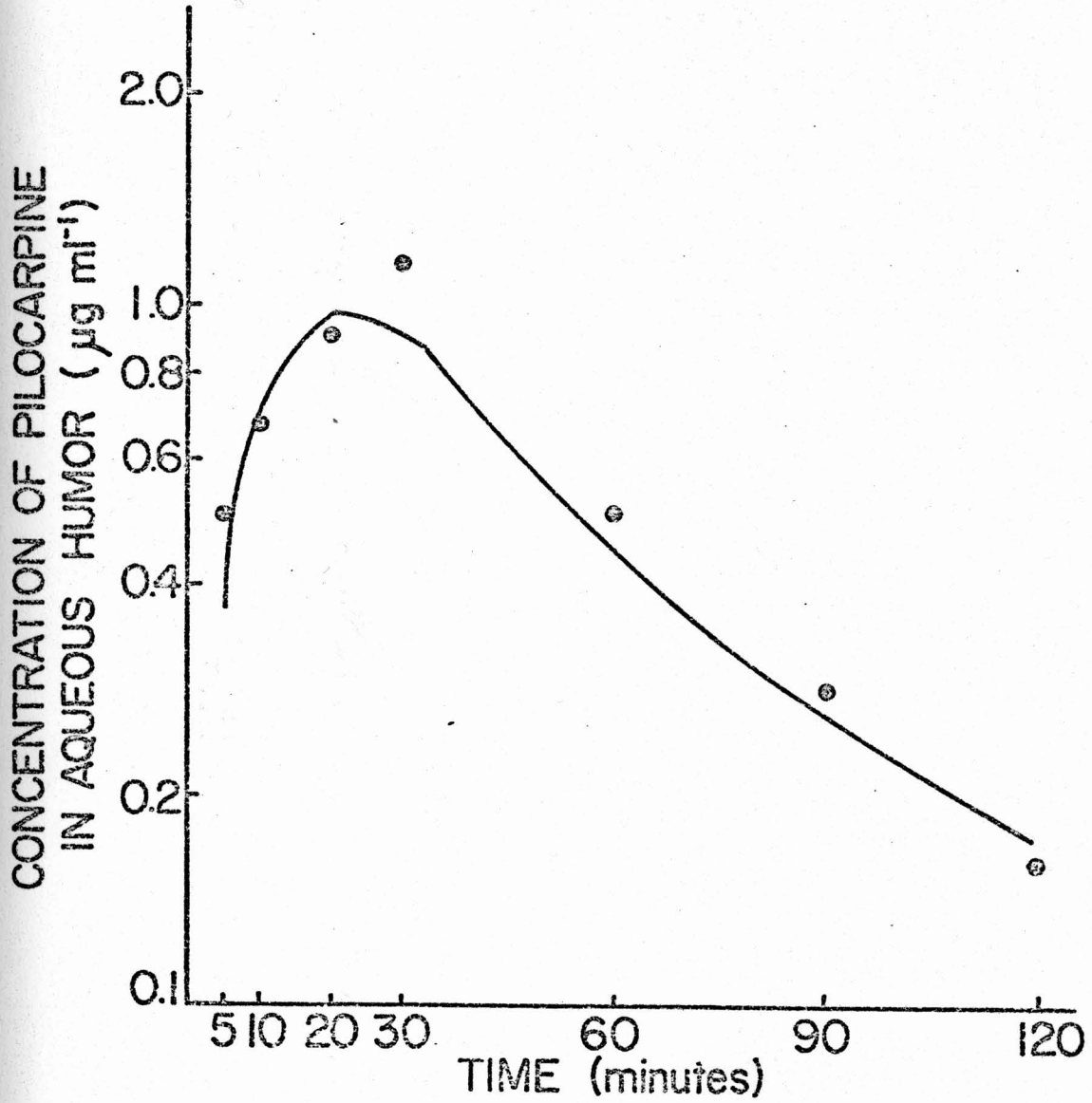
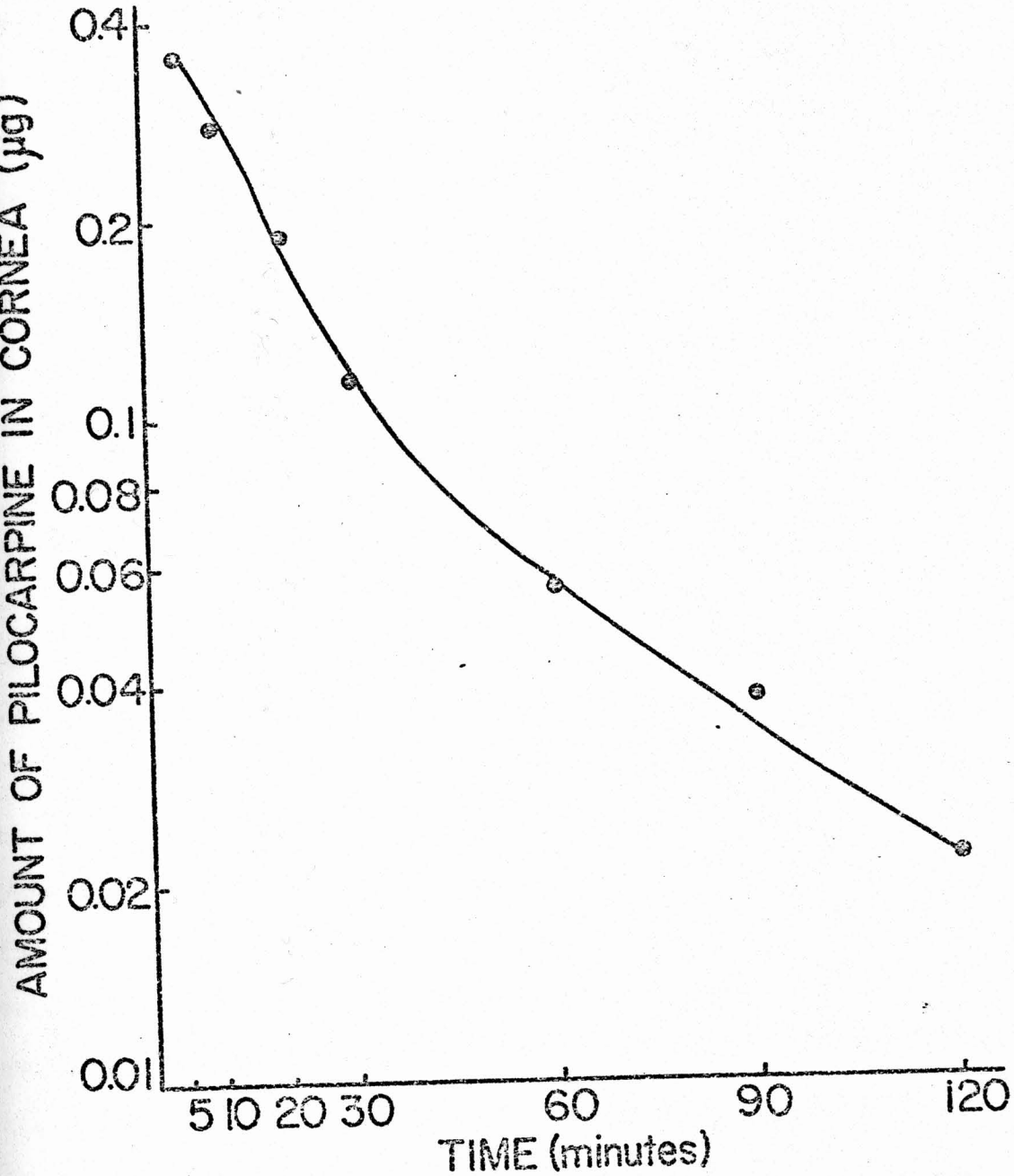

CONCENTRATION OF PILOCARPINE
IN AQUEOUS HUMOR (μg ml⁻¹)
2.0
1.0
0.8
0.6
0.4
0.2
0.1
5 10 20 30
60
90
120
TIME (minutes)

Figure 11. Amount of pilocarpine in cornea of pigmented rabbits following topical instillation of its solution.

Key: ● Experimental data

—— Simulated profile


AMOUNT OF PILOCARPINE IN CORNEA (µg)
0.4
0.2
0.1
0.08
0.06
0.04
0.02
0.01
5 10 20 30
60
90
120
TIME (minutes)

additional data points about 30 minutes are needed to resolve this apparent discrepancy. Furthermore, although the experimental data are not sufficiently precise to permit one to state that at least a biexponential decline in pilocarpine level is present, this is rather distinct in the computer simulated results.

Turning to the cornea profile (Figure 11), it can be seen that the experimental data and simulated results are in remarkably good agreement. This indicates that the "feedback" criterion value of 0.12 is a reasonable one to use. A careful examination of the simulated profile reveals that the 60, 90, and 120 minutes points do not describe a straight line, suggesting a triphasic decline of levels. However, the scatter of experimental data would probably only permit a biphasic decline to be discerned.

A disturbing aspect of this simulation is its prediction of a very much lower level of pilocarpine in the early portion of the iris and ciliary body (the reservoir) profile (Table XVII). This indicates that either: (a) the value assigned to the rate constants (k_r, k_{-r}, and k_{ro}) associated with the movement of pilocarpine into and out of the reservoir is incorrect, or (b) the model is not adequate in its accounting for the distribution of pilocarpine into these tissues. Alternative (a), in turn, implies that either k_r is too small or k_{-r} and k_{ro} are too large. However, increasing the magnitude of k_r will cause a reduction

Table XVII. A Comparison of Predicted and Experimentally Observed Amount of Pilocarpine in the Iris-Ciliary Body.

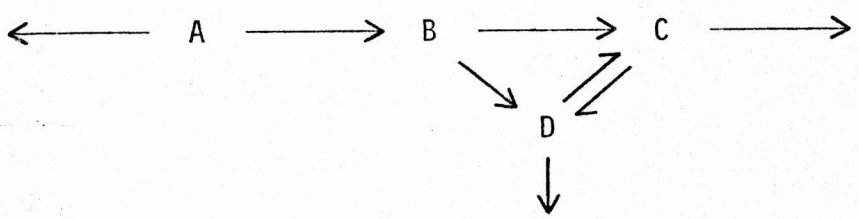
	Microgram of Pilocarpine		
Minutes	Experimental	Predicted	Experimental/Predicted
5	0.08	0.003	26.7
10	0.12	0.02	6.0
20	0.14	0.05	2.8
30	0.23	0.09	2.6
60	0.24	0.15	1.6
90	0.28	0.16	1.8
120	0.18	0.15	1.2

in the aqueous chamber level and at the same time a shortening of its peak time. Decreasing the magnitude of k_{-r} will bring about a similar effect. On the other hand, a further diminishing of the already small value, 0.002 min^{-1}, assigned to k_{ro} will not be sufficient to cause an approximately eighteen-fold increase in the amount of pilocarpine in the reservoir. In short, alternative (a) is not a plausible one insofar as bringing the predicted and experimental values into agreement.

In examining alternative (b), we have to focus on that portion of the model concerned with the transfer of pilocarpine into and out of the reservoir. According to this model, pilocarpine can enter the reservoir only via the aqueous humor. However, an examination of the anatomical arrangement of the cornea and ciliary body indicates that the latter structure is in close association with the corneoscleral junction (38). It is possible that pilocarpine is transferred across this junction into the ciliary body, giving rise to a high initial level there. This proposed route of drug entry into the ciliary body may appear to be farfetched when one considers that the region beyond the corneoscleral junction is well vascularized. One must realize, however, that drug molecules upon successfully penetrating the corneoscleral junction can be carried away by the circulation there only if they manage to cross the capillary endothelium. As this region offers little

resistance to physical diffusion of solutes with molecular weights less than 500 (see a later section), these drug molecules are subjected to competition by these two pathways leading to drug removal from this area. In short, one cannot discount the notion of transfer of drug across the corneoscleral junction into the ciliary body. So, to include this source of drug for the iris-ciliary body in addition to that via the aqueous humor, Scheme III becomes:

Scheme IV



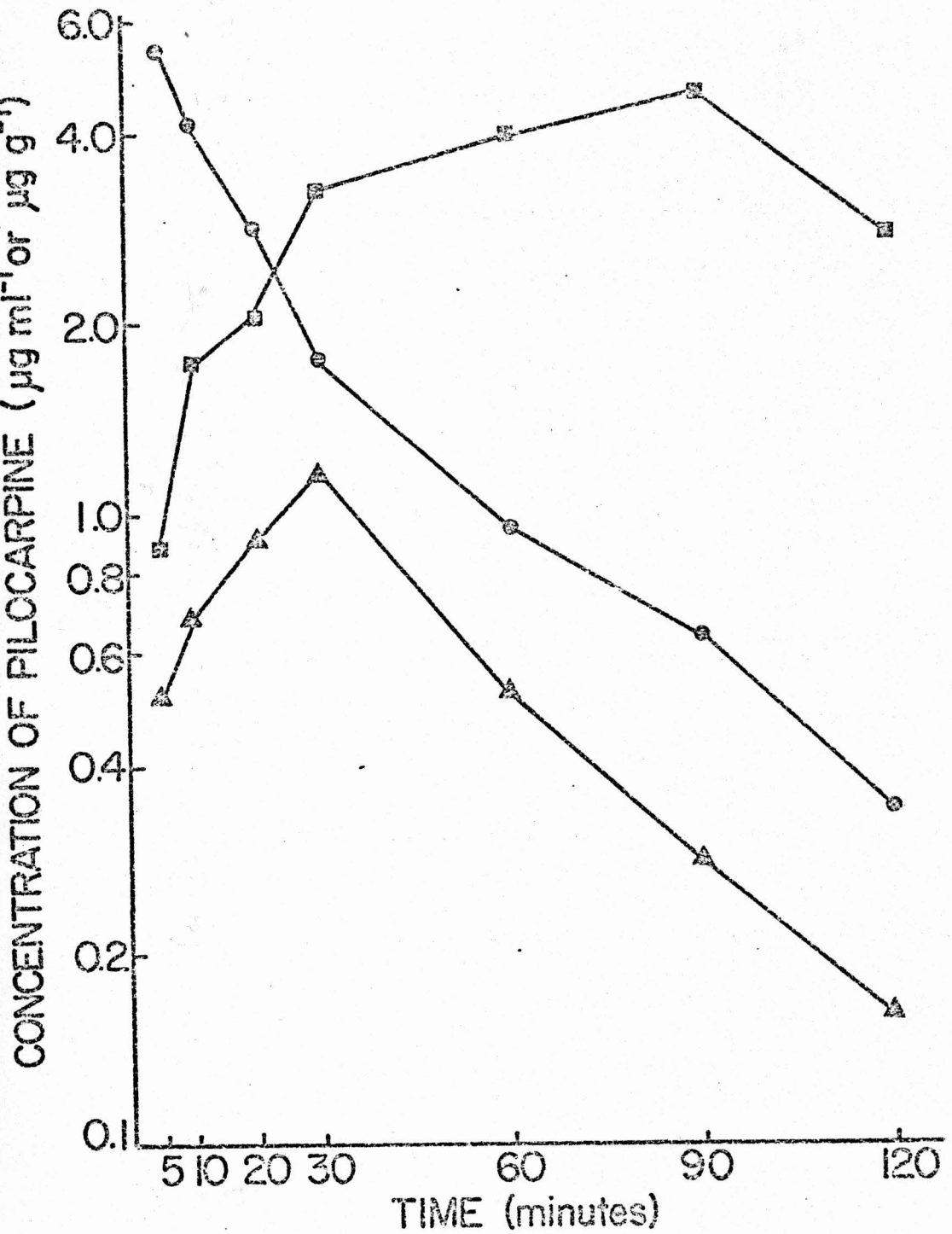
The adoption of Scheme IV would mean some, but not substantial, changes in the initial input values selected for k_p, k_a, k_r, k_{-r}, and k_{ro}. In order to provide additional drug for the ciliary body-iris, the magnitude of k_p must exceed its present value of 0.0045 min^{-1}. The simulated disposition of pilocarpine according to Scheme IV is currently being examined. Naturally, further experimental studies are needed to confirm and then to quantitate this mode of drug transfer to the iris-ciliary body.

IV. DISCUSSION

A. Disposition of Pilocarpine in Pigmented Rabbits

Figure 12 is a composite of concentration-time profiles in the cornea, aqueous humor, and iris-ciliary body. As in the case of albino rabbits, at no times does the aqueous humor drug level exceed that in the cornea. Unlike the albino rabbit, the aqueous humor level is lower than that in the iris-ciliary body. Provided that all of the radioactivity in the iris and ciliary body is due to undegraded pilocarpine, then beyond 25 minutes these tissues achieve the highest level of drug. Even if the radioactivity is due to a mixture of pilocarpine and its metabolite(s), it can still be said that a substantial amount of drug reaches the iris and ciliary body. The important point is that the aqueous chamber is surrounded by regions (cornea and iris-ciliary body) with concentrations higher than itself; yet, no sustaining of its drug level is apparent within the period of this study. This raises some interesting questions regarding the distribution of pilocarpine once it penetrates the corneal epithelium, and particularly the transfer of drug into the iris-ciliary body and its fate in these tissues once it gets there. As a starting point in our discussion of disposition kinetics of pilocarpine in pigmented rabbits, let us focus our attention on the iris and ciliary body.

Figure 12. Concentration of pilocarpine in the cornea (●), aqueous humor (▲), and iris-ciliary body (■) of pigmented rabbits following topical instillation of its solution. It is expressed as μg ml^{-1} for aqueous humor and as μg g^{-1} for cornea and iris-ciliary body.


CONCENTRATION OF PILOCARPINE (μg ml⁻¹ or μg g⁻¹)
6.0
4.0
2.0
1.0
0.8
0.6
0.4
0.2
0.1
5 10 20 30 60 90 120
TIME (minutes)

1. Binding of Pilocarpine to Pigments in the Iris and Ciliary Body. The iris is bathed in aqueous humor and it roots into the ciliary body. The iris and ciliary body are similar in at least two aspects. In the first place, they are porous, thereby allowing free movement of aqueous humor through them (33-35, 39). Secondly, they contain pigments, the amount of which varies from one individual to another and from race to race. Presumably such pigments influence the intensity of pharmacological response to a given drug. The following examples illustrate this point.

In the late 1920's, Chen et al. (10, 11) reported that pseudoephedrine, cocaine and ephedrine were more effective mydriatics in individuals with light irides than in those with dark ones. Subsequent studies with such drugs as neosynephrine hydrochloride and scopolamine hydrobromide produced similar results (14, 40). Harris and Galin (15) monitored the intraocular pressure in patients whose irides possessed varying degrees of pigmentation only to find that relative to patients with blue eyes, the hypotensive effect of pilocarpine was diminished in patients with brown eyes.

The preceding examples should leave little doubts that pigmented and non-pigmented individuals show a different intensity of response to a given drug. However, the mechanism underlying all these observations is not so clear. As a first step in the search for an answer to this problem much attention has been paid to the aspect of

drug-pigment interaction.

Upon converting concentration to amount in Figure 9, it can be seen that a considerable amount of pilocarpine accumulates in the iris-ciliary body. The same phenomenon has been observed in topically applied atropine (17) and epinephrine (41) as well as intravenously administered phenothiazines (42) and chloroquine (43). Further support for the accumulation of drugs in pigmented irides comes from incubation studies involving a variety of drugs including pilocarpine and imipramine (14, 17, 18, 41, 44, 45). For instance, Lyons and Krohn (46) demonstrated that a 48-hour incubation of iris and ciliary body of pigmented rabbits in 0.15 ml of 500 μg ml^{-1} pilocarpine nitrate solution resulted in approximately 10% of the drug being taken up by the tissues, as compared with the 5% observed in albino rabbits. The difference in extent of uptake was attributed to binding to pigments. Note that the concentration used, 500 μg ml^{-1}, is about 100 times higher than the peak concentration achieved in aqueous humor following topical instillation of a 1×10^{-2} M pilocarpine solution. It is not known if this high concentration lies within the region where saturation of binding sites occurs.

Regarding the strength of binding it has been shown qualitatively that far more drug is retained in the pigmented than non-pigmented irides when the tissues are subjected to repeated washing (17, 41, 44). An important

aspect concerning strength of binding is whether the drug that is bound is eventually released into aqueous humor and other ocular tissues at its therapeutic concentration. This, in turn, dictates the duration of pharmacological response and therefore the dosing regimen. The answer to this question probably rests on studies on release kinetics of pilocarpine from the iris and ciliary body predosed with drug.

As far as studies such as the one described in this report are concerned, it is important to recognize that the amount-time profile obtained in the iris and ciliary body entails at least two aspects: uptake of drug by the tissue and its elimination from the tissue. Upon comparing the AUC's from zero to 120 minutes for iris-ciliary body in pigmented and non-pigmented rabbits, it is found that the AUC in pigmented rabbits exceeds that of their counterpart by a factor of approximately ten. However, this parameter provides no information about how accumulation is brought about. Table XVIII compares the amount of pilocarpine in the iris-ciliary body at selected times in the two types of rabbits. On the assumption of little or no degradation of pilocarpine, the larger ratios at 60 and 120 minutes implies that accumulation of pilocarpine occurs as a result of slow elimination. Conversely, the ratio for 10 and 20 minutes implies considerable amount of drug being taken up by the tissues. Although the iris and ciliary

Table XVIII. Ratio of Amount of Pilocarpine in the Iris and Ciliary Body at Selected Times in Albino and Pigmented Rabbits.

Time (Minutes)	Ratio[a,b]
10	5.5
20	3.8
60	13.9
120	18.0

[a]Ratio is defined as ratio of µg pilocarpine in iris-ciliary body of pigmented rabbits to that in non-pigmented rabbits.

[b]Data on albino rabbits are those of Makoid (25).

body weigh more in the pigmented than non-pigmented rabbits (70.3 ± 1.6 mg vs. 33.5 ± 0.8 mg), the increased level of drug at these early time points cannot be accounted for by a weight difference alone. An expanded capacity of the pigmented iris and ciliary body to accomodate as well as to retain pilocarpine is a likely explanation for the high drug level observed in pigmented rabbits. This, in turn, must be related in some fashion to the chemical nature of the pigment.

It is generally believed that the pigment is a melanin, a complex non-homogeneous quinonoid entity (44, 47, 48). Larsson et al. (49), for instance, presented indirect evidence for its presence in the pigmented mice. More recently, Valenzuela et al. (50) detected the presence of phenol oxidase, the enzyme responsible for melanin formation, in the iris and ciliary body in rats. Naturally occurring melanins seldom exist alone; instead, they exist as pigment-protein conjugates (51). Therefore, it would be interesting to see if pilocarpine binds specifically to the pigment or to its protein carrier. Potts (48) reported that the accumulation of phenothiazines by pigmented granules found in the uveal tract was a function of the melanin itself, not of the rest of pigment granule. Moreover, the fact that melanin exists as both reduced and oxidized forms makes one wonder if pilocarpine would show preferential affinity for one form of the pigment to the

other. In short, the molecular mechanism for the binding on melanin has not been conclusively established (49).

To put the preceding discussion into proper perspective it cannot be overemphasized that the mechanism underlying a reduction in miotic response to topically applied pilocarpine as a result of pigmentation in the iris and ciliary body remains to be carefully studied. Current thinking is that pigment binding of drugs is responsible for the differential effects observed, but this is likely to be a simplistic view of an inherently complex picture. To cause miosis pilocarpine must reach its receptors in the iris at the proper concentration. Considering that (a) the iris is a porous tissue (33-35, 39) and therefore very accessible to aqueous humor, hence the pilocarpine contained in it, and (b) the pigment is concentrated in the two layers bordering the posterior surface of the iris (52), a pilocarpine molecule entering the iris from its anterior surface and escaping interaction with the relatively small amount of pigments there (53) must have to traverse some distance into the tissue before it reaches the pigment granules on the posterior surface. On this basis, one may rationalize a reduction in the degree of miosis by invoking the following possibilities.

The first possibility deals with the spatial arrangement of the receptors relative to the pigmented layers. A reduction in miotic response implies that the receptors and

the pigmented layers are in the immediate vicinities of one another. Indeed, the band of smooth muscle fibers comprising the pupillary sphincter, which presumably contains the receptors for cholinergic drugs such as pilocarpine, lies just anterior to the pigmented epithelium (54). Secondly, it is possible that the pigmented layers are more accessible to pilocarpine than are the receptors. The third alternative concerns with a competition phenomenon; either the drug shows a preferential affinity for the pigments or there is a greater abundance of pigment cells than of receptors. The second possibility is intimately related with how the iris is supplied with pilocarpine. It is conceivable that most of the pilocarpine travels with the aqueous humor to the posterior chamber and then enters the iris from its posterior surface. This mechanism implies non-uniform distribution of drug in aqueous humor, which is difficult to visualize. Consideration of the flow dynamics of aqueous humor in the aqueous chamber lends additional support to the rejection of this proposed mechanism.

From the above analysis it is clear that identifying the receptors for pilocarpine including their location in the iris and their properties holds the key to a critical evaluation of the possibilities just mentioned as explanations for the differential response to pilocarpine as a result of pigmentation in the iris and ciliary body.

2. Relationship Between Aqueous Humor and Iris-Ciliary Body Drug Levels. While awaiting the results from such ventures, we may ask ourselves to what extent are aqueous humor drug level data useful in gaining information on the distribution of drug into the iris and ciliary body. To this end we shall examine the aqueous humor and iris-ciliary body data for albino as well as pigmented rabbits, and also the alteration in pilocarpine concentration in these tissues of pigmented rabbits with varying instilled concentration. To simplify our discussion let us assume that iris-ciliary body tissue components other than their pigments do not bind pilocarpine to any appreciable extent. This is a reasonable assumption to make in the light of Makoid's data (25) obtained in albino rabbits. It follows that across the spectrum of pigment-pilocarpine binding the albino rabbit represents one extreme of no binding occurring. The data in the present study indicate that with the exception of the 30 -minute point pilocarpine concentrations in the aqueous humor of dark-eyed rabbits are not statistically different from those in the albinos, despite a substantial difference in the amount of drug taken up by the iris-ciliary body of pigmented rabbits. Therefore, on the basis of aqueous humor level alone one would be inclined to predict that the iris-ciliary body pilocarpine levels should be similar in both rabbits. This is an example of the danger inherent in the usual assumption

made to the effect that aqueous humor (or in general terms, sampling tissue) levels reflect those in other ocular tissues including iris-ciliary body. Again, we cannot overemphasize that we are comparing albino rabbits which possess no pigments in their iris-ciliary body with pigmented rabbits which do possess varying amounts of pigments.

Although miotic response was not monitored in this study, numerous reports (9-19) indicate that for a given dose, the intensity of miosis is reduced in patients with heavily pigmented irides. This observation, coupled with our preliminary data on iris-ciliary body concentration, suggests that the high levels observed are mostly due to bound drug. At this point it is appropriate to employ the data obtained in the dose-tissue level portion of the study to see how aqueous humor level, [AH], is related to the concentration of free drug, $[I_f]$, in the iris and ciliary body of pigmented rabbits.

If [I] represents the total concentration of drug in these tissues and assuming that rapid equilibrium exists between bound and unbound drug, then:

$$[I] = [I_f]\left(1 + \frac{[T]K_A}{1 + K_A[I_f]}\right) \qquad \text{Eq. 10}$$

Here [T] is the total concentration of pigment and

receptors, and K_A is the apparent equilibrium constant associated with the process:

$$\text{free drug} + \text{pigment and receptor} \underset{}{\overset{K_A}{\rightleftharpoons}} \text{bound drug}$$

From regression results of the dose-tissue level study shown in Table XV, we obtain the approximate relationship:

$$[I] = 221[AH]^{1.3} \qquad \text{Eq. 11}$$

Substituting this result into Equation 10 and solving the resulting expression in $[I_f]$ will lead to an unwieldy expression. A simplification can be made if $K_A[I_f] << 1$ so that Equation 10 becomes:

$$[I] \simeq [I_f](1 + [T]K_A)$$

This condition probably holds for the linear segment of the dose-response curve, which is the region that applies in this experiment. Therefore, we can apply the relationship given by Equation 11 and obtain an expression relating iris-ciliary body free drug concentration and aqueous humor drug concentration (Equation 12).

$$[I_f] = \frac{221[AH]^{1.3}}{1 + [T]K_A} \qquad \text{Eq. 12}$$

Provided that the numerical value of $(1 + [T]K_A)$ is much greater than 221, the free pilocarpine concentration in the iris-ciliary body is less than that in the aqueous humor, in contrast to the similarity in levels in these same tissues of albino rabbits (25). It is also important to note in Equation 12 that like the total drug concentration in the iris-ciliary body, the free drug concentration in these tissues parallels that in aqueous humor.

Proceeding to the other extreme of the spectrum where saturation of binding sites in the pigment is achieved, it can easily be seen that the concentrations of both bound and unbound drug, hence total drug concentration, are a constant. Under this condition aqueous humor drug concentration increases without a corresponding increase in iris-ciliary body levels; in other words, aqueous humor drug levels, once again, do not reflect those in the iris-ciliary body.

Suppose we are in the region where the binding sites in the pigment of iris-ciliary body are far from being saturated. Relative to the albino rabbit and for a given intensity of miotic response, how should instilled drug concentration be adjusted to account for this phenomenon? As before, suppose that equilibrium conditions are attained

so that competition between receptors and pigments for drug can be described by:

$$D_f + R_f \underset{}{\overset{K_R}{\rightleftharpoons}} DR \qquad \text{for drug-receptor interaction, and}$$

$$D_f + P_f \overset{K_P}{\rightleftharpoons} DP \qquad \text{for drug-pigment interaction,}$$

where $[D_f]$ denotes free drug; $[R_f]$, free receptor concentration; and $[P_f]$, free pigment concentration. The corresponding equilibrium expressions are:

$$K_R = \frac{[DR]}{[D_f][R_f]} \qquad \text{and}$$

$$K_P = \frac{[DP]}{[D_f][P_f]}$$

Let y be the proportion of receptors occupied by the drug for a given response, i.e., $y = [DR]/[R_T]$, $[R_T]$ being the total receptor concentration given by:

$$[R_T] = (1 + K_R[D_f])[R_f]$$

An equivalent expression for y in terms of $[D_f]$ and K_R is:

$$y = \frac{K_R[D_f]}{1 + K_R[D_f]}$$

whence $\frac{y}{1 - y} = K_R[D_f]$ Eq. 13

Note that Equation 13 applies regardless of drug-pigment binding. Assuming that the intensity of miosis is directly proportional to the fraction of receptors occupied, Equation 13 indicates that the free drug concentration in the iris-ciliary body must be identical for a given miotic response in both pigmented and non-pigmented rabbits, which is an expected result. Now let z be the fraction of pigment bound. Then the total drug concentration $[D_T]$ can be expressed as:

$$[D_T] = [D_f] + z[P_T] + y[R_T]$$

whence $[D_f] = [D_T] - y[R_T] - z[P_T]$

If $[D_f]'$ is the free drug concentration in the absence of pigment, then:

$$[D_f]' = [D_T]' - y[R_T]$$

As shown earlier $[D_f]$ and $[D_f]'$ must be equal for a given

miotic response, hence:

$$[D_T] = [D_T]' + z[P_T] \qquad \text{Eq. 14}$$

Therefore, according to Equation 14, the total drug concentration in the iris and ciliary body must be increased by an amount equal to the concentration of bound drug, which is another expected result. In the same equation $[D_T]'$ can be estimated from aqueous humor data of albino rabbits. Unfortunately z is a function of $[D_T]$ making it difficult to determine a priori what $[D_T]$ should be. In the limit of saturation of binding sites z assumes a value of one so that:

$$[D_T] = [D_T]' + [P_T]$$

Once $[D_T]$ is known the instilled concentration required to elicit a given response can be calculated from the relationship given in Table XV. In fact, analysis of miosis-time data led Yoshida and Mishima (16) to predict an approximately three-fold increase in the least effective instilled concentration for individuals with dark irides over those with light ones. Likewise, Harris and Galin (15) noted that an approximately eight-fold increase in instilled concentration was necessary to obtain the same

degree of intraocular pressure lowering in patients with brown irides relative to those with blue ones.

3. Degradation of Pilocarpine in the Iris-Ciliary Body. Up to this point, our discussion on the role played by the pigment in the miotic response to pilocarpine has centered on the binding aspects without making reference to the possibility of enzymatic degradation of the drug in the iris and ciliary body. In the present study, only total radioactivity is measured so that if pilocarpine degradation does occur in these tissues, the intact drug concentration can be overestimated. Several years ago Ellis et al. (55, 56) reported some evidence for the presence of a pilocarpine hydrolyzing enzyme in the uveal tissues including iris, but its pharmacological significance is unknown. More recently, Sendelbeck et al. (57) monitored pilocarpine levels in the cornea and aqueous humor following topical multiple dosing as well as insertion of a membrane-controlled delivery system only to find that at the end of four days, only 40% of the radioactivity in the aqueous humor and 50% of the radioactivity in the cornea were due to intact drug. The source of the responsible enzymes is unknown. In the iris and the ciliary body they may be contained in the granules housing melanin, which are known to contain a variety of enzymes (51). If the latter conjecture is

proven to be correct, then binding of pilocarpine to the pigment constitutes an irreversible route of drug loss since less intact drug will now be available for release into the surrounding tissues. By inference, the extent of drug metabolism is expected to be greater in the iris and ciliary body of dark-eyed rabbits than in those of the albinos. Naturally, in the absence of data on the extent and rate of metabolism of pilocarpine in the iris and ciliary body, one must be cautious in interpreting the profile displayed in Figure 9. Thus, one cannot attribute the prolonged peak time observed either to a slow absorption step or to a slow elimination step alone. This is because if metabolism of pilocarpine occurs the peak time receives additional contributions from the formation of the metabolite(s), as well as from its (their) elimination. As mentioned earlier, there is greater scatter of data in iris-ciliary body than in aqueous humor or cornea. It is not clear if this suggests that the iris and ciliary body are sufficiently different to justify their being considered as a single tissue, or if this suggests that the profile is a composite of intact drug and its metabolite(s), or if it reflects variation in the degree of pigmentation from one rabbit to another.

4. Some Possible Factors Responsible for Accumulation of Pilocarpine in Iris and Ciliary Body. To reiterate, we

do not know the relative contribution of uptake of drug and its degradation products, if any, to the ascending branch of the concentration-time profile. The data for the early time points (5, 10 and 20 minutes) suggest that the amount of drug taken up by the pigmented irides is increased. Moreover, the long peak time seems to speak against a rapid rate of degradation for pilocarpine. Undoubtedly, resolving this issue is central to understanding the aqueous humor concentration-time profile, especially when one considers the observation that there is no statistical difference in aqueous humor pilocarpine levels between pigmented and non-pigmented rabbits. This leads to the important question of how accumulation of drug in the iris and ciliary body is brought about. We can explore two possibilities: one deals with the uptake aspect and the other the elimination aspect of this accumulation process.

Without making reference to the corneal drug level for the moment, let us assume that the same fraction of drug entering the aqueous humor is taken up by the iris and ciliary body in both types of rabbits (Mechanism A). The lesser amount of drug detected in the iris-ciliary body of albino rabbits implies that the drug is efficiently removed, perhaps by the circulation supplying these tissues. Conversely, some mechanism exists in the pigmented rabbit resisting its removal, perhaps by binding to pigments. Moreover, as pilocarpine at therapeutic

concentration causes increased blood-aqueous barrier permeability (58), its binding to pigment means that less free drug will now be available for mediating the permeability effect, thereby intensifying resistance to its removal. Quantitatively, the approximately ten-fold difference in AUC from zero to 120 minutes observed in pigmented and non-pigmented rabbits suggests that the rate constant of elimination differs by the same order of magnitude, provided that one-compartment analysis applies. The net result is that the time at which peak iris and ciliary body levels are reached is prolonged. At the same time, the amount of drug retained by these tissues is increased.

Ignoring the effect of pilocarpine on blood-aqueous barrier permeability, the retention of pilocarpine by pigments in the iris and ciliary body can be treated mathematically as follows. We shall make three assumptions. The first is that the rate of decline in total drug concentration $[D_T]$ at any given moment is proportional to the concentration of free drug $[D_f]$ in these tissues at that moment, i.e.,

$$\frac{d[D_T]}{dt} = -k[D_f] \qquad \text{Eq. 15}$$

where k is a constant. The second assumption is that equilibrium between free pigment P_f, free drug D_f, and

drug-pigment complex DP is sufficiently rapid so that the law of mass action applies:

$$\frac{[DP]}{[D_f][P_f]} = \frac{1}{K_D} \qquad \text{Eq. 16}$$

where K_D is the dissociation constant governing the process:

$$DP \underset{\longleftarrow}{\xrightarrow{K_D}} D_f + P_f$$

The third assumption is that the contribution of drug-receptor complex to total drug concentration is negligible. Equation 16 can then be written as:

$$\frac{[D_T] - [D_f]}{[D_f]([P_T] - [D_T] + [D_f])} = \frac{1}{K_D}$$

Solving this equation in $[D_f]$ and substituting the corresponding value in Equation 15 would yield a differential equation of little practical use. Provided that there is a large excess of pigment relative to drug ($[P_T] - [D_T] + [D_f]$) can be approximated by $[P_T]$. The above equation then simplifies to:

$$\frac{[D_T] - [D_f]}{[D_f][P_T]} \simeq \frac{1}{K_D}$$

whence $$[D_f] = \frac{[D_T]}{1 + \frac{[P_T]}{K_D}}$$ Eq. 17

Combining Equations 15 and 17 yields:

$$\frac{d[D_T]}{dt} = -k'[D_T]$$ Eq. 18

where $k' = k/(1 + \frac{[P_T]}{K_D})$

Note that k is also the rate constant governing the decline of iris-ciliary body drug concentration in the absence of pigment. It therefore becomes smaller when pigment is present, and the reduction in its magnitude can be substantial when $[P_T]/K_D$ is large. Also note that a small k' can result even when dissociation of the drug-pigment complex is favorable (i.e., large K_D) so long as there is a high concentration of pigment $[P_T]$. The relationship depicted by Equation 18 has been verified by Silhavy et al. (59) in studies on dialysis of maltose in the presence of varying concentrations of periplasmic maltose-binding protein of Escherichia coli.

Turning to the other side of the argument, let us assume that it is a difference in uptake of drug rather than that of its elimination which is responsible for its accumulation in the iris and ciliary body (Mechanism B). Under this condition, aqueous humor pilocarpine level in the pigmented rabbit will fall below that of the albino rabbit unless its removal via aqueous humor turnover is reduced or perhaps even eliminated. Elimination of aqueous humor turnover would involve a shutdown in aqueous humor production as well as its outflow, but this is unlikely to occur in the presence of pilocarpine (60). Hence we can assume that there is a source of drug supplying the aqueous humor at a rate similar to that governing its uptake by iris and ciliary body. A logical choice of this source is the cornea, but this requires an increased amount of drug being taken up by the cornea of pigmented rabbits. Table XIX illustrates this point.

If we were to assume that pigmented rabbits absorb the same amount of pilocarpine as albino rabbits and that the elimination process in the cornea is the same in both rabbits, then by approximately 30 minutes the cornea will be exhausted of drug (Table XIX). On the contrary, this study shows that with the exception of the 30-minute point, the amount of pilocarpine in the corneas of pigmented rabbits exceeds that of non-pigmented rabbits by a factor of approximately 1.5. In addition, the slopes associated with

Table XIX. Hypothetical and Actual Amount of Pilocarpine in Cornea and Aqueous Humor of Pigmented Rabbits Following Instillation of a 1×10^{-2} M Solution.

Time (Minutes)	Actual Cornea Level (µg)	Hypothetical Cornea Level (µg)[a,b]	Hypothetical Aqueous Humor Level (µg/ml)[a,c]	Actual Aqueous Level (µg/ml)
5	0.35	---[d]	0.84	0.13
10	0.28	0.16	1.16	0.17
20	0.19	0.08	1.48	0.23
30	0.12	0	2.08	0.29
60	0.056	0	1.48	0.13
90	0.038	0	1.40	0.07
120	0.022	0	0.88	0.04

[a]Assume volume of anterior chamber to be 250 µl.

[b]Assume that pigmented rabbits absorb the same amount of drug as albino rabbits.

[c]Assume that drug is not taken up by iris and ciliary body.

[d]Insufficient information.

its decline in the cornea are similar in both types of rabbits. On the one hand this observation reinforces the previous assumption that elimination of pilocarpine from the cornea is unchanged; more importantly, it indicates that the level of pilocarpine in the cornea increases as a result of increased uptake rather than reduced elimination. If the only route of loss of drug from the cornea is entry into the aqueous humor, then one should expect an increased aqueous humor level of drug due to increased uptake of drug by the cornea (Table XIX). To achieve aqueous humor drug levels comparable to those in albino rabbits, there must be an accelerated loss of drug from this fluid, suggesting a larger rate constant governing this process. Comparison of the terminal slopes of aqueous humor concentration-time profiles fails to reveal statistical differences in this regard between the two types of rabbits and hence appears to contradict what one may expect. The answer to this issue is contained within the 30-minute point, which is statistically higher in concentration in the pigmented than non-pigmented rabbits ($p < 0.05$ by the F-test). Together with the 60-minute point, it implies biphasic elimination from the aqueous humor. In other words, during this period distribution into the iris and ciliary body is complete. Obviously, additional data points in the time interval between 20 and 60 minutes are needed to better define the behavior of this portion of the profile not only in the

aqueous humor but also in the cornea. To summarize, according to this picture, no drastic reductions are apparent in aqueous humor levels despite an increased uptake of drug by the iris and ciliary body because increased uptake of drug into the cornea occurs.

Note that the picture just presented is not the only explanation for the similarity in aqueous humor profiles in pigmented and non-pigmented rabbits. One may argue that the major route of drug transfer to iris-ciliary body is via direct movement of drug across the cornea-ciliary body junction, thereby bypassing the aqueous humor as a vehicle of delivering drug to these tissues. Although this route is implied by computer simulations, it is unlikely to be the major one since the iris and ciliary body are porous and are therefore readily accessible to aqueous humor. Only if binding to protein in the aqueous humor is strong and/or extensive would this route serve as a major source of pilocarpine for the iris and ciliary body. Besides, this picture requires the presence of a mechanism to limit the amount of drug that can enter the anterior chamber in spite of a large amount of drug in the cornea, for example, a saturable carrier-mediated transfer process.

Thus, the cornea can exercise an important influence on the uptake of pilocarpine by the iris and ciliary body. To investigate this alleged role of the cornea, one can study the disposition profile of pilocarpine in the aqueous

humor and iris-ciliary body as a result of direct injection of the drug into the anterior chamber. In the absence of a source of drug in the cornea, one would anticipate the pigmented rabbits to give lower aqueous humor drug levels than albino rabbits. Indeed, Conrad and Robinson (32) in their volume of distribution study obtained data which indicated that the level of pilocarpine in aqueous humor was significantly less in the pigmented than non-pigmented rabbits for the five- and ten-minute points, the time period deemed crucial to the distribution of pilocarpine into other tissues including iris and ciliary body. Therefore, this portion of the profile appears to support Mechanism B. On the other hand, the similarity in levels for the remaining sampling times appears to support Mechanism A; that is, the same fraction of drug in the aqueous humor is carried to the iris-ciliary body in the two types of rabbits. However, this is insufficient evidence for a justifiable conclusion to be made regarding the role of cornea in the accumulation process in the iris and ciliary body for two reasons. First, the study by Conrad and Robinson (32) was carried out only to 60 minutes. Secondly, in the same study the level of pilocarpine in the iris, ciliary body as well as the cornea was not determined.

5. Uptake of Pilocarpine into the Cornea. In reviewing the literature it becomes apparent that almost

all of the reported work on the response of pigmented individuals or rabbits to topically applied autonomic drugs examined just the single aspect of binding to pigments in the iris and ciliary body. Little attention has been paid to the events taking place in the precorneal area which are of utmost importance in determining the amount of drug ultimately absorbed and therefore the magnitude of pharmacological response. Even though the extent and rate of metabolism that may occur in the iris and ciliary body are not determined in this study, a comparison with data on albino rabbits clearly indicates an increase in bioavailability of a topically applied dose of pilocarpine. Among the factors that may contribute to this result include a diminished influence of any one, or combination, of the parallel loss factors, and an increased permeation rate constant across the epithelium. The fact that the peak in corneal levels is reached within five minutes post-instillation favors the notion of an increased permeation rate constant. Unfortunately, the data do not allow judgement to be made on whether the observed increase in uptake is due to increased surface area for absorption, increased diffusion coefficient, reduced diffusional pathlength, or a combination of such factors. As noted in the computer simulation portion of the study, a modest increase in permeability rate constant from 0.004 min^{-1} to 0.0045 min^{-1} with the parallel loss rate constants unchanged reproduced

the cornea data well.

At this point we will digress from our discussion on the events taking place in the precorneal area as well as intraocular tissues of pigmented rabbits and turn our attention to the concept of amount versus concentration. In speaking of levels of pilocarpine achieved in the cornea or any other tissue, one has the liberty of expressing it in terms of concentration or amount. Since passive transport of drugs is governed by concentration but not amount, the concentration designation is more meaningful from the standpoint of understanding the rate at which processes occur; hence it is more often employed. However, it is important to recognize that for tissues such as cornea and iris-ciliary body concentration is a derived quantity; it is obtained from two measurable quantities, namely, amount of drug and amount of tissue. Two assumptions are implicit in this procedure: (a) the drug is uniformly distributed in the tissue and (b) the tissue is homogeneous. Moreover, the concentration thus obtained refers to, at best, an average concentration of drug. It does not distinguish between bound and unbound drug, but it is the unbound drug that is able to move through tissues. Closely associated with this phenomenon is the extent of hydration of tissues which affects its relative distribution between the proteins making up the tissue and the fluid accompanying it. In short, "concentration" plays the role of a weighted

amount. These points must be kept in mind in expressing or interpreting drug levels in tissues.

The subtle differences between these two designations become transparent while comparing the levels of pilocarpine achieved in the cornea of pigmented and of non-pigmented rabbits. Except for the 30-minute point, the concentration in terms of microgram of pilocarpine per gram of cornea are statistically indistinguishable; however, the microgram of drug in the cornea of pigmented rabbits is greater than that in non-pigmented rabbits by a factor of 1.5. These observations take us back to our original discussion on corneal level of pilocarpine achieved in albino and pigmented rabbits. Since the cornea is composed of two major tissues, epithelium and stroma, the corneal data alone provide no information on whether the increase is evenly distributed between epithelium and stroma. Concentration, being a weighted quantity, implies that this is the case. This study observes an increase in weight of the cornea by a factor of approximately 1.5, which is of the same magnitude as that observed in the increase in amount of pilocarpine in the cornea. As the weight of epithelium was not determined, it is not known if there is a proportional increase in weight of the epithelium and stroma. For the moment, let us examine what the drug levels in the epithelium and stroma will be, relative to albino rabbits, under different conditions of weight increase in these

corneal components for a given weight increase of the cornea. In particular, let us identify the following three cases:

(a) Case 1: the weight increase in cornea in the pigmented rabbit is due to an increase in weight of stroma alone,

(b) Case 2: the stroma and the epithelium undergo an increase in weight in direct proportion to that of the cornea, and

(c) Case 3: an increase in weight of epithelium is solely responsible for the increase in weight of cornea.

Table XX displays the theoretical ratios of amount of drug in (a) the epithelium (E_P/E_A) of and (b) the stroma (S_P/S_A) of pigmented to albino rabbits. Here, subscript P refers to pigmented rabbits while subscript A refers to albino rabbits. The meaning of the remaining symbols in Table XX is as follows:

E = amount of drug in the epithelium,

S = amount of drug in the stroma,

[E] = concentration of drug in the epithelium,

[S] = concentration of drug in the stroma,

W_E = weight of epithelium in the albino rabbit, and

W_S = weight of stroma in the albino rabbit.

These expressions are derived on the assumption that as in albino rabbits, the stroma and aqueous humor of pigmented

Table XX. Theoretical Ratios of Amount of Drug in (a) the Epithelium (E_P/E_A) of and (b) the Stroma (S_P/S_A) of Pigmented to Albino Rabbits.

Case	E_P/E_A	S_P/S_A
1	$1.5 - 0.5(\frac{[S]_A}{[E]_A})$	$1.5 + 0.5(\frac{W_E}{W_S})$
2	1.5	1.5
3	$1.5 + 0.5(\frac{S_A}{E_A})$	1.0

rabbits are kinetically indistinguishable insofar as pilocarpine is concerned. In addition, they acknowledge the observation that with the exception of the 30-minute point, there are no statistical differences in the aqueous humor concentration-time profiles between pigmented and non-pigmented rabbits.

From the data of Sieg and Robinson (7), the numerical value of E_P/E_A can be calculated to be approximately 1.5 for Case 1 and 1.7 for Case 3. The significance of these values is that an increased uptake of drug by the epithelium gives rise to an enhanced reservoir effect. Prince (61) reported the presence of pigment granules in the basal cells of epithelium of pigmented rabbits. It is conceivable that these granules bind pilocarpine just as their counterparts in the iris-ciliary body do, thereby adding to the already present reservoir effect. Also note that in Case 3, whereas the ratio of amount of drug in the epithelium is increased, the ratio of concentration is reduced, and its value is now 0.5.

It is clear from the above deliberations that the ratio of amount of drug in the epithelium of pigmented and non-pigmented rabbits is rather insensitive to the manner in which the increase in weight of cornea is partitioned between stroma and epithelium. This insensitivity may be a consequence of the mechanism proposed for the intraocular disposition of pilocarpine, which requires that stroma and

aqueous humor be kinetically indistinguishable. Computer simulation results appear to support Case 1, however. This ambiguity can only be removed by additional studies involved with careful, direct determination of drug levels in the epithelium as well as in the stroma. Only then will more insight be gained on the mechanism of intraocular disposition of pilocarpine in the pigmented rabbit.

6. Tritium Exchange. Note that the above discussions tacitly assumed no or negligible exchange of the tritium label on a pilocarpine molecule with labile hydrogens of the yet to be identified carrier protein for the pigments in the iris and ciliary body. Inevitably drug levels in these tissues will be overestimated if tritium exchange occurs without the pilocarpine being bound. On the other hand, tritium exchange will be hindered if the labile hydrogens are remote from the region on the pigment in which a pilocarpine molecule undergoes binding. Sieg (22) demonstrated that the tritium label remained as a stable entity during passage through the cornea and existed as such in the aqueous humor. A similar attempt was not made in the present study insofar as monitoring the fate of the tritium label in the iris and ciliary body. Consequently due caution must be exercised in interpreting these data. However, according to Hvidt and Nielsen (62) tritium exchange between labile hydrogens of a native protein and

hydrogen atoms of the surrounding water molecules is slow; in fact, the associated first-order rate constant at 37° C can be as small as 1×10^{-5} sec^{-1}. In view of the state of pigment within the cells forming the ciliary epithelium, and taking into account the observation of Hvidt and Nielsen (62), it is probably safe to assume that tritium exchange is minimal.

7. Summary. Several aspects of the disposition of pilocarpine in the pigmented rabbit eye are apparent from these preliminary data. A topically applied dose of pilocarpine encounters essentially the same parallel loss factors which are present in the precorneal area of the albino rabbit. More of the dose is absorbed, however, due possibly to an increased permeability of the limiting membranes of the cornea, namely, the epithelium. Specifically the amount of pilocarpine in the cornea is increased. It is interesting to note that this increase parallels an increase in the weight of cornea resulting in a concentration-time profile that is statistically indistinguishable from that of albino rabbits. Results from computer simulation studies suggest that the change in weight of cornea is primarily that of the stroma. Like the cornea concentration-time profiles, the aqueous humor concentration-time profiles are virtually indistinguishable in pigmented and non-pigmented rabbits. The exception lies in the region

between 20 and 60 minutes, and appears to suggest biphasic elimination from the aqueous humor. The most striking difference in the behavior of pilocarpine in pigmented and non-pigmented rabbits is the remarkable accumulation of "drug" in the iris and ciliary body, realizing that it has yet to be resolved what fraction of the observed radioactivity is due to tritium exchange between ^{3}H on a pilocarpine molecule and the labile hydrogens of pigment carrier-proteins. To date, surprisingly little is known about the route of drug entry into these tissues as well as the interactions it undergoes with the same tissues once it reaches there.

Several aspects of the distribution of pilocarpine in pigmented rabbits remain to be investigated. To name a few, they include: (a) the relative distribution of drug between epithelium and stroma, (b) to what extent do pigment granules in the epithelium influence accumulation of drug in the cornea, (c) the role played by the cornea in the accumulation of drug by the iris-ciliary body, (d) whether metabolism takes place in the iris and ciliary body and if so, at what rate and to what extent, and (e) the affinity of these metabolites for pigments and how would their binding interfere with the binding of the intact drug.

To point the way to additional studies on the role of pigment in influencing drug action we would like to pose

the following questions:

(1) Until now almost all of the studies focus on therapeutic agents whose sites of action presumably reside in the iris and ciliary body, how do such drugs as antibiotics and steroids behave under similar circumstances?

(2) Chemically speaking, the same drugs that were used are amines, do acidic or neutral compounds show similar affinity for the pigments?

Of course, it cannot be overemphasized that the distribution, quantity, and nature of pigmentation must be specified before a clear picture of drug-pigment binding emerges.

B. Evaluation of Precorneal Factors

1. Analysis of the Equations Describing Time Dependence of Amount and of Concentration of Drug in the Precorneal Area. The peculiar influence of volume of dose instilled on ocular drug bioavailability has been noted for several years (1, 4, 63). However, it has never been put on a satisfactory, quantitative basis due to apparent difficulties in obtaining integrated forms of the expressions describing the disposition of drugs in the various segments of the eye including the precorneal area. The volume of dose instilled affects not only the initial concentration established in the cul-de-sac but also the magnitude of

drainage that results (1). The drainage process operates as long as the volume of fluid in the precorneal area is in excess of the resident volume. This means that the amount of drug in the precorneal area should exhibit biphasic decline with the initial phase reflecting a combined influence of drainage and the other parallel loss factors enumerated in previous paragraphs. Until now, the rate constant associated with parallel loss of an instilled dose lumps the contribution of drainage with those due to the remaining parallel loss factors, but it has never been clear as to what fraction of the rate constant is due to drainage alone. The apparent rate constant associated with uptake of pilocarpine into the epithelium is thought to mirror parallel loss (37); for a 25-μl dose it has a typical value of 0.57 min^{-1}, which is of the right order of magnitude as the drainage rate constant for that dose size. This treatment is, however, inadequate in accounting for the decline of amount of drug in the precorneal area for two reasons. First, it does not predict biphasic behavior. Secondly, it implicitly assumes that drainage operates over the entire time course of drug action. This latter point does not constitute a serious limitation, however, since drainage removes a substantial portion of a 25-μl dose over a five-minute interval so that shortly thereafter, the amount of drug in the precorneal area is essentially zero and parallel loss including drainage ceases.

This study refines the treatment just described by explicitly identifying the role of instilled dose size on drug disposition kinetics in the eye. As noted before, the model applies specifically to pilocarpine. Insofar as the precorneal portion of the model, it holds for both pigmented and non-pigmented rabbits.

Equation 6 describes the time dependence of drug concentration in the precorneal area following topical instillation.

$$[A] = [A]_i\left(\frac{V_i}{V_i + V_o}\right)\left(\frac{V_i + V_o}{V_o + V_i \exp(-k_d t)}\right)^{\frac{Q_T}{V_o k_d}} \exp\left(-\left\{k_p + k_n + \frac{Q_T}{V_o}\right\}t\right) \qquad \text{Eq. 6}$$

From this equation plots such as those shown in Figure 1 can be made. On the other hand, Figure 13 depicts the biphasic decline in amount of drug in the tear film predicted by Equation 19 for the hypothetical case of only tear turnover and drainage being responsible for loss of drug from the precorneal area.

$$A = A_o\left(\frac{V_o + V_i \exp(-k_d t)}{V_o + V_i}\right)^{\left(1 - \frac{Q_T}{V_o k_d}\right)} \exp(-k't) \qquad \text{Eq. 19}$$

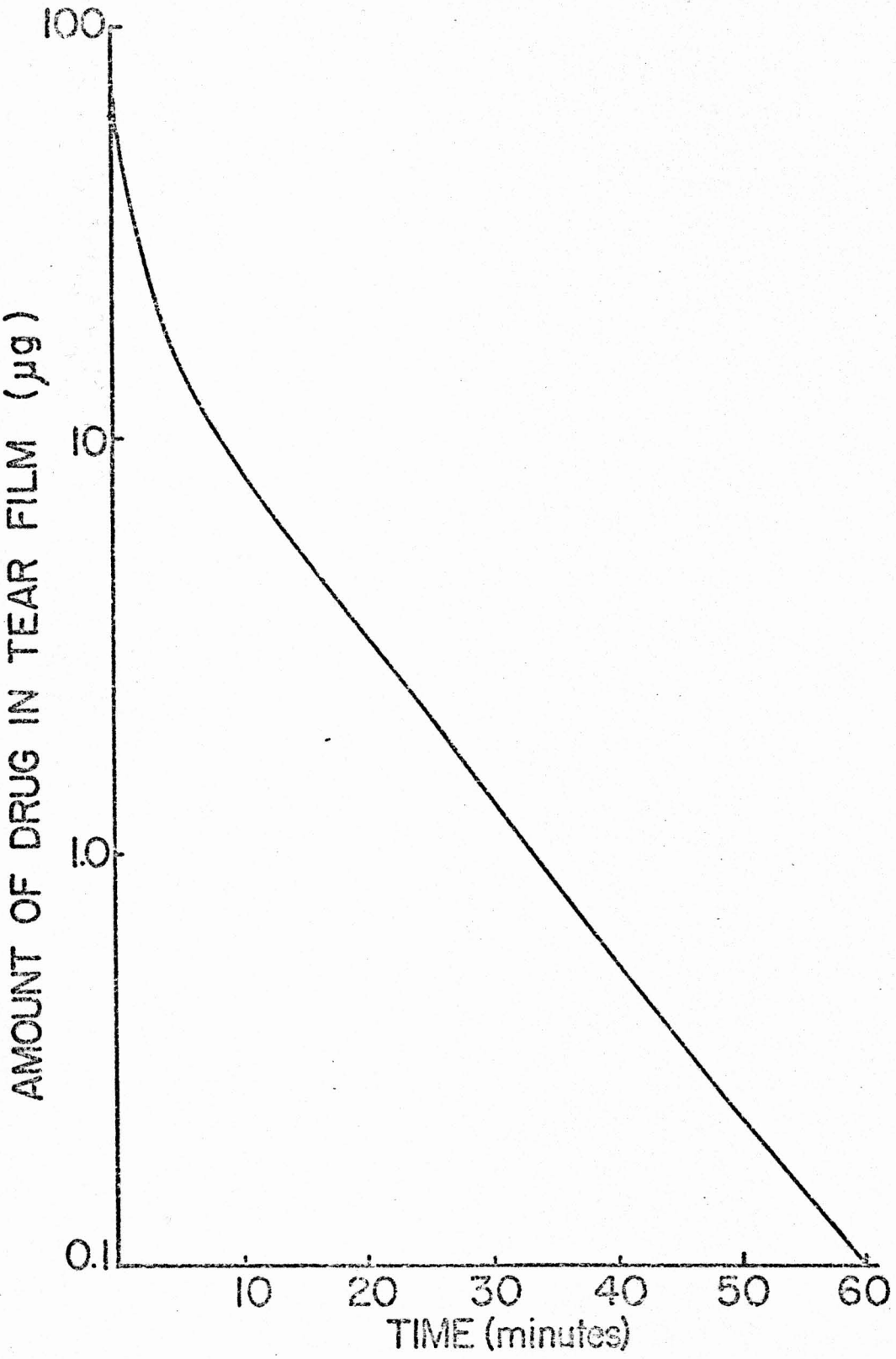
where: A is the amount of drug in the tear film at a given time, A_o is the amount instilled, k' is the rate constant governing overall loss of drug, excluding drainage, from the tear film, and the other symbols are as described previsouly. From Equation 19 it can be seen that the terminal slope is, in this particular case, the first-order rate constant associated with tear turnover. On the other hand, the slope of the residual is equal to the sum of all the factors responsible for loss of drug from the tear film, i.e., $(k_d + k_t)$ in this instance. The proof is as follows.

The linear, terminal portion of amount versus time plot on semilogarithmic paper is given by Equation 20.

$$A' = A_o\left(\frac{V_o}{V_o + V_i}\right)^{\left(1 - \frac{Q_T}{V_o k_d}\right)} \exp(-k't) \qquad \text{Eq. 20}$$

Subtraction of Equation 20 from Equation 19 leads to:

Figure 13. Biphasic decline in amount of drug in tear film for the hypothetical case of tear turnover (Q_T) and drainage (k_d) being the only parallel loss factors. $k_d = 0.545\ min^{-1}$ and $Q_T = 0.66\ \mu l\ min^{-1}$.


100
10
1.0
0.1
AMOUNT OF DRUG IN TEAR FILM (μg)
10
20
30
40
50
60
TIME (minutes)

$$A - A' = A_o\left(\frac{V_o}{V_o + V_i}\right)^{\left(1 - \frac{Q_T}{V_o k_d}\right)} \left[\left(1 + \frac{V_i}{V_o} e^{-k_d t}\right)^{\left(1 - \frac{Q_T}{V_o k_d}\right)} - 1\right] \times e^{-k' t} \qquad \text{Eq. 21}$$

Upon expanding the term within brackets and simplifying we obtain

$$\left(1 - \frac{Q_T}{V_o k_d}\right)\left(\frac{V_i}{V_o}\right) e^{-k_d t} + \frac{\left(1 - \frac{Q_T}{V_o k_d}\right)\left(\frac{Q_T}{V_o k_d}\right)}{2!}\left(\frac{V_i}{V_o}\right)^2 e^{-2k_d t} + \ldots$$

For a 25-µl dose, the second and higher order terms can be ignored. Equation 21 then becomes:

$$A - A' \simeq A_o\left(1 - \frac{Q_T}{V_o k_d}\right)\left(\frac{V_o}{V_o + V_i}\right)^{\left(1 - \frac{Q_T}{V_o k_d}\right)} \left(\frac{V_i}{V_o}\right) \times \exp[-(k_d + k')t] \qquad \text{Eq. 22}$$

According to Equation 22, the slope of the residual gives the overall loss rate constant of which drainage is a part. This is to be contrasted with the concentration-time plot for which a satisfactory slope of a residual cannot be obtained. The proof for this is analogous to that for the

amount case, as is evident from the following.

Equation 23 gives the linear terminal portion of the curve plotted on semilogarithmic paper in accordance with Equation 6.

$$[A]' = \left(\frac{V_i + V_o}{V_o}\right)^{\frac{Q_T}{V_o k_d}} e^{-k't}[A]_i \qquad \text{Eq. 23}$$

Subtracting Equation 6 from Equation 23, Equation 24 results.

$$[A]' - [A] = \left(\frac{V_i + V_o}{V_o}\right)^{\frac{Q_T}{V_o k_d}} e^{-k't}\left[1 - \left(1 + \frac{V_i}{V_o} e^{-k_d t}\right)^{-\frac{Q_T}{V_o k_d}}\right][A]_i \qquad \text{Eq. 24}$$

Upon expanding the term within brackets and simplifying we obtain:

$$\left(\frac{Q_T}{V_o k_d}\right)\left(\frac{V_i}{V_o}\right)e^{-k_d t} - \left(1 + \frac{Q_T}{V_o k_d}\right)\left(\frac{Q_T}{V_o k_d}\right)\left(\frac{V_i}{V_o}\right)^2 e^{-2k_d t} + \ldots$$

This expression will be greatly simplified if second and higher order terms can be ignored. Unfortunately, this is not so, at least for a 25-μl dose. In other words, when ([A]' - [A]) is plotted against time on semilogarithmic paper, a straight line will not be obtained. Consequently, the drainage rate constant would be more readily determined from an amount-time plot rather than a concentration-time plot.

2. The Precorneal Drug Disposition Picture Implied by Computer Simulation Results. Analysis of the relative importance of precorneal factors controlling bioavailability produces some puzzling results. In the first place, drainage does not play a role to the extent that it was once ascribed. To account for the efficient parallel loss observed experimentally, a non-conjunctival loss route is postulated and a combined rate constant of non-productive loss of 0.258 min^{-1} is assigned. In so doing we have assumed that the rate constant governing non-productive loss determined by Patton and Robinson (6) is due solely to conjunctival absorption. The only piece of evidence supporting this, which is also indirect, is Maurice's (64) data on passive diffusion of sodium and chloride ions across the conjunctiva. Hence it is necessary to explore other possibilities which serve to explain the large parallel loss rate constant implied by computer simulation

results.

In order to confine our discussion within a manageable framework, we shall consider only those instances where a single factor is varied, with the remaining ones kept constant.

Table XXI summarizes the adjustment in parameter values needed to bring the predicted and experimentally observed tear film profiles into agreement. It can be seen that an approximately four-fold increase in the magnitude of tear turnover rate constant is sufficient for this purpose. According to Mishima and his co-workers (65) such an increase is easy to accomplish. In this instance, tear turnover rate increases due to the local effects, as yet nondocumented, that pilocarpine may have on lacrimal glands. The vehicle itself is not likely to play a role. At least this is the case in the studies by Conrad et al. (66) in which they monitored the decline of radioactivities due to technetium in the tear film. This mechanism assumes that the alteration in tear turnover rate is maintained over the period of experimental study. However, the data of Patton and Robinson (6) on the aqueous humor concentration profile for anesthetized rabbits appear to speak against it as a viable explanation for the large parallel loss rate constant observed. Also, by a fluorescein dilution technique Yoshida and Mishima (16) obtained in humans tear turnover rate constant in the presence of pilocarpine

Table XXI. Adjusted Precorneal Parameter Values to Yield an Overall Parallel Loss Rate Constant (Excluding Drainage) of 0.346 min^{-1}.

Parameter	Value (min^{-1})		Factor of Increase
	Original	Adjusted	
k_t [a]	0.088	0.324	3.7
k_n	0.0217	0.258	11.9

[a] $k_t = Q_T/V_o$.

that was not different from its basal value. Nonetheless, to test this mechanism would involve, for example, the determination of the rate constant associated with uptake of pilocarpine into the epithelium of anesthetized rabbits. If this mechanism is operative, this uptake rate constant should be substantially less than that observed in unanesthetized rabbits.

Unlike the tear turnover case, an approximately 12-fold increase in the rate constant associated with conjunctival absorption is necessary to yield an overall parallel loss rate constant of 0.346 min^{-1}. This is equivalent to saying that the value for this rate constant as reported by Patton and Robinson (6) underestimated the true value by the same amount. It is difficult to visualize a discrepancy of such a magnitude. Suppose conjunctival absorption is the only mode of non-productive loss. Then, since this rate constant was obtained from rabbits whose drainage ducts were plugged prior to the experiment, it is conceivable that the very act of plugging the duct altered the mechanism of conjunctival loss, possibly by changing the physical dimensions of this membrane as well as its properties. It follows that to resolve this uncertainty, determination of tear film pilocarpine concentration in rabbits with functional drainage apparatus is needed. Also, comparing uptake of pilocarpine by the conjunctiva in the presence as well as in the absence of drainage may provide

additional information. In any event, based on the difference in surface area of the cornea and conjunctiva available for absorption (67), the value of 0.0217 min^{-1} for the rate constant of conjunctival loss appears to be a reasonable one.

If we were to assume, and we will, that pilocarpine has no influence on lacrimation to cause a significant deviation of tear turnover rate from its basal value, and that the value reported for the rate constant of conjunctival absorption is of the right order of magnitude, it is necessary to invoke a route of drug loss other than those already identified experimentally. Based on the data of Patton and Robinson (6) a requirement that has to be satisfied by this yet to be defined route is that it must be connected with the drainage process in some fashion. If this is not the case, then its contribution to parallel loss would have been detected during sampling tear fluids under the condition of plugged drainage duct. It is possible that this additional route of parallel loss involves absorption of drug during its passage through the nasolacrimal apparatus. Presumably the fluid contained in the nasolacrimal apparatus mixes uniformly with the fluid found in the precorneal area so that alteration in drug concentration in the fluid of one region is immediately and quantitatively mirrored by that of the other.

Thus, according to this simplistic picture, blocking

the drainage duct should remove this additional route of drug loss. However, computer simulation studies yield results that tend to contradict this contention. Referring to Ratio B for Cases II and X in Table IX and comparing them with the data of Patton and Robinson (6), it is evident that the increase in AUC predicted by this model exceeds that observed experimentally. While the data of Patton and Robinson (6) indicated that plugging the duct caused an approximately four-fold increase in AUC over the unplugged duct case, the model predicts a 15-fold increase when anesthesia is present (Case II) and a 28-fold increase when it is absent (Case X). Better agreement results when the additional route of loss is allowed to operate even when the duct is plugged (Case VIII). In this light, uptake of drug by the nictitating membrane or even the sclera may constitute this additional route of drug loss. Indeed, Doane et al. (68) observed no significant passage of pilocarpine into the anterior chamber following its application onto the bare scleral surface around the entire limbus. Maurice and Polgar (69) inferred from their studies on diffusion of solutes, including pilocarpine, across bovine sclera that the findings of Doane et al. could not be attributed to a scleral barrier. Instead they offered removal of the drug by the blood in the capillaries of iris, ciliary body or perilimbal circulation as an explanation. In any event, the question remains, why wasn't this

route observed in the study conducted by Patton and Robinson (6)? At this point there appears to be no immediate explanation forthcoming for the difference in value of the non-productive loss constant determined by the two procedures, i.e., experimental data versus computer simulations. Nevertheless, until this mystery is solved we shall in the remainder of this report continue to refer to non-conjunctival absorption as an additional route of drug loss, obviously with the uncertainty of its existence being kept in mind.

Summarizing then, there are five factors that are responsible for loss of an instilled dose from the precorneal area: corneal absorption, drainage, tear turnover, conjunctival absorption, and non-conjunctival absorption. Among these, the role of the first three is well established.

3. Relationship Between Instilled Dose Parameters and Ocular Drug Bioavailability. Earlier in the discussion, the concept of concentration versus amount was introduced in the context of specifying drug levels in intraocular tissues. Because of the presence of drainage it is also important to decide whether amount or concentration is the more suitable designation of drug levels in the precorneal area. As drainage exercises no influence on concentration, two tear film concentration profiles can be identical

although the profiles for the intraocular tissues are different. This point is exemplified by the case where drainage is the only parallel loss factor present and the case where no parallel loss factors are present at all. As a corollary, it should be the amount rather than concentration in the tear film which correlates with such indicators of bioavailability as AUC in the epithelium and in the aqueous humor. In this light, the amount designation should be preferred.

A related question to raise at this point is, "What do the concentration-time profiles in a given intraocular tissue look like for cases in which a fixed amount of drug is delivered in various combinations of volume and concentration?" Contrary to what one may expect from the role of concentration as a driving force for permeation, pharmacokinetics treatment as it is employed today will predict identical profiles for the various combinations of instilled concentration and volume as long as the same amount of drug is administered. The essence of the preceding statement is contained in Equation 25, which

$$C = \frac{FD}{V_d}\left(\frac{k_a}{k_a - k_e}\right)\left(e^{-k_e t} - e^{-k_a t}\right) \qquad \text{Eq. 25}$$

expresses the concentration, C, of drug in the central compartment as a function of time, for a drug that obeys

one-compartment model kinetics. In Equation 25, 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 predicts characteristic intraocular tissue concentration-time profiles for each combination of instilled concentration and volume. Figure 14 illustrates this point. In this figure, two curves (Curves 2 and 3) are included for the five-microliter case. Curve 3 represents the case where the drainage rate constant, k_d, possesses a value characteristic of this volume, i.e., $k_d = 0.31\ min^{-1}$, whereas 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 1). This indicates that the differences that arise 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 turnover, Q_T, to parallel loss, it is necessary to see if the profiles would remain to be different even when Q_T is set equal to zero. The answer is provided by the profiles displayed in Figure 15, both of which are generated from the same set of parallel loss factors. Again, the five-microliter dose gives a higher concentration than the 25-µl dose, indicating that the

Figure 14. Effect of varying instilled concentration and volume while keeping amount of drug administered fixed on aqueous humor levels. Tear turnover present. Key: V_i = instilled volume, $[A]_o$ = initial concentration of drug in tear film, k_d = drainage rate constant. The instilled concentration is 13.56 μg μl^{-1} for an instilled volume of 5 μl and is 2.71 μg μl^{-1} for 25 μl.

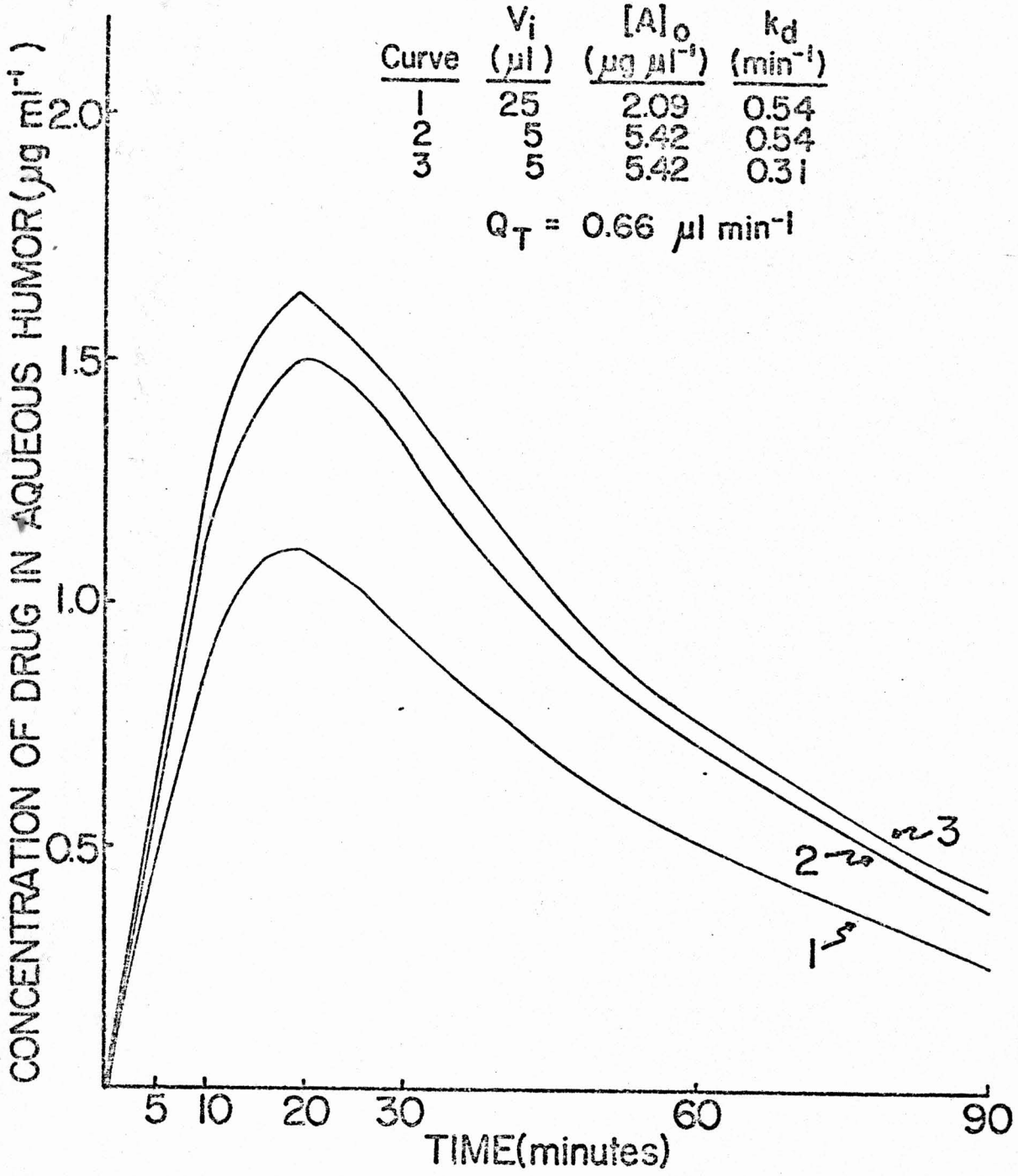
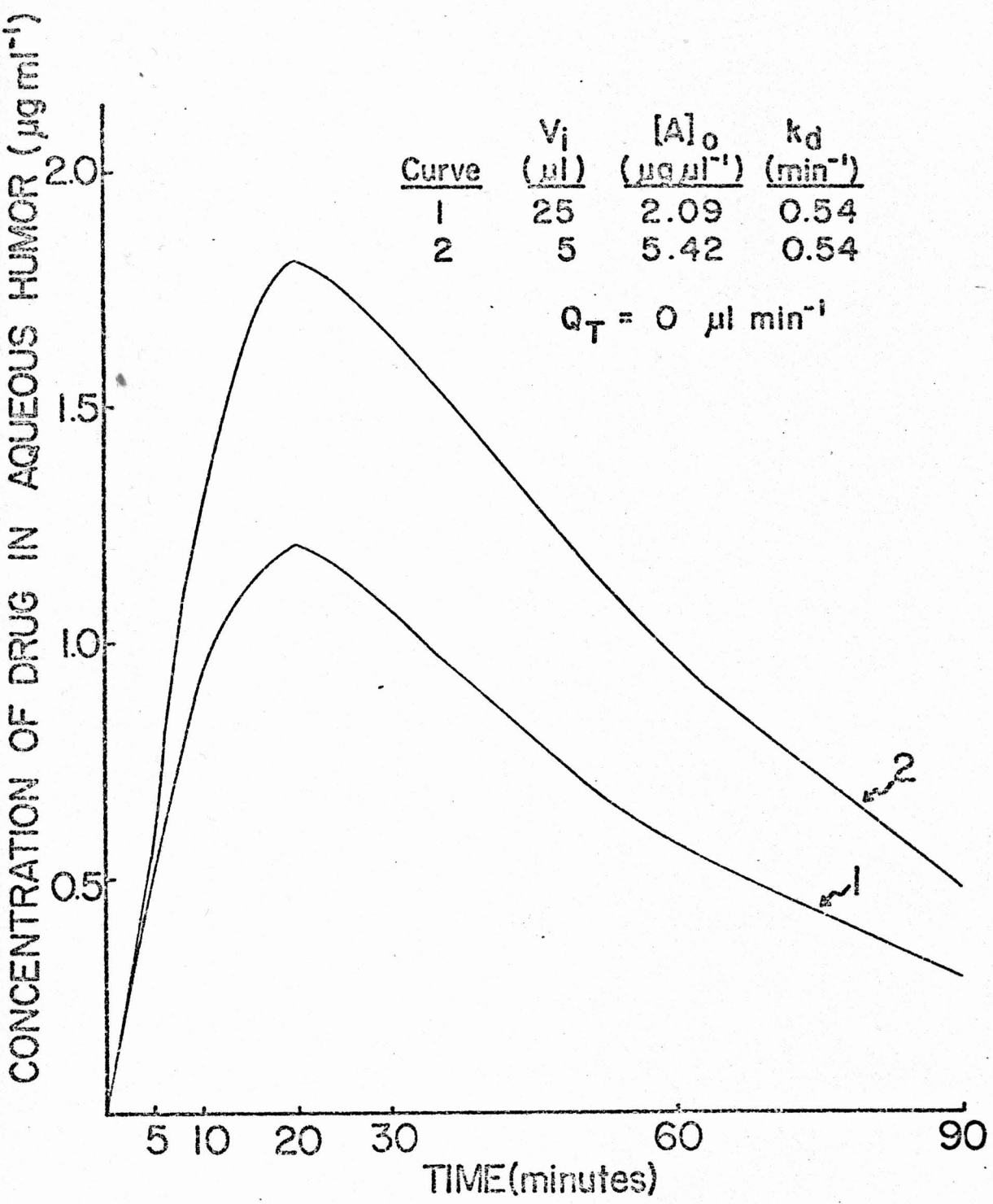

Curve | V_i (µl) | $[A]_o$ (µg µl^{-1}) | k_d (min^{-1})
1 | 25 | 2.09 | 0.54
2 | 5 | 5.42 | 0.54
3 | 5 | 5.42 | 0.31
Q_T = 0.66 µl min^{-1}
CONCENTRATION OF DRUG IN AQUEOUS HUMOR (µg ml^{-1})
2.0
1.5
1.0
0.5
3
2
1
5
10
20
30
60
90
TIME(minutes)

Figure 15. Effect of varying instilled concentration and volume while keeping amount of drug administered fixed on aqueous humor levels. Tear turnover absent. Key: V_i = instilled volume, $[A]_o$ = initial concentration of drug in tear film, k_d = drainage rate constant. The instilled concentration is 13.56 $\mu g\ \mu l^{-1}$ for an instilled volume of 5 μl and is 2.71 $\mu g\ \mu l^{-1}$ for 25 μl.


CONCENTRATION OF DRUG IN AQUEOUS HUMOR (μg ml⁻¹)
2.0
1.5
1.0
0.5
Curve | V_i (μl) | $[A]_o$ (μg μl⁻¹) | k_d (min⁻¹)
1 | 25 | 2.09 | 0.54
2 | 5 | 5.42 | 0.54
Q_T = 0 μl min⁻¹
2
1
5 10 20 30 60 90
TIME(minutes)

increase in extent of drug absorption from a smaller volume of dose cannot be largely due to the indirect influence exerted by drainage on tear turnover. It should be noted that drainage is present in all these instances. Hence, when it ceases to be a parallel loss factor, the results given by our model coincide with those given by the pharmacokinetics treatment described earlier in this paragraph. In essence, the volume of dose administered exerts an influence on the tissue concentration-time profiles only if the volume of fluid at the absorption site undergoes a time-dependent change in response to the volume administered. Therefore, this volume effect will only be observed in regions of the body, e.g., precorneal area and muscles (70, 71), which have a limited capacity to accomodate volume and/or 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. Suffice it to say that this influence is a complex one as illustrated by studies on aspirin (72) and on amoxicillin and ampicillin (73).

Before proceeding further let us explore the role of initial concentration as it relates to drug bioavailability from topical instillation. We have just seen that for a given amount of drug, a small volume of dose favors extent of drug absorption due to a reduction in loss via drainage as well as to a higher initial concentration established in

the tear film. What results would emerge if a given initial concentration is achieved by suitable combinations of volume and concentration ? According to Table XXII the AUC in aqueous humor, hence the amount of drug absorbed, is greater for a 25-µl dose than for a five-microliter 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 five-microliter 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. On the other hand, for a given initial or instilled concentration, the opposite is the case. However, in terms of fraction of dose absorbed, as reflected by the value obtained upon dividing AUC by instilled amount, a small volume of dose is still more efficient than a large one (Table XXII). Such a volume effect has been implied by previous studies in this laboratory (1, 4) and, more recently, has been confirmed experimentally by Patton (63).

Similar statements can be made regarding the rate of change in concentration, dC/dt, in a tissue, e.g., aqueous

Table XXII. Effect of Initial Concentration in Tear Film on Area Under the Curve (AUC) in Aqueous Humor.

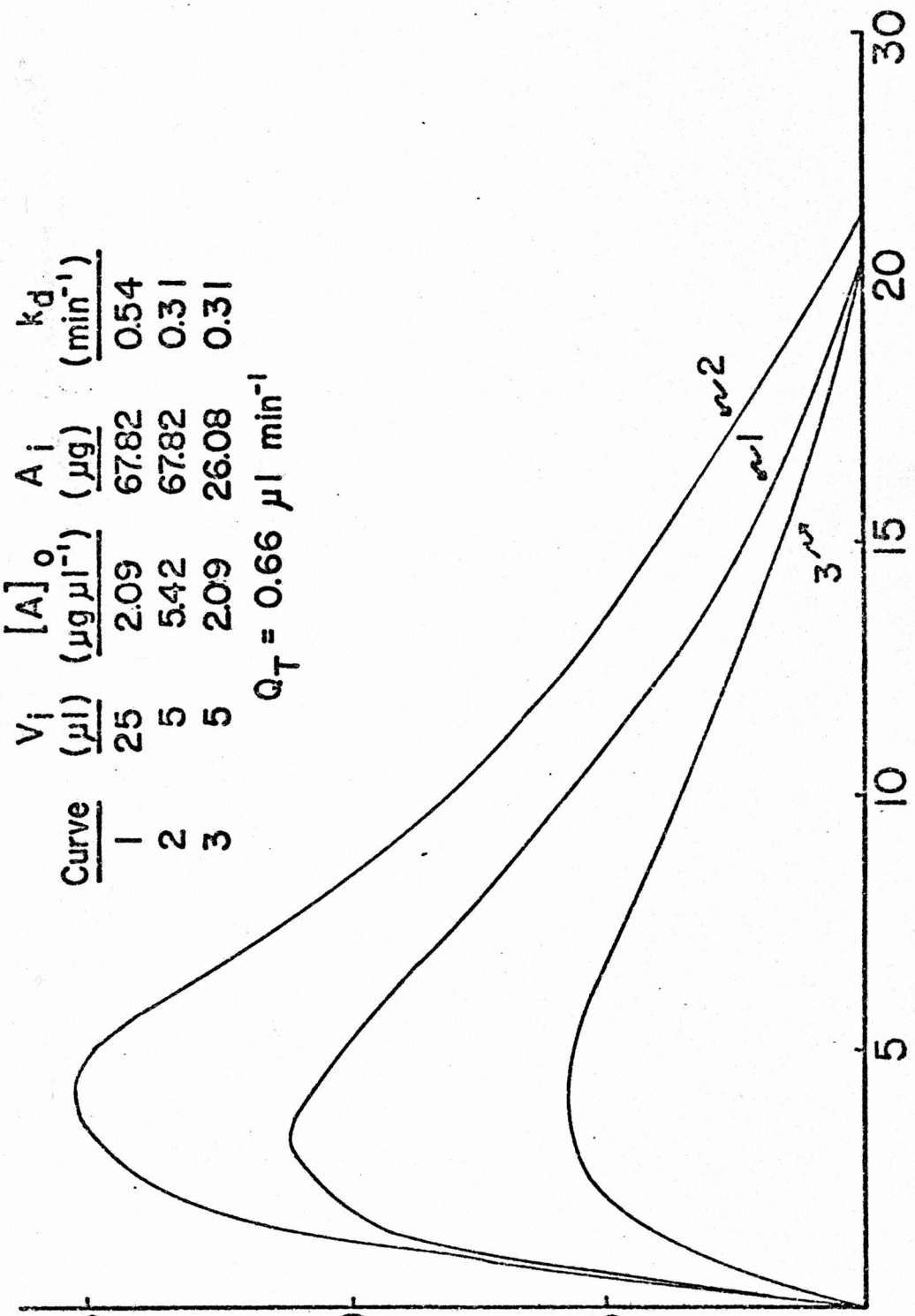
Volume Instilled (µl)	Instilled Concentration (µg/µl)	Instilled Amount (µg)	Initial Concentration (µg/µl)	AUC in Aqueous Humor[a] ($\mu g\ ml^{-1}\ min$)	AUC ÷ Instilled Amount ($\mu g\ ml^{-1}\ min/\mu g$)
5	5.22	26.08	2.09	39.14	1.50
25	2.71	67.82	2.09	69.22	1.02

[a]From zero to 120 minutes.

humor as a function of time. Referring to Figure 16, it can be seen that over the time interval from zero to 20 minutes (i.e., prior to the peak time in aqueous humor), for a fixed amount of drug, i.e., Curves 1 and 2, dC/dt is greater for the five-microliter dose having a higher initial concentration than the 25-μl dose. That the amount of drug administered also exerts some influence on dC/dt is illustrated by Curves 1 and 3, which are characterized by the same initial concentration achieved in the tear film differing only in the volume of dose instilled. A feature shared by all these three curves representing various combinations of instilled volume and concentration is that the maximum rate of change of concentration in the aqueous humor is reached within five minutes post-dosing, which is also the time at which peak levels in the epithelium are attained.

4. Relative Importance of Parallel Loss Factors on Amount and Concentration of Drug in the Tear Film. It is interesting to note that in ranking the various parallel loss factors according to their influence on tear film drug levels, the order differs depending on whether it is the concentration or the amount which is being considered. In particular, the rank order is:

Figure 16. Rate of change (dC/dt) in aqueous humor drug concentration for various combinations of instilled volume (V_i), initial concentration in tear film ($[A]_0$), and amount of drug administered (A_i).



Curve	V_i (μl)	$[A]_o$ (μg μl^{-1})	A_i (μg)	k_d (min^{-1})
1	25	2.09	67.82	0.54
2	5	5.42	67.82	0.31
3	5	2.09	26.08	0.31

Q_T = 0.66 μl min^{-1}

non-conjunctival loss > tear turnover > conjunctival absorption > drainage for tear film concentration, and

non-conjunctival loss > tear turnover > drainage > conjunctival absorption for amount of drug in the tear film.

Regarding tear film drug concentration, the relative importance of parallel loss factors is in accord with the magnitude of the value assigned to them. The exception is drainage, but this is expected since it has no influence on concentration. Interestingly, concerning amount of drug in the tear film, drainage ranks just above conjunctival absorption in its effectiveness in removing drug from the precorneal area. Indeed, Cases V and IX (Table IX) together strengthen the belief that tear turnover is far more important than drainage in causing drug loss from the precorneal area over the entire time course of action, despite the fact that drainage removes a fraction of the instilled dose to the extent of $V_i/(V_i + V_o)$ which, for a 25-µl dose, amounts to 77%. Thus, if conjunctival absorption and non-conjunctival loss are to be considered as a single parallel loss factor, then drainage will be the least efficient one, be it concentration or amount.

Note that the arguments outlined in the preceding two paragraphs refer to the entire time course of drug action. When one considers just the first five minutes post-

instillation, drainage plays the predominant role in removing drug from the tear film. The appropriate rank order is:

drainage > non-conjunctival loss > tear turnover > conjunctival absorption.

In drug therapy it is sometimes desirable to maintain drug levels in tissues, within a certain range, over a period of time. In terms of ocular drug delivery by topical instillation, an obvious approach is to eliminate all the parallel loss factors with a concomitant adjustment in dose. The small size of corneal permeation rate constant, k_p, permits sustaining of levels to occur (Figure 17). The same result can be accomplished by eliminating all the parallel loss factors except drainage, but this procedure is acceptable only if the toxicity of drug upon systemic absorption is not a serious concern. In either approach, it is necessary to predose the system in order to rapidly achieve therapeutic levels. In short, the peculiar influence of drainage on drug loss can potentially be exploited as a therapeutic tool such as prolonging ocular drug levels.

5. Meaning of the Slope of Semilogarithmic Plot of Percent Reduction in AUC Versus Rinse Time. Not only does

Figure 17. Concentration-time profiles in aqueous humor under various conditions existing in the precorneal area.

CONCENTRATION OF DRUG
IN AQUEOUS HUMOR(μg ml⁻¹)
12
10
8
6
4
2
10
30
60
90
120
150
180
TIME (minutes)
1
2
3
Curve
Dose (μg)
Condition
1
67.82
Physiological
2
2.51
Drainage Alone
3
11.30
No Parallel Loss

it exercise a definite role in controlling the fraction of instilled dose available for absorption, drainage also complicates the interpretation of the meaning of the slope of a semilogarithmic plot of percent reduction in AUC versus rinse time. Table XXIII lists the value of the slope of such a plot for three cases. They are:

(A) Case of drainage being absent, i.e., $k_d = 0$,

(B) Case of drainage being the only parallel loss factor, i.e., $k_{nl} = k_{cj} = k_t = 0$, and

(C) Case of all parallel loss factors being present.

In Case A, the slope gives a value which equals the sum of the rate constants associated with corneal permeation, k_p (0.004 min^{-1}); conjunctival absorption, k_{cj} (0.0217 min^{-1}); non-conjunctival loss, k_{nl} (0.2363 min^{-1}); and tear turnover, k_t (0.088 min^{-1}). This result can be predicted mathematically by manipulating the integrated forms of appropriate rate equations.

Thus, Case A suggests that the slope is related to the overall rate constant governing the loss of drug from the precorneal area. On this basis, one would anticipate the slope for Case B to possess a value which is in the neighborhood of the drainage rate constant, 0.54 min^{-1}. As can be seen in Table XXIII, this is not the case. Instead, the value obtained is approximately 20 times less than the rate constant governing the drainage process and is approximately 10 times larger than the corneal permeation rate

Table XXIII. Slopes of Plots of Percent Reduction in AUC in Epithelium Versus Rinse Time.[a]

Case	Slope (min^{-1})
A. $k_d = 0$	-0.35
B. k_d only	-0.03
C. All parallel loss factors present	-0.52

[a]Rinse times used are 0, 1, 2, 3, and 5 minutes.

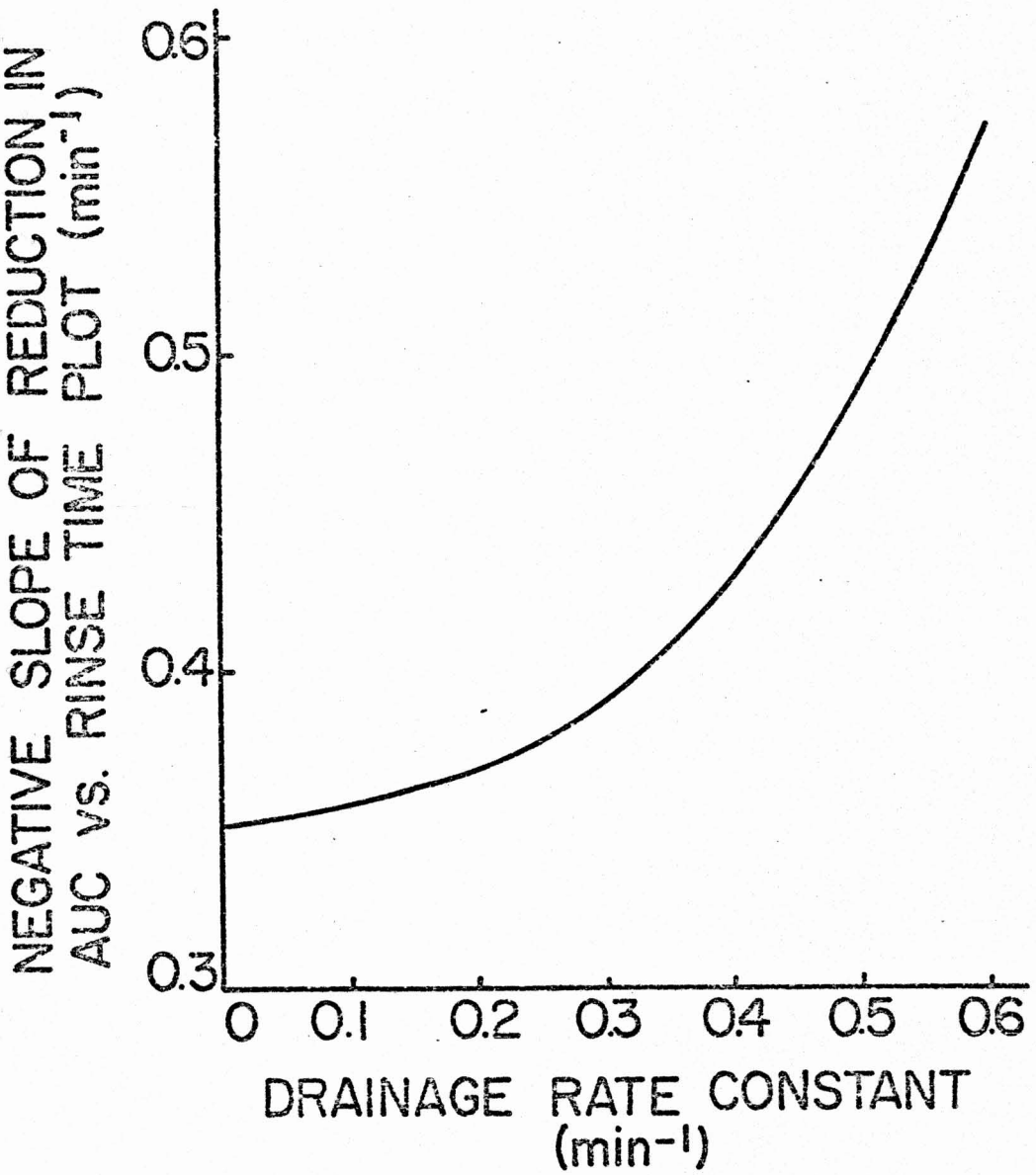
constant. At the very least the slope reflects the tremendous impact of rinsing on reducing bioavailability in this hypothetical case. This is a surprising result to note at first glance but is not so when one considers that drainage operates for approximately five minutes, after which time approximately 23% of the instilled dose is still available for absorption. Table XXIV lists the percent reduction in AUC as a function of rinse time for all the three cases considered.

As expected, when drainage is allowed to operate in addition to non-conjunctival absorption, conjunctival absorption, and tear turnover (Case C), the slope obtained exceeds that obtained under conditions in which it is absent (Case A). In fact, it exceeds the sum of the slopes given by Cases A and B, but falls short of the sum of drainage rate constant (0.54 min^{-1}) and the slope characterizing Case A (0.35 min^{-1}). This indicates that drainage exhibits a synergistic, but not strictly additive effect with the other parallel loss factors. It is interesting to note, however, that the value of the slope in Case C (0.52 min^{-1}) is rather close to that of the drainage rate constant (0.54 min^{-1}). Similar results are obtained for a five-microliter dose, for which the drainage rate constant is 0.31 min^{-1}, as compared with a value of 0.39 min^{-1} obtained for the slope of a plot of percent reduction in AUC versus rinse time. Still, based on the result obtained

Table XXIV. Percent Reduction of AUC Versus Rinse Time.

Rinse Time (min)	% Reduction in AUC		
	A. $k_d = 0$	B. k_d Only	C. All Parallel Loss Factors Present
1	70.31	95.80	56.41
2	49.52	92.89	33.95
3	35.11	90.74	21.99
5	17.20	87.60	8.88

Figure 18. Relationship between drainage rate constant and negative slope of percent reduction in area under the curve (AUC) versus rinse time plot.


NEGATIVE SLOPE OF REDUCTION IN AUC vs. RINSE TIME PLOT (min⁻¹)
0.6
0.5
0.4
0.3
0
0.1
0.2
0.3
0.4
0.5
0.6
DRAINAGE RATE CONSTANT (min⁻¹)

for Case B, it cannot be said that the slope of such plots represents the drainage rate constant. What can be said is that the two are not linearly related and this is demonstrated by Figure 18. In contrast with the instance where drainage is absent, equivalent analytical expressions cannot be obtained, making it difficult to define the contribution of drainage to the slope of a plot of percent reduction in AUC versus rinse time.

6. Role of Drainage in Determining the Early Peak Time Observed in the Epithelium. One other question concerning drainage that need to be answered is: "To what extent is the early peak time (within five minutes of instillation) observed in the epithelium due to drainage?" A partial answer is provided by comparing Case IV, where drainage is the only parallel loss factor present, and Case IX, where drainage is the only parallel loss factor absent (Table XXV). Contrary to what one might expect from the magnitude of the drainage rate constant, the peak time obtained for Case IV is about four times longer than that obtained for Case IX, 20 minutes versus five minutes respectively. The short peak time observed in Case IX is due primarily to non-conjunctival loss. 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

Table XXV. Influence of Parallel Loss Factors on Peak Time in Epithelium, With Special Reference to That of Drainage.

Parameter	Peak Time (min.)
k_{nl} (Case III)	6.5
k_t	10.5
k_{cj}	18.5
k_d (Case IV)	20.0
k_{cj}, k_t, and k_{nl} (Case IX)	5.0
k_d and k_{nl}	4.0
k_d and k_t	6.5
k_d and k_{cj}	9.0

when drainage is allowed to act in conjunction with at least one other parallel loss factor. For example, in the presence of tear turnover, drainage gives a peak time of 6.5 minutes, as compared with the 20 minutes obtained when it is otherwise the case. Similar examples are listed in Table XXV. All of them indicate the dramatic reduction in peak time due to the mere presence of another parallel loss factor in addition to drainage.

7. Interaction Between Drainage and Tear Turnover. At various points in the above discussion the intimate relationship between tear turnover and drainage in effecting loss of an instilled dose from the cul-de-sac has been alluded to or pointed out. Consequently, we will not elaborate upon it in great detail here.

An example which is illustrative of this relationship centers on a comparison between Cases VIII and IX (Table VIII). In either case, drainage is not a factor in causing drug loss, but this result is accomplished in divergent ways. In Case VIII, drainage is eliminated by physical means such as blocking the nasolacrimal duct, whereas in Case IX drainage is not favored because the drug is introduced in zero volume. Yet, relative to the case where all the precorneal factors are functional, the case of no volume of dose is actually inferior to the case of blocking the drainage apparatus insofar as increasing the area under

the curve in the epithelium or aqueous humor (Table IX). The cause underlying this difference is obvious and is due to the effect of resident volume on tear turnover. As pointed out earlier, when the drainage duct is plugged, the resident volume increases from 7.5 µl to 32.5 µl, thereby diminishing the influence of tear turnover, whose associated first-order rate constant decreases from 0.088 min^{-1} to 0.020 min^{-1}. It is also interesting to note that Case IX gives a smaller AUC than Case VIII, despite a four-fold difference in initial concentration favoring Case IX.

A particularly interesting case that must be brought to the reader's attention concerns the one in which tear turnover is absent as in anesthesia. In this instance, no curvature will be evident in a semilogarithmic plot of tear film drug concentration versus time profile, and this result is evident in Equation 26.

$$[A] = [A]_o \exp[-(k_p + k_n)t] \qquad \text{Eq. 26}$$

where the symbols are as defined previously. Another feature is that the concentration at any time is insensitive to variations in drainage rate constant brought about, for example, by instilling solutions of different viscosity. Furthermore, Chrai *et al.* (1) observed that in the presence of anesthesia, the drainage rate constant is independent of

volume instilled, at least for a dose size within the range of five and fifty microliters. The significance of this observation in terms of amount of drug in the tear film is summarized in Equation 27.

$$A = [A]_o(V_o + V_i e^{-k_d t})\exp[-(k_p + k_n)t]$$

or its equivalent,

$$A = A_o\left[1 - \frac{V_i}{V_i + V_o}(1 - e^{-k_d t})\right]\exp[-(k_p + k_n)t] \qquad \text{Eq. 27}$$

where A is the amount of drug in the tear at a given time and A_o is the amount of drug administered. Equation 27 predicts that for a fixed amount of drug administered, a small volume of dose instilled causes less of the drug to be lost than when a large volume is employed (Figure 15). In contrast to those instances where drainage is volume dependent, the improvement of bioavailability observed here is solely due to a higher initial concentration attained by the smaller volume instilled.

For several years drainage has occupied the center stage of research on precorneal drug disposition. Only very recently have studies been initiated in an attempt to quantitate the influence of tear turnover on drug

bioavailability (66). Invariably they amount to determining tear turnover rate constants. Briefly, the numerical value for this parameter can be derived from the intercept obtained by extrapolating the terminal portion of a semilogarithmic plot of tear film drug concentration versus time. The details are contained in Equation 28. Obviously, uncertainties in the values of drainage rate constant, k_d, and resident tear volume, V_o, will be reflected in the accuracy with which Q_T is known. Once Q_T is determined and

$$Q_T = V_o k_d \left[\frac{\ln \left(\frac{\text{Intercept}}{\frac{[A]_i V_i}{V_i + V_o}} \right)}{\ln \left(\frac{V_i + V_o}{V_o} \right)} \right] \qquad \text{Eq. 28}$$

provided that an estimate of corneal permeation rate constant is available, the rate constant governing nonproductive loss ($k_n = k_{nl} + k_{cj}$) can be calculated from the slope. More importantly, Equation 28 enables one to determine if the instillation of a dose alters tear turnover.

8. Binding of Drugs to Proteins in the Tear Fluid. Thus far, our treatment on precorneal drug disposition factors has not identified binding of drugs to proteins in the tear fluid explicitly (2, 3, 20). Needless to say this

very phenomenon affects the mathematical description of decline in tear film drug concentration. At the outset it is reasonable to assume that drainage and tear turnover affect both the bound and unbound forms of a drug to the same extent. Conjunctival and corneal absorption, on the other hand, are processes limited to the unbound form; such is also the case if some sort of absorptive process is involved in non-conjunctival loss. Let [A] denote total concentration of drug in the precorneal area; $[A_f]$, concentration of unbound drug; and $[A_b]$, concentration of bound drug. The rate of change of concentration in the precorneal area d[A]/dt can be partitioned into one due to the unbound drug and one due to the bound drug. Hence,

$$\frac{d[A]}{dt} = \frac{d[A_f]}{dt} + \frac{d[A_b]}{dt}$$

Treating the role of the precorneal disposition factors in the manner prescribed at the beginning of this paragraph, we obtain:

$$\frac{d[A]}{dt} = -\frac{Q_T}{V_A}[A_b] - (k'_p + k'_n + \frac{Q_T}{V_A})[A_f] \qquad \text{Eq. 29}$$

where k'_p and k'_n are the true rate constants governing corneal permeation and non-productive loss of <u>unbound</u> drug,

respectively, and are to be distinguished from the corresponding rate constants, k_p and k_n, referred to total concentration of drug. Provided that the conditions specified earlier for drug-pigment binding (see Equation 17) prevail, Equation 29 can be expressed as:

$$\frac{d[A]}{dt} = -\left(\frac{Q_T}{V_A} + \frac{k'_p + k'_n}{1 + K_A[P_T]}\right)[A] \qquad \text{Eq. 30}$$

where $[P_T]$ is the total concentration of protein in the precorneal area, and K_A is the equilibrium constant governing the process:

$$\text{unbound drug} + \text{protein} \underset{}{\overset{K_A}{\rightleftharpoons}} \text{bound drug}$$

Upon comparing Equation 30 with Equation 4 it is clear that $k_p = k'_p/(1 + K_A[P_T])$ and $k_n = k'_n/(1 + K_A[P_T])$. It follows that $k_p = k'_p$ and $k_n = k'_n$ only when $K_A[P_T] << 1$. Likewise, for $K_A[P_T] >> 1$, the apparent rate constants governing corneal permeation and non-productive loss will be less than the true values by a factor of $K_A[P_T]$. In other words, if protein binding is extensive and, at the same time, unrecognized, the true permeation rate constant will be underestimated, and the difference is substantial when $K_A[P_T]$ is large. Thus, a small k_p does not necessarily mean low

permeability of the epithelium to the drug. Note that K_A is an average quantity since several protein fractions exist in the tear fluid and each exhibits different capacities towards binding drug (20).

A logical question to ask at this point is: "How likely are these limits to be satisfied in practice?" Considering that (a) the total protein concentration in tears is approximately 3×10^{-5} M, and (b) the association constant (K_A) for many drugs falls within the range of 10^3 M^{-1} and 10^5 M^{-1}, the lower limit of $K_A[P_T] << 1$ is the more likely one to be encountered.

In spite of this, the upper limit is still useful in the sense that it simplifies the answer to the question: "To what extent is the small value reported for the nonproductive loss rate constant under the condition of blocked drainage apparatus due to an increase in $[P_T]$, an increase in K_A, or both?" Let us suppose that the value inferred from computer simulations is the one being applicable under physiological conditions. Then, to account for the magnitude of discrepancy ($\sim$ten-fold) observed in its value, there must be at least a ten-fold increase in protein concentration when the drainage apparatus is blocked, assuming further that K_A is unchanged. This implies that plugging the duct represents an insult to the eye, which reacts to it by elaborating a protein-rich tear fluid. However, this is inconsistent with what one usually

observes under similar circumstances. Besides, the albino rabbit under anesthesia has been shown to have a zero or negligible tear turnover rate (1). Therefore, this mechanism of protein content increase is untenable. On the other hand, tear protein concentration can increase if the tear volume is reduced, say, through evaporation and/or absorption into the tissues surrounding the tear chamber. The fact that the drug solution was instilled one minute after the plug was inserted (6) would require a remarkable rate of evaporation and/or absorption of tear fluid to achieve a ten-fold reduction in its volume. On this basis, this mechanism is also untenable.

We are thus left with the alternative of an increase in K_A with little or no change in total protein content. This simply means a perturbation in the composition of tear proteins, which has to take place within the time constraint of one minute. Again, it is difficult to visualize how such a change can be realized, let alone the fact that under anesthesia tear secretion is suppressed (1). Simply stated, it remains to be seen whether an enhancement in protein binding when the drainage apparatus is blocked is indeed the cause underlying a small non-productive loss rate constant.

C. Transcorneal Penetration of Pilocarpine

Following the scheme proposed by Sieg and Robinson (7),

the computer simulation studies treat the cornea as a composite of two tissues: epithelium and stroma-endothelium. It is found that the biphasic behavior observed experimentally in the epithelium is not predicted by the system of differential equations written in accordance with the proposed scheme. To reconcile this discrepancy, we introduce a "feedback" criterion which is based on the ratio of concentrations between the epithelium and the stroma-endothelium. An alternative treatment would involve the subdivision of the epithelium into two components, perhaps the tissue proper itself and the mucin coat postulated to be present on its surface (74, 75, 76). In this instance, the mucin coat will allow rapid uptake of drug to occur while the tissue proper functions in the capacity of a reservoir. Note that the epithelial concentration profile obtained by Sieg and Robinson (7) is based on a tissue scraping technique, which by its very nature does not distinguish between drug in the epithelium and drug in the mucin layer. Consequently, to confirm this alternative scheme of drug disposition in the cornea, it is necessary to determine drug concentration in the presence and also in the absence of the mucin layer.

D. The "Volume" Parameter in the Context of One-Compartment Pharmacokinetics and in the Context of the Proposed Model

Before proceeding further let us focus our attention on the apparent distinction between the two approaches in treatment of the "volume" parameter. For simplicity let us consider the one-compartment model case in which the compartment is composed of two tissues. According to the pharmacokinetics approach, appropriate equations are written to describe the rate of change in amount in a compartment and are then integrated. To get the corresponding expression for concentration in, say, the sampling tissue, the amount expression is divided by a parameter with dimensions of volume, whose value is such that the concentration so calculated matches that observed in the sampling tissue. In contrast, the rate equations generated in accordance with our approach incorporate the volume of individual tissues or combinations of them. On this basis, one can expect differences to arise in the expressions describing the time dependence of concentration in the sampling tissue, for example. However, at least in those instances where the volume of fluid at the absorption site does not change appreciably with time, either approach gives expressions that differ only in the volume term. In pharmacokinetics, the volume employed, so-called apparent volume of distribution, V_d, may not have physiological correspondence.

Such is not the case in our approach but, as will be discussed shortly, there is an inherent problem with estimating the value of this parameter.

The one-compartment model is the simplest pharmacokinetic model. Since it depicts the body as a single homogeneous unit, the average concentration, $\overline{C}$, in the body can be expressed as:

$$\overline{C} = \frac{C_1V_1 + C_2V_2}{V_d} \qquad \text{Eq. 31}$$

where C refers to concentration, V refers to volume, and the subscripts 1 and 2 refer to the two tissues making up the compartment. Furthermore, it is inherent in the one-compartment model that the concentration in the sampling tissue equals the average concentration. Therefore, if tissue 1 is the sampling tissue, Equation 31 becomes:

$$C_1 = \frac{C_1V_1 + C_2V_2}{V_d} \qquad \text{Eq. 32}$$

which upon rearrangement yields:

$$V_d = V_1 + (\frac{C_2}{C_1})V_2 \qquad \text{Eq. 33}$$

Two implications arise from Equation 33. First, since C_1 and C_2 are not required to be the same (77), the apparent volume of distribution in a one-compartment model need not be the sum of the volumes of the two tissues. Nevertheless, it is at least as large as the volume of the sampling compartment. Secondly, the value of V_d varies depending on whether tissue 1 or tissue 2 is being sampled. Usually only one of the tissues is accessible to sampling so that a consistency check on the value of V_d is lacking. Nonetheless, Equation 33 can be modified to suit instances in which the single compartment is composed of more than two tissues. The appropriate expression is Equation 34.

$$V_d = C_1 + \sum_{i=2}^{n} \left(\frac{C_i}{C_1}\right) V_i \qquad \text{Eq. 34}$$

where tissue 1 is the sampling tissue. Obviously, the problem becomes increasingly complex as soon as one departs from the one-compartment situation.

As mentioned earlier, our approach has its share of problems. First of all, the volume of tissue is difficult to determine. Next, even when this information is available, it may not be an appropriate one to use because the drug may not be homogeneously distributed throughout the tissue. However, since the "feedback" criterion employed in our simulation studies is a function of concentration

differentials, it is necessary to have good estimates of the volumes of tissues involved. This, admittedly, is a weakness of our approach.

E. Meaning of the Slope of Residual in Aqueous Humor Obtained by Feathering Technique

An issue that has never been resolved, to our knowledge, is whether the rate constant governing disappearance of drug from one compartment or tissue and the rate constant governing its appearance in the succeeding compartment or tissue are one and the same, assuming that no parallel loss processes interpose between the two tissues. The computer simulation studies provide a unique opportunity to explore this issue. The process being considered is the transfer of drug from the epithelium to the stroma-aqueous humor. We will utilize the results generated for the several cases concerning the evaluation of relative importance of precorneal disposition factors on drug bioavailability. As a first approximation we shall employ one-compartment pharmacokinetics technique to obtain the slope of the residual. Table XXVI displays the results for such an analysis. In no case is the coefficient of determination, R^2, less than 0.94. It can be seen that the value obtained for the slope of the residual is somewhat different from the theoretical value of 0.10 min^{-1}. The deviation cannot be due to the fact that the rate constant governing the decline in drug

Table XXVI. Slope of Residual of Aqueous Humor Concentration-Time Profile and Peak Time in Aqueous Humor as a Function of Parallel Loss Factors.

Case	Parallel Loss Factors Present	Slope of Residual	Peak Time in Aqueous Humor (min)
I	k_t*	0.037	32.0
II	k_{cj}	0.038	51.5
III	k_{nl}	0.13	23.5
IV	k_d	$\sim$0	96.75
V	k_{nl}, k_{cj} and k_d	0.16	21.5
VI	k_t, k_{nl} and k_d	0.18	21.0
VII	k_t, k_{cj} and k_d	0.13	27.0
VIII	k_{nl}, k_{cj} and k_t*	0.14	23.0
IX	k_{nl}, k_{cj} and k_t†	0.16	22.0
X	k_{cj} and k_t*	0.054	41.5
XI	k_{cj}, k_t†, k_{nl} and k_d	0.13	20.75

*$k_t = 0.020\ min^{-1}$.

†$k_t = 0.088\ min^{-1}$.

levels in the epithelium undergoes a change in value from 0.10 min^{-1} to 0.018 min^{-1} as required by "feedback". This is because by the time this process occurs absorption into the aqueous humor is virtually complete.

Several general statements can be made. First, with the exception of drainage, the slope of the residual appears to increase with the magnitude assigned to the parallel loss factors. Secondly, the marked departure of the slopes for Cases I, II and X (Table XXVI) may be reflecting the existence of a "flip-flop" model in the epithelium. This comes about because the apparent rate constant associated with parallel loss has a magnitude which is less than that associated with transfer of drug from the epithelium to the stroma-aqueous humor. Thirdly, whereas drainage by itself gives an approximately zero slope, its contributions is a positive one when present with at least one other parallel loss factor. Unfortunately, its influence is not an entirely predictable one, as can be seen by comparing Cases IX and XI. In short, the slope of the residual of an aqueous humor plot is not a simple function of the rate constant governing the disappearance of drug from the epithelium, but is likely to be influenced in some manner by parallel loss factors in the precorneal area. An additional piece of evidence supporting this statement is the peak time observed in aqueous humor under the circumstances listed in Table XXVI. It

can be seen that qualitatively, the peak time in aqueous humor increases with a decrease in the magnitude of overall parallel loss rate constant.

We must re-emphasize that we have just employed the methods of residuals in the context of one-compartment pharmacokinetics to obtain the slope of residuals based on figures that are generated by a model not derived from one-compartment pharmacokinetic principles. Therefore, it is conceivable that deviations of the slope of the residual from the theoretical value is related to the very procedure employed to obtain it. However, this is precisely the situation one is confronted with in treating experimental data of this kind.

F. How Complete Does the Model Have To Be?

A question that bears similar significance to the one concerning whether the disappearance rate constant in one tissue and the appearance rate constant in the adjoining tissue are one and the same is: "From a practical point of view, how complete does the model have to be?" The model we just put forth is undoubtedly more complete than the one-compartment model (see Scheme I), although both of them are based on the same assumption of drug transfer processes being first-order in nature. Our model is more complete not only because it considers the important influence of dose size on drug bioavailability, but also because it

considers the behavior of drug in the components of the cornea. In this sense, our model provides some insight into the mechanism of drug movement within the cornea, whereas the one-compartment model does not. Since our model is constructed on what is known of the fate of pilocarpine in the cornea at this point in time, how should the model be modified when additional information on the behavior of pilocarpine is available at the cellular level, for example, within the cells making up the epithelial tissue? Although a model incorporating information at the cellular level is more complete than the one at the tissue level in describing the fate of drug, the mathematics involved can conceivably be cumbersome. To generalize, we are usually confronted with the situation in which a conflict exists between having a simple model that is, within limits, mathematically easy to handle but is perhaps too unrealistic to be convincing, and a very complicated model that has a far greater degree of realism but is too difficult to manipulate and interpret. In either case, the basic question remains, is the real situation readily attainable?

Perhaps the foregoing arguments refer to the extreme, yet ultimate level of understanding of drug disposition that one should strive for. Suppose we are content with working at the tissue level. Should we treat each tissue that drug molecules encounter individually? One can easily

see that this situation is quite analogous to the one discussed in the preceding paragraph. In our case, we identify the epithelium and stroma of the cornea, aqueous chamber, and reservoir. The reservoir consists of tissues within and/or surrounding the aqueous chamber. Specifically they include iris and ciliary body, and theoretically also include those tissues outside the eye that drug molecules are likely to travel to! The rationale behind identifying specifically the epithelium, stroma and aqueous humor is that we want to focus on drug disposition in these tissues.

In general terms then, the first step in constructing a model is to identify the system and treat the remainder of the body as its surroundings. Within the system we can have phases between which drug transfer occurs in a specified manner. Obviously drug transfer is also allowed to occur between the system and its surroundings. In our case, the system consists of the phases epithelium, stroma and aqueous humor. However, if we are also interested in the transfer of drug between aqueous humor and iris-ciliary body, the latter tissues should also be included as a phase within the system, which now assumes a new meaning. It is easy to see that the principles just presented bear some resemblance to those underlying the two-compartment pharmacokinetic model.

As a final remark, we should note that by making a

model more complete, we remove some of the assumptions made in the simpler model. For example, by considering the epithelium and stroma as distinct entities of the cornea, we no longer assume the cornea to be a single membrane. Now when we speak of the concentration gradient governing transfer of drug to the aqueous chamber, it no longer is cornea to aqueous chamber, rather it is stroma to aqueous chamber, for example.

V. CONCLUSIONS

From the preliminary data on pilocarpine levels in the cornea, aqueous humor, and iris and ciliary body, it is evident that the behavior of pilocarpine in the pigmented rabbit is not as simple as what one might anticipate from the mere aspect of binding of the drug to pigments. Specifically a larger fraction of dose is recovered in the pigmented rabbit eye not because of an impairment in parallel loss processes in the precorneal area but probably because of certain favorable physical characteristics, as yet undisclosed, of the corneal epithelium. Quite unexpectedly the aqueous humor levels from five to 120 minutes in the pigmented rabbit are statistically indistinguishable from those of albino rabbits, despite a larger amount of drug present in the cornea of the pigmented rabbit as well as accumulation of drug in its iris and ciliary body. Admittedly, an unambiguous interpretation of the iris and ciliary body data in terms of accumulation of intact pilocarpine is made difficult by lack of data on metabolism of the drug in these tissues as well as on possible tritium exchange between the ^{3}H label on a pilocarpine molecule with the labile hydrogens of the carrier protein for the pigment. If the radioactivity observed is due to intact drug alone, then a reduction in the intensity of miosis from a given dose signifies a corresponding reduction in

free drug concentration available for binding with the receptors. It is found that within the instilled concentration range from 0.5×10^{-2} M up to 4×10^{-2} M the intraocular tissue levels and instilled concentration are, roughly speaking, related logarithmically. This means that compared with albino rabbits, a given miotic response is obtained in the presence of elevated aqueous humor levels in the pigmented rabbits.

On the basis of these preliminary data it becomes apparent that additional studies are needed: (a) to clearly define the role of the components of the cornea in the drug accumulation process in the iris and ciliary body; (b) to evaluate the extent and rate of metabolism of pilocarpine in the iris and ciliary body; (c) to identify the extent of tritium exchange in these tissues or, more simply, to see if the accumulation pattern is reproduced when a ^{14}C label is employed; and (d) to obtain data in the iris and ciliary body beyond 120 minutes so that a complete picture of accumulation of pilocarpine in these tissues emerges.

Turning to computer simulations, an important finding is that the role of drainage in causing drug loss from the precorneal area is now seen in a new light. At the very least, the results reinforce previous observations which have suggested that despite its magnitude, drainage does not play a predominant role in the parallel loss process.

In fact, over the entire time course of action, tear turnover is a more effective parallel loss factor than drainage. The second important finding that emerges from computer simulations concerns the value obtained for the rate constant associated with non-productive loss, k_n. In particular they indicate that the experimentally determined value is approximately ten times too small. In view of the findings with viscous solutions and plugged duct (6, 78, 79), it is unlikely that this discrepancy in the value for k_n is an artifact of the model. Consequently, a non-conjunctival loss route is postulated, and the value determined by Patton and Robinson (6) for k_n is considered as the rate constant governing conjunctival absorption. Unfortunately, at the present level of our knowledge, the physical reality of this non-conjunctival loss route cannot be defined with certainty. The logical step, then, is to re-evaluate the parameter of non-productive loss experimentally. Nonetheless, according to this model, non-productive loss is the most efficient parallel loss factor. It is followed by tear turnover and lastly drainage.

In treating parallel loss it is customary to add the rate constants governing the processes concerned and then call this sum the parallel loss rate constant. Indeed, the computer simulation studies indicate that this is an acceptable practice for treating all the precorneal parallel loss factors except drainage. It is clear that drainage does

not exhibit an additive effect towards the other parallel loss factors. Unfortunately, the fraction of the observed parallel loss rate constant due to drainage is still unknown. At first glance this combined rate constant appears to be dominated by drainage, but a careful analysis of the slope of a semilogarithmic plot of percent reduction in area under the curve versus rinse time and of the peak time observed in the epithelium rules this notion out.

The volume of dose instilled is of immense importance in influencing ocular drug bioavailability not only because of the volume-dependent drainage effect, but also because by affecting the initial concentration established in the tear film it influences the driving force governing corneal permeation. These two effects cannot be satisfactorily accounted for by traditional pharmacokinetics treatment but are adequately treated in our model. Furthermore, there is no reason to believe that this "volume" effect is restricted to the ocular system. Inevitably it also exists in absorption sites elsewhere in the body and therefore should be treated accordingly in the interpretation of concentration-time profiles in the sampling compartment.

Although our model adds to our understanding of the relative importance of precorneal factors in influencing drug bioavailability, there is certainly room for improvement or refinement to be made in its treatment of precorneal drug disposition. Most important of all it does not

explicitly treat the initial rapid uptake of drug that can occur at the moment a drop is deposited onto the corneal surface before it mixes with the rest of the tear fluid in the entire tear chamber. By making this modification, agreement between the predicted and observed five-minute value in aqueous humor may be improved. Also, the model does not allow a variable tear turnover rate over the period of simulation, and therefore does not apply to those situations in which the eye reacts to an instilled dose of drug by altering the lacrimation rate momentarily.

As the model was developed expressly for solutions, modifications have to be made to adapt to other forms of drug delivery including suspensions, ointments, therapeutic devices such as contact lenses and the OCUSERT®, and pro-drugs. It is easy to see that such models are of practical value in aiding dosage form design and formulation.

As mentioned earlier, the system of differential equations as originally written does not reproduce the biphasic behavior observed in the epithelium. Agreement between predicted and observed values improves only when a "feedback" criterion is introduced, but it is still far from satisfactory. This indicates that either the simple kinetics scheme of the form A → B → C → D is not appropriate for small systems such as the epithelium, or the epithelium cannot be treated as a single, homogeneous tissue. Herein lies another area where improvements in the model

can be made. On the experimental front, the role of mucin layer in uptake of drug from the tear fluid must be defined.

Except for these shortcomings, the model strengthens the scheme proposed for the movement of pilocarpine from the tear chamber, through the cornea and into the aqueous chamber. The model applies equally well to both pigmented and non-pigmented rabbits and undoubtedly lays down a framework upon which the disposition of other drugs can be built.

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APPENDIX

LISTING OF COMPUTER PROGRAM

```
.E2(1),DRUGCONCNTRT
      PARAMETER ISVDM1 = 100,ISVDM3 = 200
      PARAMETER MXEVNT =  20
      PARAMETER NVARSP = 14
      PARAMETER NAVARP = 6,NCMPSB = 6
      PARAMETER LASTSV = NVARSP + 1
      PARAMETER NTGRLV = 1
      PARAMETER ISVDM2 = NVARSP + 1
      PARAMETER ISVDM4 = NVARSP + 1
      DOUBLE PRECISION VALINT,VALACC
      DIMENSION SAVE(ISVDM1,ISVDM2),SAVE2(ISVDM3,ISVDM4)
      DIMENSION TEMP(ISVDM3)
      DIMENSION ISCAL(NVARSP)
      DIMENSION IPLOT(NVARSP)
      DIMENSION VALINT(NVARSP),VALACC(NVARSP)
      DIMENSION ITGRIX(NVARSP)
      DIMENSION YINIT(NVARSP),YFINAL(ISVDM2)
      DIMENSION NAMVAR(NVARSP)
      DIMENSION ITGVAR(NVARSP)
      DIMENSION IPLNAM(NVARSP)
      DIMENSION ITIME(MXEVNT),DOSE(MXEVNT),VOL(MXEVNT)
      DIMENSION ICHAR2(14)
      COMMON PN,PP,PM,P1,P2,P3,P4,P5,VI,QT,VO,V11,V12,V2,NVARS,NBVARS,
     1  NAVARS,NSVARS,NCOMPN(NCMPSB),ICASE,IVCOMP(NAVARP,NCMPSB),
     2  P12,P31,P13,V13,V3,V4,X,P30
      DATA HMIN/1.E-3/,HMAX/1.0/,HSTART/.5/
      EXTERNAL DERIVS
      READ 3310,ICASE
 3310 FORMAT(A6)
      PRINT 3312,ICASE
 3312 FORMAT(25X,'TYPE OF PROBLEM =  ',A6)
 3316 READ 3,NVARS
      PRINT 14,NVARS
   14 FORMAT(10X,'NUMBER OF VARIABLES = ',I5)
      IF (NVARS .LE. NVARSP)GO TO 3330
      PRINT 3328
 3328 FORMAT(25X,'NVARS TOO LARGE ')
      STOP
 3330 READ 3333,(NAMVAR(K),K = 1,NVARS)
 3333 FORMAT(14A5)
      PRINT 15
   15 FORMAT(20X,'VARIABLE NAMES FOLLOW')
      PRINT 3337,(NAMVAR(K),K = 1,NVARS)
 3337 FORMAT(5X,14A5)
      READ 3,NBVARS,NSVARS
      PRINT 3339,NBVARS,NSVARS
 3339 FORMAT('    NUMBER OF BASIC VARIABLES = ',I6,'      NUMBER OF SPEC
     *IAL VARIABLES = ',I6)
```

```
      IF (NBVARS +NSVARS .EQ. NVARS)GO TO 3372
      PRINT 3345
 3345 FORMAT('     ERROR.  NVARS .NE. NBVARS + NSVARS ')
      STOP
 3372 IF (NSVARS .EQ. 0)GO TO 3380
      PRINT 3373
 3373 FORMAT('      SPECIAL VARIABLES FOLLOW')
      IFIRST = NBVARS + 1
      LAST = NBVARS + NSVARS
      DO 3378 I = IFIRST,LAST
      READ 3376,ICHAR2
 3376 FORMAT(13A6,A2)
      PRINT 3377,ICHAR2
 3377 FORMAT(10X,13A6,A2)
 3378 CONTINUE
 3380 READ 3,AA
      READ 3,VV
      PRINT 2,AA,VV
    2 FORMAT('     INITIAL CONCENTRATION =  ',F10.5,'   VOLUME = ',F10.5)
      READ 3,PN,PP,PM,P1,P2,P3,P4,P5,P12,P31,P13,VI,QT,VO,V11,V12,V13,
     1V2,V3,P30
    3 FORMAT()
      PRINT 4,PN,PP,PM,P1,P2,P3,P4,P5,P12,P31,P13,VI,QT,VO,V11,V12,
     1V13,V2,V3,P30
    4 FORMAT('      PN = ',F10.5,'  PP = ',F10.5,'   PM = ',F10.5,
     1  '  P1 = ',F10.5,'  P2 = ',F10.5,/,'  P3 = ',F10.5,
     2  '  P4 = ',F10.5,'  P5 = ',F10.5,' P12 = ',F10.5,
     3  '  P31 = ',F10.5,/,'P13 = ',F10.5,'  VI = ',F10.5,
     R  '  QT=  ',F10.5,'  VO=  ',F10.5,'  V11=  ',F10.5,/,
     S  '  V12 =  ',F10.5,'  V13 =  ',F10.5,'  V2 =  ',F10.5,
     T  '  V3 =  ',F10.5,/,'  P30 =  ',F10.5)
      READ 3,X
      PRINT 41,X
   41 FORMAT('  FEEDBACK CRITERION : X =  ',F10.5)

      READ 3,NEVENT
      PRINT 5,NEVENT
    5 FORMAT('       NUMBER OF EVENTS  = ',I5)
      IF (NEVENT .GT. 0 .AND. NEVENT .LE. MXEVNT)GO TO 6
      PRINT 3411
 3411 FORMAT(25X,'TOO MANY EVENTS OR NO EVENTS')
      STOP
    6 READ 3,(ITIME(I),I = 1,NEVENT)
      PRINT 7
    7 FORMAT(25X,'EVENT TIMES FOLLOW  ')
      PRINT 8,(ITIME(I),I = 1,NEVENT)
    8 FORMAT(10I10)
      READ 3,(DOSE(I),I = 1,NEVENT)
      PRINT 11,(DOSE(I),I = 1,NEVENT)
   11 FORMAT(25X,'DOSES FOLLOW')
      READ 3,(VOL(I),I = 1,NEVENT)
      PRINT 1101,(VOL(I),I = 1,NEVENT)
 1101 FORMAT(25X,'VOLUMES FOLLOW')
C
   20 READ 3,STEP,IPRSTP
      PRINT 21,STEP,IPRSTP
   21 FORMAT(10X,'STEP = ',F10.5,'  PRINT EVERY  ',I8,'  STEPS')
```

```
      HPRINT = STEP/2.0
      READ 3420,ISIZE,MODEPL
 3420 FORMAT(A6,4X,A6)
      PRINT 3422,ISIZE,MODEPL
 3422 FORMAT(5X,'GRAPH SIZE = ',A6,'   MODE = ',A6)
      READ 3,NMPLOT
      READ 3,(IPLOT(I),I = 1,NMPLOT)
      PRINT 22,NMPLOT,(IPLOT(I),I = 1,NMPLOT)
   22 FORMAT(10X,'PLOT ',I5,'  VARIABLES ',/10X,'NUMBERED  ',15I5)
      IF (NMPLOT .LE. NVARSP)GO TO 23
      PRINT 3457
 3457 FORMAT(25X,'NMPLOT TO LARGE!')
      STOP
   23 LASTCL = NMPLOT + 1
      DO 24 I = 1,NMPLOT
      K = IPLOT(I)
      IPLNAM(I) = NAMVAR(K)
   24 CONTINUE
      READ 3,(ISCAL(I),I = 1,NMPLOT)
      PRINT 25
   25 FORMAT(//,25X,'PLOTTING SCALE FACTORS FOLLOW ')
      PRINT 3390,(IPLNAM(I),ISCAL(I),I = 1,NMPLOT)
 3390 FORMAT(4(3X,A6,'  = ',I5))
      READ 3,NMITGT
      IF (NMITGT .LE. NVARSP)GO TO 3392
      PRINT 3388,NMITGT
 3388 FORMAT(10X,'NMITGT TOO LARGE,  NMITGT = ',I5)
 3392 READ 3,(ITGRIX(K),K = 1,NMITGT)
      DO 26 I = 1,NMITGT
      K = ITGRIX(I)
      ITGVAR(I) = NAMVAR(K)
   26 CONTINUE
      PRINT 27
   27 FORMAT('          INTEGRATE THE FOLLOWING VARIABLES')
      PRINT 3337,(ITGVAR(K),K = 1,NMITGT)
      PRINT 28
   28 FORMAT(10X,'THEIR NUMBERS ARE ')
      PRINT  8,(ITGRIX(K),K = 1,NMITGT)
      READ 3,IOUT
      PRINT 31,IOUT
   31 FORMAT(10X,'DE OUTPUT OPTION = ',I5)
      READ 3,EPS,ERLEVL
      PRINT 33,EPS,ERLEVL
   33 FORMAT('    EPS = ',E18.8,' ERLEVL = ',E18.8)
C
C     ***** SET INITIAL CONDITIONS *****
   50 DO 55 I = 1,NVARS
      YINIT(I) = 0.0
   55 CONTINUE
      DO 53 I = 1,NVARS
      VALACC(I) = 0.0
   53 CONTINUE
      YINIT(1) = VV
      YINIT(2) = AA
      IPNTR2 = 1
      NUMPTS = 0
      T2 = 0.0
```

```
      CALL INTGRT(NPOINT,HPRINT,SAVE(1,IX),VALINT(J))
      VALACC(J) = VALACC(J) + VALINT(J)
   86 CONTINUE
      PRINT 87,HPRINT
   87 FORMAT(10X,'INTEGRALS FOLLOW.  HPRINT = ',F10.5)
      PRINT 88,(ITGVAR(K),VALINT(K),VALACC(K),K = 1,NMITGT)
   88 FORMAT(3X,A6,'  = ',F10.5,'  ACCUMULATED INTEGRAL = ',F10.5)
C     ***** COPY POINTS TO BE GRAPHED FROM ARRAY SAVE TO ARRAY SAVE2 ****
C
C     ***** DOUBLE PRINT INTERVAL BECAUSE HPRINT = .5 * STEP *****
      INCRMT = IPRSTP + IPRSTP
   90 NUMPTS = NUMPTS + 1
      IF (NUMPTS .LE. ISVDM3)GO TO 91
      PRINT 3441
 3441 FORMAT(25X,'TOO MANY PRINTING POINTS')
      STOP
   91 SAVE2(NUMPTS,1) = SAVE(IPNTR2,1)
      DO 92 L = 1,NMPLOT
      JJ = IPLOT(L) + 1
      SAVE2(NUMPTS,L+1) = SAVE(IPNTR2,JJ)
   92 CONTINUE
      IPNTR2 = IPNTR2 + INCRMT
      IF (IPNTR2 .LE. NPOINT)GO TO 90
C
C     ***** ADJUST POINTER FOR NEXT TIME INTERVAL *****
      IPNTR2 = IPNTR2 - NPOINT + 1
  200 CONTINUE
      PRINT 230,NUMPTS
  230 FORMAT(25X,'NUMPTS = ',I5)
      PRINT 234
  234 FORMAT(//)
      PRINT 237
  237 FORMAT(25X,'RESULTS FOLLOW')
      PRINT 238,(IPLNAM(K),K = 1,NMPLOT)
  238 FORMAT('        TIME     ',13(2X,A5,2X))
      DO 250 I = 1,NUMPTS
      PRINT 235,(SAVE2(I,K),K = 1,LASTCL)
  235 FORMAT(F10.5,2F9.5,10F11.7)
  250 CONTINUE
      DO 270 I = 1,NUMPTS
      TEMP(I) = FLOAT(ISCAL(1)) * SAVE2(I,2)
  270 CONTINUE
      NPTS = NUMPTS
      IF (MODEPL .EQ. 'POINT')NPTS = -NPTS
      CALL GRAPH2(SAVE2(1,1),'R', TEMP,'R',NPTS,ISIZE,'AUTO',
     1 'DRUG CONCENTRATIONS IN THE EYE..',
     2 '   TIME..','   CONCENTRATION..',IPLNAM(1))
      IF (NMPLOT .LT. 2)GO TO 300
      DO 285 J = 2,NMPLOT
      DO 284 I = 1,NUMPTS
      TEMP(I) = FLOAT(ISCAL(J)) * SAVE2(I,J+1)
  284 CONTINUE
      CALL GPPH2V(SAVE2(1,1),'R', TEMP,'R',NPTS,'SAME',IPLNAM(J))
  285 CONTINUE
  300 CALL GRPHND
      STOP
  600 PRINT 601,NOTIFY
```

```
C       ***** BEGIN SIMULATION*****
        DO 200 IP = 1,NEVENT
        IL = IP - 1
C       ***** SET INITIAL TIME FOR THIS INTERVAL *****
        T1 = T2
C
C       ***** SET FINAL TIME FOR THIS INTERVAL *****
        T2 = FLOAT(ITIME(IP))
        IF (IP .EQ. 1)GO TO 70
C       ***** COPY FINAL CONCENTRATIONS FROM PREVIOUS INTERVAL*****
        DO 60 I = 1,NVARS
        YINIT(I) = SAVE(NPOINT,I+1)
   60 CONTINUE
C
C       ***** ADD NEW DOSE OR REMOVE DRUG OR LEAVE ALONE *****
        IF (DOSE(IL) .EQ. 0.0)GO TO 70
        IF (DOSE(IL) .GT. 0.0)GO TO 65
        IF (DOSE(IL) .EQ. -5.0 .AND. ICASE .EQ. 'REMOVE')GO TO 62
        PRINT 61,IL,DOSE(IL),ICASE
   61 FORMAT(25X,'DOSE NUMBER ',I8,' IS ILLEGAL.  IT = ',F10.5,'  ICASE
     1 = ',I5)
        STOP
C       ***** REMOVE DRUG *****
   62 YINIT(1) = VO
        YINIT(2) = 0.0
        GO TO 70
C       ***** ADD NEW DOSE *****
   65 YINIT(2) = (YINIT(2) * YINIT(1) + DOSE(IL) * VOL(IL))
     1  / (YINIT(1) + VOL(IL))
        YINIT(1) = YINIT(1) + VOL(IL)
        VI = YINIT(1) - VO
        GO TO 70
C
C       ***** CALL DIFFERENTIAL EQUATIONS ROUTINE *****
   70 CALL DEPC(NBVARS,T1,YINIT,T2,YFINAL,DERIVS,EPS,ERLEVL,HMIN,HMAX,
     1 HSTART,IOUT,HPRINT,'DEPC OUTPUT..',SAVE,ISVDM1,NVARS + 1,NPOINT,
     2 NOTIFY,3600)
   74 PRINT 75,T1,T2,ITIME(IP)
   75 FORMAT(10X,'FINISHED INTERVAL FROM TIME ',F10.5,'  TO ',F10.5,
     1  5X,'ITIME = ',I5)
        PRINT 78
   78 FORMAT(25X,'TIME VALUES USED  ')
        PRINT 79,(SAVE(K,1),K = 1,NPOINT)
   79 FORMAT(8(F12.7,1X))
        PRINT 80
   80 FORMAT(//10X,'SOLUTION VALUES FOLLOW')
        PRINT 79,(YFINAL(K),K = 1,NVARS)
        DO 85 J = 1,NVARS
        PRINT 81,NAMVAR(J)
   81 FORMAT(10X,A6,'   VALUES')
        JP1 = J + 1
        PRINT 79,(SAVE(K,JP1),K = 1,NPOINT)
   85 CONTINUE
C
C       ***** COMPUTE THE INTEGRAL OVER THAT INTERVAL *****
        DO 86 J = 1,NMITGT
        IX = ITGRIX(J) + 1
```

```
  601 FORMAT('  DE FAILURE , NOTIFY = ',I8)
      END
2(1),DERIVS
      SUBROUTINE DERIVS(T,Y,DY,STORE,ITEST)
      PARAMETER NAVARP = 6,NCMPSB = 6
      DIMENSION DY(1),Y(1),STORE(1)
      COMMON PN,PP,PM,P1,P2,P3,P4,P5,VI,QT,VO,V11,V12,V2,NVARS,NBVARS,
     1  NAVARS,NSVARS,NCOMPN(NCMPSB),ICASE,IVCOMP(NAVARP,NCMPSB),
     2  P12,P31,P13,V13,V3,V4,X,P30
      V4=V12+V2
      DY(1)=-(P4+P5*VI)*(Y(1)-VO)
      DY(2) =-(PP+PN+QT/Y(1))*Y(2)+P3*(V11/Y(1))*Y(3)
      DY(3)=PP*(Y(1)/V11)*Y(2)-(P1+P3+PM)*Y(3)
      IF(NBVARS .GT. 5)GO TO 5
C  RESERVOIR REPRESENTS IRIS, LENS AND CILIARY BODY
C  MODEL A RESERVOIR PRESENT
      DY(4)=P1*(V11/V4)*Y(3)+P13*(V3/V4)*Y(5)-(P2+P31)*Y(4)
      DY(5)=P31*(V4/V3)*Y(4)-(P13+P30)*Y(5)
      B = X*Y(3)
      C = Y(4)
      IF(C  .GT.  B)P1 = P2
      GO TO 8
C  MODEL B 'BOWMAN MEMBRANE AND RESERVOIR PRESENT'
    5 DY(4)=P1*(V11/V13)*Y(3)-P12*Y(4)
      DY(5)=P12*(V13/V4)*Y(4)+P13*(V3/V4)*Y(6)-(P2+P31)*Y(5)
      DY(6)=P31*(V4/V3)*Y(5)-P13*Y(6)
    8 STORE(1) = T
      DO 10 K = 1, NBVARS
      STORE(K+1) = Y(K)
   10 CONTINUE
   35 IF (NSVARS .EQ. 0)RETURN
C     ***** SET IX TO INDEX OF FIRST SPECIAL VARIABLE T *****
      IX = NBVARS + 2
C     ****** SET T = A * V *****
      STORE(IX) = Y(1) * Y(2)
      IX = IX + 1
C     ***** SET K = (B*V11 + E*V13 + C*V12)/(V11 + V12 + V13 )
      IF(NBVARS .LE. 5)STORE(IX)=(Y(3)*V11+Y(4)*V12)/(V11+V12)
      IF (NBVARS .GE. 6)STORE(IX)=(Y(3)*V11+Y(4)*V13+Y(5)*V12)/(V11+V12
     1+V13)
      RETURN
      END
2(1),INTGRT
      SUBROUTINE INTGRT(NP,STEP,Y,ANS)
C     ***** INTGRT USES THE COMPOSITE SIMPSON RULE TO INTEGRATE OVER THE
C           VALUES Y(1),Y(2),...,Y(NP).  Y IS THE FUNCTION AND STEP IS THE
C           INTERVAL BETWEEN THEM. ANS IS SET TO THE VALUE OF THE COMPUTED
C           VALUE OF THE INTEGRAL *****
      DOUBLE PRECISION ANS,SUM
      DIMENSION Y(NP)
      IF (NP .GE. 3 .AND. NP/2 + NP/2 .NE. NP)GO TO 10
      PRINT 5,NP
    5 FORMAT(25X,'NP .LT. 3 OR NP IS EVEN,  NP = ',I5)
      CALL UPWALK('INTGRT',1)
      RETURN
```

```
   10 SUM = DBLE(Y(1)) + DBLE(Y(NP))
      NPM1 = NP - 1
      DO 20 K = 2,NPM1,2
      SUM = SUM + 4.0D0 * DBLE(Y(K))
   20 CONTINUE
      NPM2 = NP - 2
      IF (NPM2 .LT. 3)GO TO 50
      DO 30 K = 3,NPM2,2
      SUM = SUM + 2.0D0 * DBLE(Y(K))
   30 CONTINUE
C
   50 ANS = DBLE(STEP) * SUM/3.0D0
      RETURN
      END
```