of I and lesser amounts of conjugates were found in the mouse feces at all intervals of sampling. No significant amount of II was obtained from the feces.

Radioassay results from the blood, urine, and feces generally correlated well with those from the electron-capture GLC procedure. Radioassays of the residual substrates (after hydrolysis and extraction), column adsorbents, and discarded solvents indicated that essentially all radioactivity had been extracted from the samples and that losses during the analytical procedure were negligible.

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Mechanistic Studies on Transcorneal Permeation of Pilocarpine

JAMES W. SIEG and JOSEPH R. ROBINSON [x]

Abstract □ The mechanism of corneal pilocarpine penetration was studied in the albino rabbit using radiochemical techniques. The apparent rate and extent of pilocarpine accumulation in the aqueous humor and the various cell layers of the cornea were determined for both intact and abraded eyes. For the first time, drug levels were monitored in the epithelium and stroma-endothelium of the intact cornea using a tissue-scraping technique. In addition, a new postinstillation rinsing method was devised to evaluate the rate of corneal uptake. The results demonstrate a dual role for the corneal epithelium, both as a barrier to drug penetration and as a reservoir for drug in the intact cornea. The transcorneal pilocarpine flux is slower than the data appear to indicate, and previous overestimates of the apparent absorption rate constant are due to parallel elimination processes occurring at the absorption site. Pharmacokinetic parameters were determined for each tissue to generate an overall mechanism for corneal permeation.

Keyphrases □ Pilocarpine—mechanism of corneal penetration, rate and extent of accumulation in aqueous humor and various cell layers, rabbits □ Corneal penetration mechanism—pilocarpine, rate and extent of accumulation in aqueous humor and various cell layers, rabbits □ Ophthalmic cholinergic agents—pilocarpine, mechanism of corneal penetration, rabbits

The cornea is an easily accessible tissue of the body. However, because of its small size and great degree of specialization, studies on corneal drug transport have been limited, with the net result that the understanding of transcorneal permeation is sketchy. Studies of inorganic ion movement have been fairly extensive (1) due to their great physiological importance to the cornea, and these ions are transported primarily through specialized transport systems associated with the epithelial potential. A relatively large number of studies also have been performed using organic molecules, both drug and nondrug, as recently described (2). However, these studies have been generally restricted to simple quantitation of drug penetration to a specific target tissue, often the aqueous humor, without elucidating the mechanism of corneal permeation. The experimental design in most of these studies does not permit anything but qualitative speculation regarding corneal penetration. The present study examines the corneal kinetics of pilocarpine in an effort to ascertain its transcorneal mechanism.

BACKGROUND

The prevailing theories on corneal penetration of pilocarpine often include, all or in part, a number of principal speculations: (*a*) the existence of a barrier to penetration in the lipophilic corneal epithelium, (*b*) rapid uptake and transport of pilocarpine by the cornea, (*c*) controlled release of pilocarpine to the anterior chamber by the endothelium, and (*d*) the presence of a depot, or reservoir, of pilocarpine somewhere in the cornea.

For most drugs, the existence of a barrier in the corneal epithelium is well documented (2, 3). This barrier is often considered to vary in magnitude according to the solubility character of the drug. A very water-soluble drug is unable to penetrate this barrier, and a very lipid-soluble drug penetrates it easily but is unable to leave; therefore, some degree of solubility in both aqueous and lipid media is deemed desirable for optimum transcorneal permeation (4, 5). Recently, the location of this barrier function was specifically attributed to the outermost cell layer of the epithelium (6, 7), and the sensitivity of this barrier to surfactants has been carefully studied (8–10).

The premise that pilocarpine has a rapid corneal penetration rate has been professed by a number of investigators whose conclusions were derived from both *in vitro* (11, 12) and *in vivo* (13) data. The basis for this assumption is usually the observation that pilocarpine exhibits early peak times in the target tissues. In addition, the flux of pilocarpine from the cornea to the anterior chamber has been attributed to some property, as yet undisclosed, of the corneal endothelium (12).

The speculation that pilocarpine accumulates somewhere in the corneal tissues arises from analogy to early studies with fluorescein (14, 15) and accurate determinations of aqueous humor drug dynamics after dosing with pilocarpine (16). In the latter case, the elimination rate constant for pilocarpine from the anterior chamber was significantly smaller than the normal aqueous humor turnover rate, and the presence of a depot in the cornea was offered as an explanation.

EXPERIMENTAL

Materials—Tritiated pilocarpine alkaloid, specific activity of 4.1 Ci/mmole, was obtained commercially[1] and purified by vacuum distillation (17). Purification of the pilocarpine was carried out immediately prior to each experimental run to minimize tritium exchange. All other chemicals were either reagent or analytical grade and were used as received.

Male albino rabbits[2], 1.8–2.4 kg, were used. Lighting was maintained on a 24-hr basis in the caging facilities. The animals were fed a regular diet, with no restrictions on the amount of food or water consumed.

Preparation of Pilocarpine Solutions—Drug solutions were prepared by addition of a solution of 10^{-2} *M* pilocarpine to the purified labeled material. The standard buffer solution was isotonic Sorensen's buffer, pH 6.24. It was determined that 0.25 mCi of tritiated pilocarpine/ml of final solution was sufficient to ensure good counting efficiency throughout the studies, *i.e.*, 150,000–175,000 cpm/μl. The small amount of labeled material did not alter significantly the molarity of the final solutions.

Removal of Corneal Epithelium—To facilitate removal of the corneal epithelium, 2 drops of a 0.5% tetracaine hydrochloride solution[3] were instilled into the eyes of test animals. Five minutes later the eye was proptosed, and the epithelial layer was removed *in toto* by scraping with a scalpel. The eye was then blotted with moist tissue to remove any loose fragments of epithelium from the precorneal area.

Aqueous Humor Drug Concentration–Time Profiles—*Pilocarpine Solutions*—The basic experimental techniques used were presented previously (18, 19). Unanesthetized animals were used, and a standard 25-μl dose was instilled.

Rinsing Studies—At various specified times following dosing, the eyes were thoroughly flushed with isotonic saline injection containing no preservatives. A polyethylene wash bottle with a fine-drawn tip was used so that a stream of saline could be directed into all precorneal parts of the eye to ensure complete removal of the instilled dose. The eyes were then left untouched, and no changes in the basic routine were made beyond the time of rinsing with saline.

Corneal Drug Concentration–Time Profiles—Corneal samples were taken immediately after the aqueous humor was aspirated from the anterior chamber. The eyes were proptosed and secured with a hemostat behind the globe. The tip of a scalpel was then inserted at the corneal margin, and the entire cornea was excised. Each corneal sample was carefully rinsed in saline and blotted on tissue. The corneas were then placed into tared combustion cones[4], and the wet weight was determined using an analytical balance. A tissue oxidizer[5] was used to burn each cornea, and samples were counted in plastic vials[6] using a commercial liquid scintillation solution[7] and scintillation spectrometer[8]. The final count for each sample then was converted to micrograms of pilocarpine per gram of cornea.

The only changes in this basic corneal sampling technique occurred in runs in which the corneal epithelium was removed just prior to taking the corneal samples. In these instances, the animals were sacrificed in the usual manner and the eyes were proptosed. A scalpel was used to scrape away the epithelium carefully. The aqueous humor was then withdrawn, and the corneal samples were removed and analyzed using the described techniques.

Throughout the studies, the surgical procedures were carried out as quickly as possible to minimize redistribution of drug during the time required to obtain samples. This precaution was especially important for very early sample times, and the eye was routinely rinsed with saline immediately at the end of the sampling interval as an added precaution against further influx of drug from the precorneal area. While some error may have been introduced due to the time required for sampling (usually less than 1 min), every effort was made to keep this source of error to a minimum.

Crossover Study to Determine Systemic Contribution to Aqueous Humor Pilocarpine—Drainage of the instilled solution into the nasolacrimal duct presents the possibility for systemic absorption of pilocarpine. A crossover study was performed to verify that all pilocarpine detected in the aqueous humor came from transcorneal penetration and was not due in part to systemic circulation. Rabbits were dosed only in the left eyes with pilocarpine solution. Aqueous humor samples were then taken from the anterior chamber of both eyes at 20, 60, and 120 min after instillation. No detectable pilocarpine was found in samples from the right eyes. On this basis, entry of pilocarpine into the anterior chamber *via* systemic circulation was discounted.

Analysis for Tritium Exchange in the Samples—A portion of the tritium label on the isotopic species was labile to exchange with water. Although careful solution purification procedures were used, there was concern that exchange could occur in the ocular tissues after dosing. This exchange could lead to misinterpretation of results due to rapid passage of tritiated water through the cornea (17). To discount this factor, corneal and aqueous humor samples were taken after dosing with purified tritiated pilocarpine solution. Corneal samples were cut into two pieces, weighed, and labeled A and B. Likewise, aqueous humor samples were divided into two 50-μl portions.

In each case, the A portion was immediately burned as a wet sample using the tissue oxidizer. The B samples were first desiccated and similarly treated. Any tritiated water present in the tissues would be expected to volatilize from the dried samples. The results showed no differences in activity between the A and B portions of each sample, indicating that the tritium label remained as a nonvolatile entity during passage through the cornea.

Table I—Concentration of Pilocarpine in Aqueous Humor following Topical Application of a 10^{-2} *M* Solution in Intact and Abraded Eyes

Minutes	Number of Eyes	Micrograms per Milliliter of Aqueous Humor
	Intact Eyes	
0.5	12	0.025 (0.001)[a]
5	18	0.60 (0.02)
10	36	0.85 (0.04)
20	36	1.06 (0.05)
30	28	0.95 (0.05)
60	32	0.52 (0.04)
90	36	0.33 (0.03)
120	32	0.20 (0.02)
	Abraded Eyes	
0.5	6	2.57 (0.17)
1	6	4.32 (0.49)
5	10	8.44 (0.61)
10	12	7.06 (0.53)
20	10	7.36 (0.53)
30	12	4.24 (0.46)
60	14	3.21 (0.60)
90	8	1.20 (0.13)
120	14	0.78 (0.07)

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

RESULTS

Aqueous Humor Drug Levels following Dosing with Pilocarpine Solution—Aqueous humor drug levels were determined at various times following topical application of pilocarpine nitrate solution (Table I). The data indicate that pilocarpine penetrated the intact cornea and reached an apparent peak concentration in the aqueous humor within 20 min, in agreement with earlier work (17, 19). Immediately following this peak concentration, the level of pilocarpine in the aqueous humor began to decline. One-compartment pharmacokinetic computer analysis of the data gave an apparent transcorneal flux rate constant of 0.08 min^{-1} and an apparent elimination rate constant of 0.017 min^{-1}.

When the corneal epithelium was removed prior to dosing, both the rate and extent to which pilocarpine penetrated to the anterior chamber increased. A comparison of the aqueous humor concentration–time profiles for intact and abraded corneas can be made (Fig. 1). Removal of the epithelium produced a seven- to eightfold increase

[1] New England Nuclear, Boston, Mass.
[2] Klubertanz, Edgerton, Wis.
[3] Pontocaine, Winthrop Laboratories, Division of Sterling Drug Inc., New York, NY 10016
[4] Combusto-Cone, Packard Instrument Co., Downers Grove, Ill.
[5] Model 306, Packard Instrument Co., Downers Grove, Ill.
[6] The Vial, Research Products International Corp., Elk Grove Village, Ill.
[7] Monophase-40, Packard Instrument Co., Downers Grove, Ill.
[8] Model 2002, Packard Instrument Co., Downers Grove, Ill.

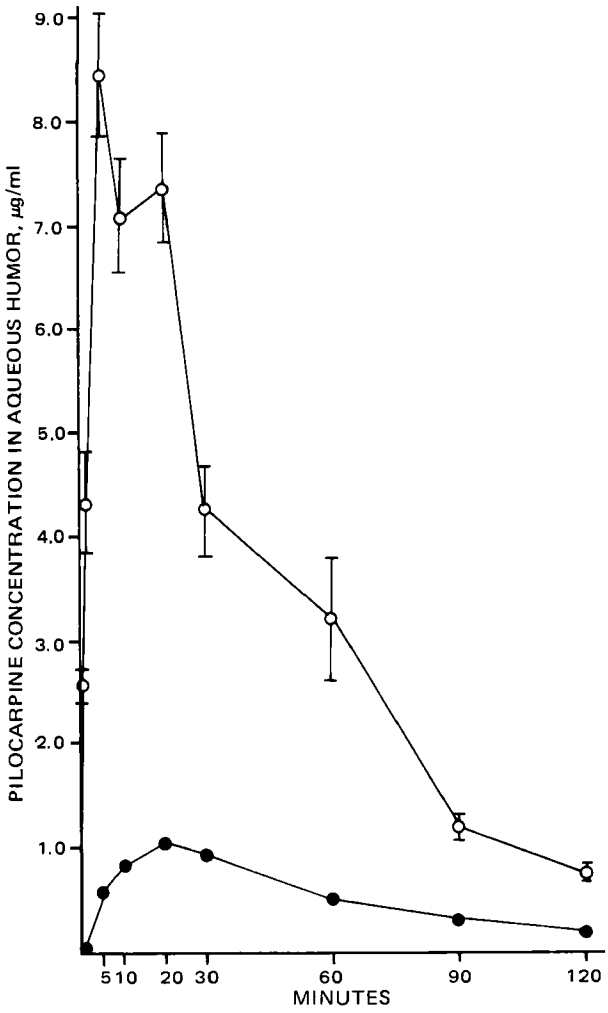


Figure 1—*Aqueous humor drug concentration after topical dosing with pilocarpine solution. Key: ●, intact eyes; and ○, abraded eyes. Error bars represent the standard error of the mean. Where no error bar is indicated, the standard error of the mean is smaller than the size of the symbol used.*

in aqueous humor levels. In addition, the drug reached a maximum level by at least 5 min. This decrease in the time required to reach a peak aqueous humor concentration suggests a change in the rate constant for transcorneal flux when the epithelium is removed. The rate constant for elimination of pilocarpine from the aqueous humor was the same as that observed for intact eyes.

Corneal Concentration of Pilocarpine following Dosing with 10^{-2} *M* Solution—The corneal pilocarpine levels for intact and abraded eyes are presented in Table II. In the intact eye, drug reached an apparent peak concentration within 5 min. If the epithelium was removed prior to dosing, the amount of pilocarpine entering the cornea markedly increased and the peak time decreased to 1 min (Fig. 2).

Analysis of whole cornea samples can only give a measure of the total amount of drug, without any indication of its distribution in the various cellular layers. To use such information, homogeneity of the cornea and uniform drug distribution must be assumed. This shortcoming is severe if an accurate mechanistic interpretation is to be attempted. The corneal epithelium has long been considered a major barrier to the penetration of drugs into and through the cornea. If this structure is to be implicated in the transcorneal mechanism for pilocarpine, the disposition of drug in the intact cornea must be determined. The current studies provide an accurate simple procedure for determining stromal and epithelial drug levels as a function of time.

The distribution of pilocarpine in the cornea at any specified time can be determined by scraping away the epithelium immediately before the corneal sample is excised. Analysis of the samples gives the

Table II—Concentration of Pilocarpine in the Cornea following Topical Application of a 10^{-2} *M* Solution

Minutes	Number of Eyes	Micrograms per Gram of Cornea
Corneas with Intact Epithelium[a]		
0.5	12	1.91 (0.20)[b]
2	12	4.31 (0.24)
5	12	5.50 (0.63)
10	10	4.77 (0.41)
20	12	3.07 (0.39)
30	10	2.72 (0.22)
60	10	0.83 (0.09)
90	12	0.50 (0.05)
120	12	0.29 (0.03)
Corneas with Epithelium Removed prior to Dosing[c]		
0.5	6	19.70 (0.98)
1	6	31.82 (1.48)
5	6	13.99 (0.69)
10	6	7.66 (1.32)
20	6	7.49 (1.37)
30	6	4.16 (0.79)
60	6	3.58 (0.57)
120	6	0.83 (0.22)
Corneas with Epithelium Removed just prior to Taking Sample[c] (Stroma-Endothelium)		
2	6	0.23 (0.03)
5	6	0.53 (0.05)
10	6	0.81 (0.08)
20	6	1.14 (0.15)
30	6	0.89 (0.11)
60	6	0.32 (0.04)
90	6	0.19 (0.08)
120	6	0.09 (0.01)
Calculated Concentration in Epithelium		
2	6	29.4
5	6	35.8
10	6	28.5
20	6	13.9
30	6	13.2
60	6	3.7
90	6	2.2
120	6	1.4

[a] Average wet weight of intact corneas was 46.1 ± 0.64 mg. [b] Numbers in parentheses represent standard error of the mean. [c] Average wet weight of corneas with epithelium removed was 39.7 ± 0.53 mg.

stroma-endothelium drug concentration of the intact cornea, since the epithelium is removed after dosing. The epithelial concentration can be determined simply by comparing the stroma-endothelium data to the data obtained for the whole intact cornea. This amount is calculated by determining the difference between the two quantities at each time point and then applying appropriate corrections for the weights of the individual tissues. The results are presented in Table II, and a graphical representation of the relative disposition of pilocarpine in the intact cornea is shown in Fig. 3.

It is clear from the data that pilocarpine concentrates in the epithelium. At very early times in the profile, up to 80% of the total pilocarpine content of the cornea was in the epithelium. This percentage decreased as the epithelium remained in contact with the stroma for longer periods. However, even up to 2 hr, the epithelium retained the major fraction of drug, indicating a reservoir and controlled release of pilocarpine to the underlying layers[9]. It is evident from these corneal studies that the epithelium plays a dual role in the mechanism for the transcorneal flux of pilocarpine, both as a barrier to penetration and as a reservoir for drug in the intact cornea.

Effect of Postinstillation Rinsing on Aqueous Humor Levels of Pilocarpine—The amount of drug ultimately reaching the anterior chamber is determined by the net result of two competitive processes: the rate of drug loss from the precorneal area and the rate of drug uptake by the cornea. The precorneal disposition of pilocarpine was extensively detailed previously (20–22). By manipulating this si-

[9] Preliminary results from this laboratory also show that scraping the endothelium does not remove a significant amount of pilocarpine, indicating no buildup of drug in this single layer of cells.

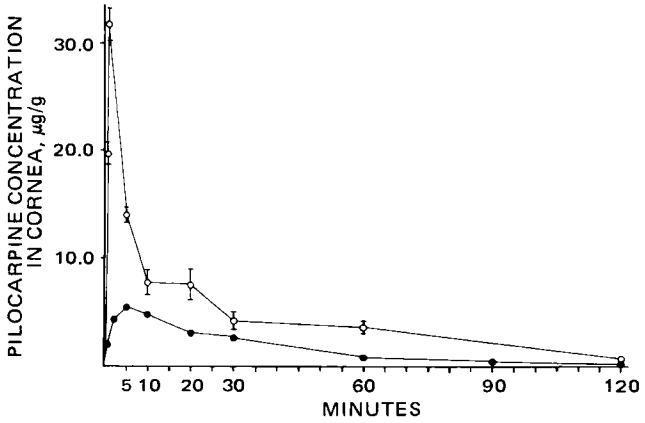


Figure 2—*Corneal drug concentration after topical dosing with pilocarpine solution. Key: ●, corneas with intact epithelium; and ○, corneas with epithelium removed prior to dosing (abraded eyes). Error bars represent the standard error of the mean. Where no error bar is indicated, the standard error of the mean is smaller than the size of the symbol used.*

multaneous process, it is possible to gain insight into the nature of transcorneal flux. Removal of drug from the precorneal area can be accelerated by rinsing the eye with saline. The changes in aqueous humor drug levels produced by varying the rinse time may then be related to the apparent rate of corneal uptake (Fig. 4).

The data in Fig. 4 show that rinsing at various times during the first 5 min caused a reduction in the amount of pilocarpine reaching the anterior chamber. Rinsing beyond 5 min did not alter the aqueous humor drug levels as compared with the nonrinsed data. This result reflects the combined influence of normal drainage, tear turnover, and nonproductive absorption and is not surprising in view of previous drainage studies (20).

The area under the curve for each rinse time was determined by graphical methods and normalized to the nonrinsed data. The percent reduction in area was then calculated and plotted (Fig. 5). The log-linear character is due to the nature of transcorneal flux since the calculated quantities are derived from measured differences between first-order processes. The slope of this line gives the change in aqueous humor pilocarpine bioavailability as a function of instilled solution contact time and, therefore, should be directly related to the apparent rate of corneal uptake. First-order kinetic analysis gave an apparent corneal uptake rate constant of 0.57 min^{-1}.

DISCUSSION

Precorneal Disposition of Pilocarpine—When pilocarpine solution was topically applied to intact eyes, the drug reached a peak concentration in the cornea within 5 min and in the aqueous humor within 20 min (Figs. 1 and 2). At first glance, these results suggest a rapid penetration rate. Similar results with pilocarpine solutions led previous investigators to this conclusion (11–13). However, it is a matter for discussion whether these early peak times are truly a reflection of a large absorption rate constant for pilocarpine.

Normal drainage of an instilled dose of solution is a function of the dosing volume and occurs immediately after instillation (20). This drainage process is essentially completed within 5 min, at which time the volume of fluid present in the precorneal area quickly returns to the normal resident volume of 7.5 μl. The rapid loss of a large volume of the dose, and a large amount of the drug, causes the precorneal drug concentration to become directly susceptible to the dilution effect of incoming tears. Loss of drug through scleral and conjunctival absorption and, to a lesser extent, *via* corneal absorption also has a direct influence (21, 22).

The precorneal drug concentration establishes a representative concentration at the epithelial surface and thus serves as the driving force for drug uptake by the cornea. It can be estimated, allowing for dilution by tears, that the initial concentration of pilocarpine in the precorneal area after instillation of 25 μl of a 10^{-2} *M* solution is about 1.6 mg/ml. The amount of drug required to establish the epithelial concentration is very small and is governed by the surface area of the

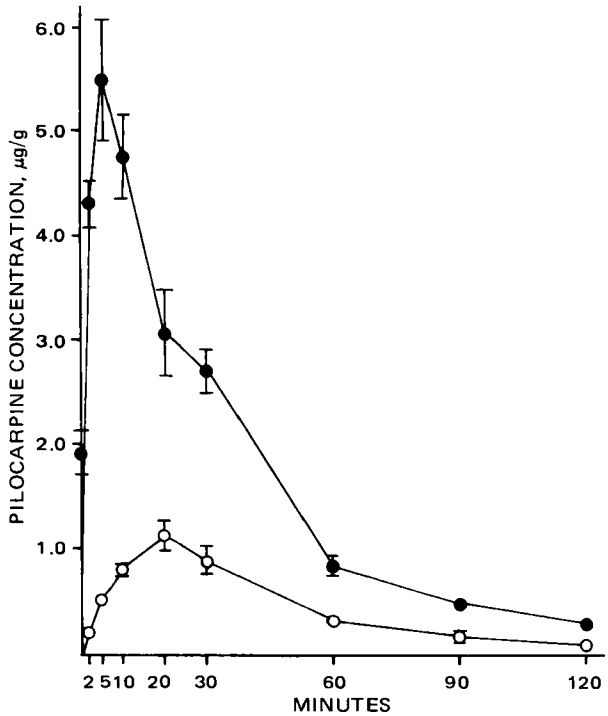


Figure 3—*Corneal drug concentration after topical dosing with pilocarpine solution. Key: ●, normal intact corneas; and ○, corneas with epithelium removed just prior to taking sample (stroma-endothelium concentration). Error bars represent the standard error of the mean. Where no error bar is indicated, the standard error of the mean is smaller than the size of the symbol used.*

epithelium. In addition, localization of drug in the outermost epithelial cells or in the bound fluid layer at short times after dosing means that a high effective concentration is achieved quickly and easily. This concentration would be much higher than the maximum bulk epithelial concentration calculated from the results of the present studies (~35 μg/g) suggests. Flux of drug into the cornea ceases as soon as the precorneal concentration falls below the epithelial surface concentration.

Studies with fluorescein (23) showed that the lacrimal fluid takes some time to equilibrate with the instilled dose; as mixing takes place, the dye concentration quickly drops to a level corresponding to only about one-fourth that of the instilled drop. The concentration in the lacrimal fluid may decrease by half as often as every 3 min. The net result is that only a small fraction of the instilled dose is absorbed and

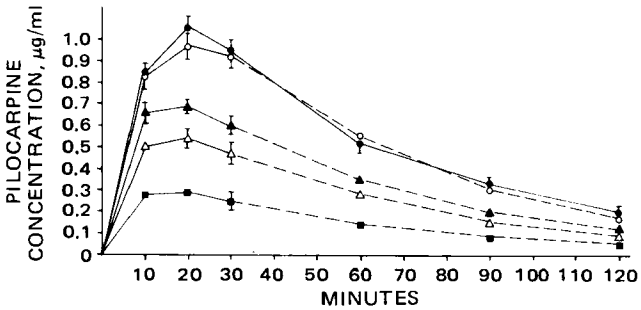
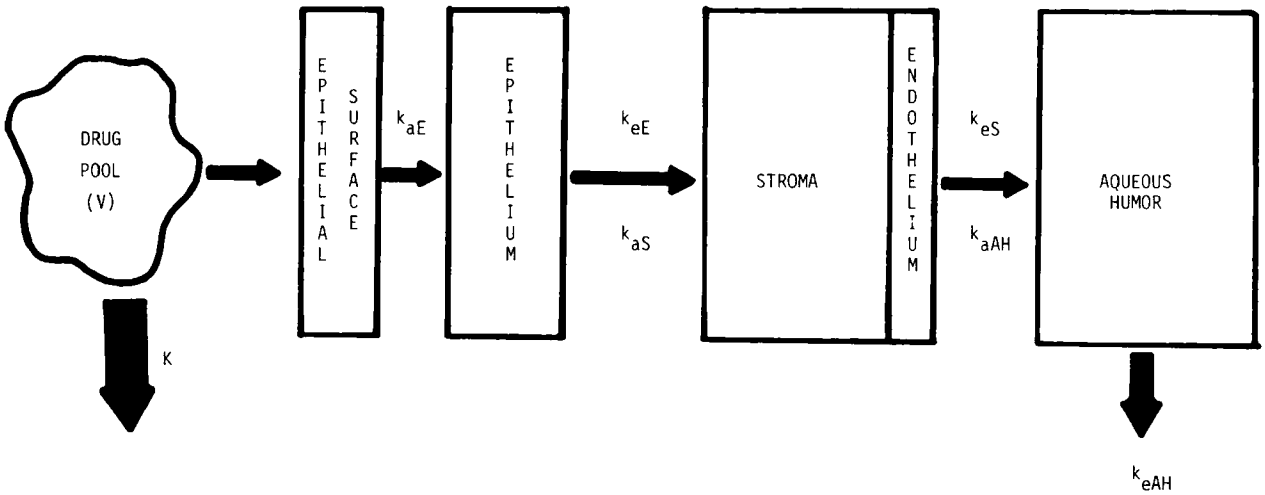


Figure 4—*Aqueous humor pilocarpine levels following rinsing with saline at various times after dosing. The horizontal axis gives the time after dosing at which drug was completely rinsed from the precorneal area. Key: ●, no rinsing; ○, 5-min rinse; ▲, 2-min rinse; △, 1-min rinse; and ■, 30-sec rinse. Solid lines connect actual data points. Dotted lines were calculated using an aqueous humor elimination rate constant of $K_e = 0.017\ min^{-1}$. Error bars represent the standard error of the mean. Where no error bar is indicated, the standard error of the mean is smaller than the size of the symbol used.*



Scheme I—Representation of transcorneal pilocarpine permeation: V = *volume of drug pool after instillation of dose,* K = *composite elimination step,* k_{aE} = *apparent absorption rate constant into epithelium,* k_{eE} = *apparent elimination rate constant from epithelium,* k_{aS} = *apparent absorption rate constant into stroma-endothelium,* k_{eS} = *apparent elimination rate constant from stroma-endothelium,* k_{aAH} = *apparent absorption rate constant into aqueous humor, and* k_{eAH} = *apparent elimination rate constant from aqueous humor.*

the peak time in the cornea appears very early, regardless of the true absorption rate constant of the drug.

The early peak time and small fraction of dose absorbed also cause an overestimate of the apparent absorption rate constant as calculated by usual pharmacokinetic methods (24, 25), and the degree of overestimation increases as the bioavailability of the drug decreases. This process was reported previously for plasma drug concentration–time data (26–28) and was recently discussed as being applicable to the eye (29).

The peak time for any drug in the cornea after topical dosing should be considered to be a false peak, generated by the parallel elimination step rather than by absorption. This will generally be true except in cases involving corneal perfusion studies and sustained-release processes where the precorneal drug concentration remains constant long enough to generate the real pharmacokinetic parameters[10]. Therefore, it can be predicted that topical solutions of most drugs will have early peak times in ocular tissues and relatively large, and somewhat similar, apparent absorption rate constants. This prediction is supported by data from studies with compounds having very low water solubility, such as steroids, where the apparent pharmacokinetic parameters are strikingly similar to those of pilocarpine (18). This aspect of transcorneal drug movement must be recognized in any mechanistic interpretation or clinical evaluation of ocular data.

The data presented in Fig. 5 also suggest an extremely rapid rate of pilocarpine uptake by the cornea. Even when the dose is completely rinsed away after a contact time of only 30 sec, a measurable amount of pilocarpine still penetrates to the anterior chamber. This finding might lead one to postulate a rapid binding mechanism of the drug to the cornea, possibly to the adsorbed mucin layer at the epithelium surface. The binding of drugs to mucosal membranes has been postulated as an alternative to the pH–partition hypothesis (30, 31). However, only about 0.3–1.0% of an instilled dose of pilocarpine is absorbed into the eye (13, 17), and preliminary evidence from this laboratory indicates almost quantitative drug transfer from the cornea to the aqueous humor.

Since the amount of drug absorbed is very small relative to the total amount instilled, it is reasonable to postulate a mechanism with a small initial absorption rate operating in the presence of a large dose. Since a first-order absorption process is a function of both the absorption rate constant and the drug concentration, this mechanism adequately explains how substantial amounts of pilocarpine can penetrate into the eye at very short contact times when the amount and concentration of drug in the precorneal area are high. It also accounts for the relative insensitivity of ocular pilocarpine penetration to increases in contact time of the dosing solution. Previous work with methylcellulose (32) and polyvinyl alcohol (33) vehicles did not show improvements in pilocarpine penetration beyond a factor of two or three. In addition, blocking of the drainage apparatus (21, 22), which reduces the magnitude of the parallel elimination step rate constant, gave similar results. If the initial absorption rate constant is as large as the data indicate, considerable increases in aqueous humor levels would be expected under these conditions. However, a small rate constant would provide only small increases in corneal pilocarpine levels, considering the time factor involved, if the precorneal drug concentration is maintained for longer period. This approach appears to be consistent with observed experimental results.

The observed insensitivity of pilocarpine flux to increases in vehicle contact time can be explained on the basis of the interplay between the precorneal drug concentration and the epithelial concentration. Increasing contact time does not increase the epithelial concentration above its initial maximum value, since this value is fixed by the concentration of the applied dose. Rather, it maintains the epithelial concentration at or near this value for an extended time, causing a shift in the peak time (increase) and a corresponding maintenance of pilocarpine flux. However, since these results are essentially an extension of the normal aqueous humor concentration–time profile (Fig. 1) for a simple solution with the same concentration, dramatic increases are not achieved. Similar reasoning applies to cases where the drainage apparatus is blocked.

The observed linear dose–response relationship for pilocarpine (17) also is easily explained since increasing the concentration of the dosing solution increases, in turn, the epithelial concentration which generates a corresponding increase in pilocarpine flux. Thus, higher levels of pilocarpine in the anterior chamber are quickly and easily achieved by increasing the concentration of the applied dose, whereas only nominal benefits are gained by increasing contact time or by fluctuation of the parallel elimination rate constant.

Mechanism of Transcorneal Pilocarpine Permeation—Scheme I shows the analysis used to determine the disposition of pilocarpine in the cornea and aqueous humor after topical dosing. The tissue scraping technique used to obtain data from the three tissues, the epithelium, stroma-endothelium, and aqueous humor, has the unique advantage that all values are representative of pilocarpine movement through the intact eye. This fact enables separate pharmacokinetic treatment of each tissue and correlation of the various absorption and elimination processes. In this manner, the overall mechanism of pilocarpine flux can be readily constructed.

As previously stated, the data in Fig. 3 show the epithelium acting as a reservoir for pilocarpine in the cornea. Pharmacokinetic analysis of these data shows that elimination of pilocarpine from this tissue was biphasic. The first phase, which occurred from the 5-min peak time until about 20 or 30 min, had an apparent elimination rate constant of 0.06 min^{-1} and an apparent attendant absorption rate constant of 0.50 min^{-1}. The second phase, which carried through the remainder of the 2-hr observation period, had an apparent elimination

[10] Perfusion studies in this laboratory with propanoic, hexanoic, and 1-octanoic acids, where the cornea was bathed with drug solution, yielded corneal uptake constants in the 0.001–0.002-min^{-1} range.

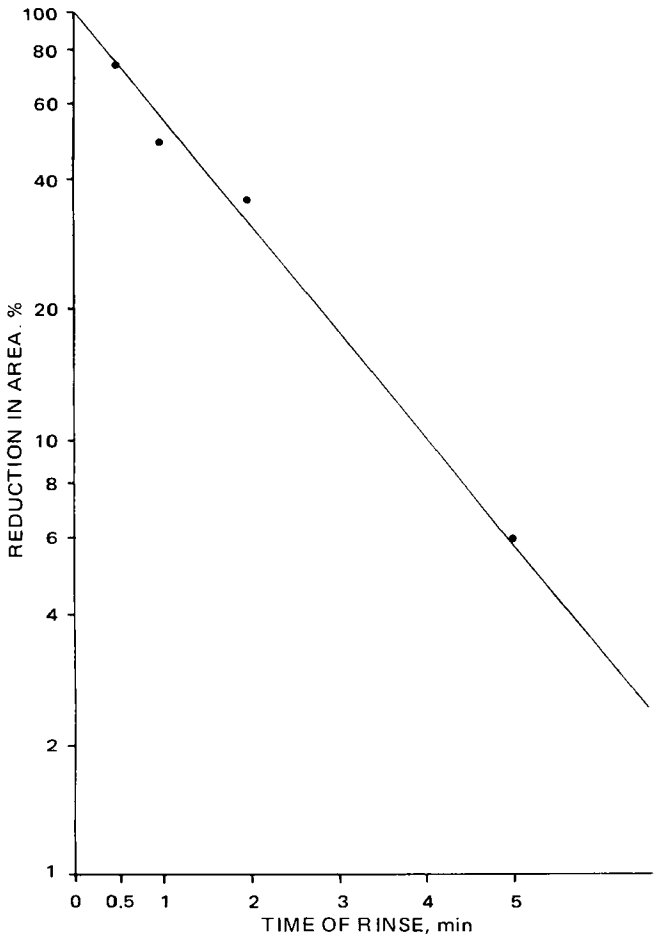


Figure 5—*Effect of rinse time on the area under the curve for pilocarpine in aqueous humor. All values were calculated by graphical methods and normalized to the nonrinsed data.*

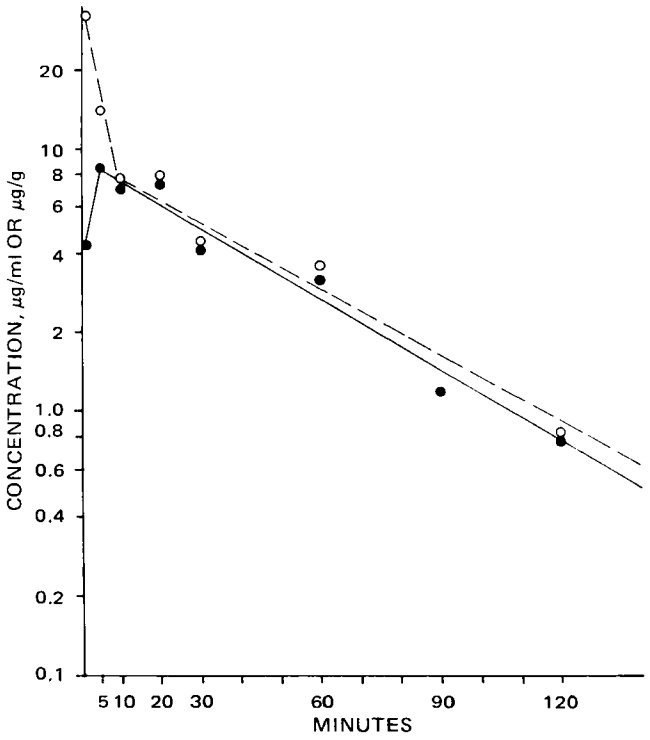


Figure 6—*Concentration of pilocarpine in ocular tissues after topical dosing of abraded eyes. Key:* ●, *aqueous humor; and* ○, *stroma-endothelium.*

rate constant of 0.016 min^{-1}. The data for the stroma-endothelium and aqueous humor were similarly treated, and their tissue concentration–time profiles consisted of a single absorption and elimination phase.

Current studies in this laboratory also showed that elimination of pilocarpine from the aqueous humor after topical dosing was multiphasic at times beyond 2 hr. This finding is in agreement with the results of other recent work (13); presumably this behavior is due to multiple equilibration of pilocarpine with the ocular tissues or possibly is a result of the pharmacological effects of pilocarpine. A summary of the apparent pharmacokinetic parameters obtained for the three tissues is presented in Table III.

The most striking feature of the results is the similarity of the stroma-endothelium and the aqueous humor. Inspection of the tissue concentration–time profiles (Figs. 1 and 3) clearly shows their near identical character. This similarity is also evidenced by the sameness of their pharmacokinetic parameters (Table III). These results indicate almost free diffusion of pilocarpine between these two tissues to the extent that they may be considered to behave as a single phase[11].

Further evidence of this behavior comes from analysis of the stroma-endothelium and aqueous humor profiles obtained for eyes when the epithelium was removed prior to dosing (Fig. 6). Again, the profiles are nearly identical at times beyond 5 or 10 min after dosing. Although early sample times at first seem to be contradictory, they are also consistent. The average wet weight of the stroma-endothelium tissue in this study was approximately 40 mg. It is well established that the corneal stroma contains about 78% water (1). If this amount is combined with an aqueous humor volume of 300 μl, the total volume of distribution of the stroma-endothelium and aqueous humor is about 330 μl. This value is in line with current data from this laboratory concerning intraocular injection of pilocarpine; the 1-min V_d was approximately 300–350 μl (34). Thus, the total initial volume of distribution was about seven to eight times greater than the volume of the stroma-endothelium alone. Since all drug that ultimately distributes in the aqueous humor must come only *via* the stroma, at some point the concentration in the stroma must exceed the aqueous humor by at least a factor of seven. This requirement is reflected well by the data in Fig. 6.

One important consequence of these results is the absence of a measurable endothelial function, at least with respect to the transcorneal mechanism of pilocarpine. It was suggested previously (12) that the endothelium is the controlling factor for movement of pilocarpine through the cornea. The current data are contrary to this premise. Although the endothelium was not separated from the stroma in these experiments, the presence of a significant barrier in the endothelium is not in any way suggested by the results. While some degree of endothelial participation cannot be discounted totally, its role is not sufficiently great to be distinguished by these techniques.

This conclusion is clearly shown by the absence of drug buildup in the stroma-endothelium combination. Also, the increased penetration of pilocarpine through the stroma and endothelium to the anterior

Table III—Pharmacokinetic Parameters for Pilocarpine Distribution in Intact Cornea and Aqueous Humor after Topical Dosing

Tissue	Peak Time, min	k_{abs}, min^{-1}	K_e[a], min^{-1}
Epithelium[b]	4	0.50	0.06, 0.02
Stroma-endothelium	20	0.09	0.021
Aqueous humor	20	0.08	0.017

[a] All pharmacokinetic parameters were calculated by the methods of Wagner and Nelson (24, 25) and are apparent quantities as discussed in the text. [b] Elimination from the epithelium is biphasic.

[11] Preliminary data from this laboratory indicate similar behavior for the iris and ciliary body. Tissue concentration–time profiles for these tissues closely parallel those for the stroma-endothelium and aqueous humor.

chamber in abraded eyes (Fig. 1) shows that there is no barrier function in the absence of the epithelium. Removal of the epithelium also eliminates the reservoir effect in the cornea, since the elimination half-life from the aqueous humor for abraded eyes was 34 min compared to 42 min for intact eyes. The longer half-life for intact eyes was due to the continuing flux of drug into the aqueous humor from the reservoir in the epithelium. This observation agrees well with the findings of other investigators (14, 16, 23) regarding the presence of a depot for pilocarpine in the cornea and is in contradiction with the conclusions of Lazare and Horlington (13).

Another feature of major significance is the consistency of the pharmacokinetic parameters (Table III) with the observed overall movement of pilocarpine through the intact cornea. The apparent absorption rate of pilocarpine into the epithelium (0.50 min^{-1}) agrees well with the apparent uptake rate obtained from the rinsing studies (0.57 min^{-1}). Also, the first-phase elimination rate constant (0.06 min^{-1}) correlates quite well with the apparent absorption rate into the stroma-endothelium (0.09 min^{-1}) and the aqueous humor (0.08 min^{-1}). In addition, the second-phase elimination rate constant equates well with the elimination rate from the stroma-endothelium (0.021 min^{-1}) and almost identically with that from the aqueous humor (0.017 min^{-1}). This overall behavior is to be expected, in fact required, if the epithelium is the controlling factor in transcorneal pilocarpine flux. The results from these experiments clearly demonstrate that the corneal epithelium is the rate-limiting tissue in the mechanism for transcorneal flux of pilocarpine.

The rate constants for the various tissues were generally obtained by graphical methods. Computer fitting of the data using a one-compartment open model also was used in several cases. However, the rate constants obtained with the two methods were comparable so that no advantage was considered to be gained from computer analysis. The rate constants so obtained are estimates only; more time points would be required to obtain precise values of the kinetic constants. The data are sufficient, however, to permit correlation of drug movement between the various tissues in the manner described.

The corneal penetration of pilocarpine can be described as a series of successive first-order absorption and elimination steps. Lateral distribution of drug does not occur in the corneal tissues, since the cornea is not vascularized. Lateral movement of drug occurs only in the precorneal area where a composite parallel first-order elimination process removes most of the instilled dose and in the anterior chamber where multiple tissue equilibrations take place. Deviations from first-order behavior are apparent only at times beyond 2 hr after dosing.

The results of these studies also suggest that the partition coefficient theory of transcorneal permeation should be reexamined in the light of true *versus* apparent pharmacokinetic parameters. Pilocarpine appears to possess optimum solubility character, being soluble in both polar and nonpolar solvents. Yet, the apparent rapid corneal penetration of this molecule, attributed to its solubility by previous investigators (13), is actually an artifact and a consequence of precorneal fluid dynamics. Further investigation of this aspect of corneal penetration of organic molecules is required before a firm relationship between solubility, partition coefficient, and corneal penetration can be established.

SUMMARY

When a topical solution of pilocarpine is instilled into eyes of albino rabbits, there appears to be a rapid uptake and distribution of drug across the cornea to the anterior chamber. This apparent rapid penetration can be attributed to a composite parallel elimination process that occurs in the precorneal area. This elimination step, in combination with a small true absorption rate constant, also accounts for the small fraction of dose absorbed into ocular tissues.

Analysis of the aqueous humor and the various cell layers of the cornea provides pharmacokinetic parameters consistent with the observed overall absorption and elimination rate constants. The corneal epithelium is the rate-limiting tissue and acts as both a barrier to penetration and as a reservoir of pilocarpine in the cornea. The stroma-endothelium and aqueous humor behave as a single aqueous phase for drug distribution, and no significant rate-determining role for the endothelium is evident from the results. The overall mechanism for transcorneal pilocarpine flux can be constructed with the apparent pharmacokinetic parameters obtained for each tissue.

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