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Mechanisms of Anterior Segment Absorption of Pilocarpine following Subconjunctival Injection in Albino Rabbits

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Abstract □ The present study was initiated to provide insight into the mechanisms of drug availability from subconjunctival injection. Pilocarpine nitrate was the probe drug, and the albino rabbit was the experimental animal. Several volumes, from 50 to 500 μl, of drug solution were injected subconjunctivally; drug levels were monitored in the tear, cornea, aqueous humor, and blood as a function of time. The injection site also was examined for residual drug postdosing. Finally, drug was placed in the subconjunctival space either by injection through the conjunctival membrane or through the eyelid. From these experiments, it was concluded that at high injection volumes, *i.e.*, >200 μl, reflux of the drug solution out of the injection site, followed by corneal absorption, is the primary mechanism to explain aqueous humor availability of the drug. At lower injection volumes, multiple mechanisms, including reflux of drug from the injection site and transconjunctival permeation, penetration of the globe of the eye, and systemic absorption followed by return *via* the vascular bed, are necessary to explain drug absorption. High aqueous humor levels of pilocarpine can be achieved from subconjunctival injection as compared to the topical route, provided that large injection volumes, *i.e.*, >200 μl, are employed and correspondingly greater amounts of drug are administered. No true sustaining of drug availability to the aqueous chamber was noted, but local sustaining at the injection site was evident.

Keyphrases □ Pilocarpine—mechanisms of distribution to cornea, tear, aqueous humor, and blood following subconjunctival injection, rabbits □ Absorption, ocular—pilocarpine, mechanisms of distribution following subconjunctival injection, rabbits □ Distribution, ocular—pilocarpine, mechanisms of absorption following subconjunctival injection, rabbits

Subconjunctival administration of ophthalmic drugs represents an important, albeit poorly understood, administration route. Some clinicians select this route because of the expected sustained drug delivery to the eye, whereas others choose it for the expected high drug levels that can be achieved in the anterior segment of the eye as compared to topical dosing. Whether a high level or a sustained effect is achieved appears to depend on the drug, the formulation, and the method of injection.

That drugs do penetrate the eye from subconjunctival injection is certain, although the penetration rate may vary considerably depending on the drug and formulation. However, it is not well understood how the drug moves from the injection site to the aqueous chamber. Until a clear mechanistic picture is established, drug release predictions for different drug entities or formulation changes will be based primarily on experience.

The purpose of the present study was to examine the mechanisms of aqueous chamber absorption from subconjunctival injection for the water-soluble drug pilocarpine.

BACKGROUND

The conjunctiva is the thin membrane covering the front of the eyeball from the lid margin to the corneoscleral junction. The subconjunctival space is the region between the conjunctiva and the eyeball, and subconjunctival injections place the drug in this area either for a local effect or as a repository to provide drug to the aqueous chamber.

Several mechanisms have been proposed to explain the movement of drugs from the subconjunctival site of injection to the anterior segment of the eye, *i.e.*, the aqueous chamber:

1. The drug is absorbed into the conjunctival blood vessels, enters the general circulation, and then enters the anterior segment of the eye.
2. The drug directly penetrates the sclera and underlying layers.
3. The drug refluxes out of the puncture hole produced in the conjunctiva by injection. It then mixes with tears and is absorbed across the cornea.
4. The drug diffuses across the conjunctiva, followed by mixing with tears and eventual corneal absorption.

One early explanation of ocular drug absorption from subconjunctival injection was systemic absorption followed by return to the eye. Without doubt, most of this injected dose is absorbed into the systemic circulation, which represents a considerable loss from the injection site. However, given the large volume of distribution of the vascular bed and the small amount of drug involved, as well as potential metabolism in the blood, it seems unlikely that this route of penetration would be major for most drugs.

The second potential route is direct scleral absorption of drug into the aqueous chamber. While this route is possible, the drug would have to permeate a relatively impermeable scleral coat (1–4) and traverse a rich vascular bed to gain entrance to the aqueous chamber. For most drugs,

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the difficulty of scleral penetration as well as loss to systemic circulation probably would cause little, if any, drug to reach the anterior segment of the eye. In addition, this mechanism would have to be compatible with the very early time of the peak drug level in the aqueous chamber that is commonly observed. Nevertheless, McCartney *et al.* (5), on the basis of autoradiographic techniques, felt that this route was a distinct mechanistic possibility for absorption of hydrocortisone.

Undoubtedly, the most widely accepted route for absorption is the needle puncture and reflux theory. Recent work (6) with small volumes (1 μl) of injected solutions raised doubts about its importance in humans. On the other hand, Wine *et al.* (7) systematically examined the mechanism of absorption of [^{14}C]hydrocortisone and found that the leakage mechanism was the most plausible. Aqueous humor drug levels from normal subconjunctival injections were compared with those achieved from injection through the upper eyelid, which kept the conjunctival membrane intact. The relatively high drug levels from subconjunctival injection compared to those from upper eyelid injection supported the puncture mechanism.

The last possible mechanism is direct permeation of the conjunctiva followed by corneal absorption. The conjunctival permeability coefficient for pilocarpine has been estimated (8) to be in the range of 10^{-3} cm/hr, which is in good agreement with the coefficient of 5×10^{-3} cm/hr found for sodium 22 (2, 9). This sufficiently large permeability coefficient explains the early peak times in the cornea and aqueous humor, provided the concentration of injected drug is very large and little or no drug is lost to the conjunctival blood vessels. Simple diffusion of drugs from subconjunctival injection has been compared with topical dosing (10). Early peaks in the cornea, sclera, and choroid can be attributed to extensive diffusion of drug across the conjunctiva, or leakage, to the tear film or to direct transfer of drug at the corneoscleral junction, presumably through limbal blood vessels.

For any individual drug entity, its physicochemical properties, such as solubility, diffusivity, and partitioning behavior, may give one mechanism precedence over another. In addition, vehicle composition, volume injected, and method of injection also may influence the mechanism. The present study examines some of these variables using pilocarpine as the drug probe.

EXPERIMENTAL

Materials—Tritiated pilocarpine alkaloid with a specific activity of 4.1 Ci/mmole was obtained commercially[1] and purified by vacuum distillation (11) immediately prior to use. All other chemicals were reagent or analytical grade and were used as received.

Male albino rabbits[2], 1.7–2.4 kg, were maintained on a regular diet with no restrictions on the amounts of food and water consumed. Lighting and auditory stimuli were kept constant on a 24-hr basis.

Preparation of Pilocarpine Solutions—Drug solutions were prepared by the addition of the purified labeled material to a 1×10^{-2} *M* (0.2%) pilocarpine solution. The buffer was pH 6.24 isotonic Sorensen buffer. Addition of [^{3}H]pilocarpine had no appreciable effect on the molarity of the final dosing solution. It was determined that 0.25 mCi of the labeled drug/ml of the final dosing solution was sufficient to give good counting efficiency. The dosing solution for injection corresponded to a final concentration of 2.08 μg of pilocarpine alkaloid/μl of solution.

Subconjunctival Injection Administration through Conjunctival Membrane—Rabbits were positioned in restraining boxes to minimize movement and to maintain the normal upright posture. Approximately 10 min prior to dosing, each eye received one drop of 0.5% tetracaine hydrochloride solution[3]. Although topical anesthetics have been shown to cause breakup of the tear film (1–3, 8, 12, 13), this step was necessary to obtain consistent injections.

Following local anesthesia, measured volumes of pilocarpine solution (50, 100, 200, and 500 μl) were injected accurately through the conjunctival membrane in the center of the upper quadrant bulbar conjunctiva, ~3–4 mm back from the corneoscleral junction. A 1-ml tuberculin syringe fitted with a 27-gauge, 1.27-cm needle was employed. Only one eye of each test animal received the dose, with the other eye serving as a control. Alternate eye dosing was used in each animal to eliminate any right to left or left to right eye bias.

Aqueous humor, blood, and corneal samples were obtained at 5, 10, 20, 30, 45, 60, and 120 min postdosing. For the 200-μl injection, samples also were obtained at 180 and 240 min postinjection. At the end of each period, the animal was sacrificed *via* rapid injection of a phenobarbital sodium overdose into the marginal ear vein.

Prior to sampling, the corneal surface was rinsed with isotonic saline and blotted dry with a tissue. Aqueous humor samples were obtained by aspirating the aqueous humor and analyzing the fluid *via* scintillation counting according to reported procedures (14–17). Corneal samples were obtained by excising the cornea immediately after aqueous humor aspiration. The method for obtaining and treating the corneal samples was described previously (14, 17).

Blood samples of 1 ml were obtained by direct cardiac puncture, and then two 100-μl samples were transferred[4] quantitatively to absorbent pads[5] contained in combustion cones[6]. These samples were allowed to dry for 24 hr prior to burning in a tissue oxidizer[7]. The oxidizer automatically deposited the tritiated sample, along with 10 ml of liquid scintillation counting solution[8], into a plastic vial[9]. The vial contents were allowed to cool to room temperature for 6 hr and then were counted and analyzed as described previously (14, 17). At least seven eyes were used for each data point. Mean values and standard errors of the mean were determined from the pooled data at each time point, and the concentration–time profile was fitted to a one-compartment pharmacokinetic model (18, 19).

Subconjunctival Injection Administration through Upper Eyelid—Rabbits were positioned in restraining boxes, and a drop of local anesthetic was instilled into each eye. Accurately measured volumes (50–100 μl) of pilocarpine solution were injected directly through the upper eyelid into the center of the upper quadrant under the bulbar conjunctiva. The injection was made so as to produce a bleb in the same location as the direct subconjunctival injection. Thus, the major difference between this study and the direct subconjunctival injection was that no puncture of the conjunctival membrane occurred.

Tissue sampling and analysis were identical to those described previously. The data treatment and analysis of data also were as described previously.

Tear Film Pilocarpine—*Single-Needle Puncture Hole*—Rabbits were placed in restraining boxes, but no local anesthetic was administered. Pilocarpine solution, 200 μl, was injected subconjunctivally; the tear film was sampled at 0, 0.5, 1, 2, 4, 5, 7, 10, 15, 20, and 30 min postdosing. The tear film samples were obtained by carefully touching a 1-μl capillary pipet[10] to the lacrimal lake at the edge of the lid and aspirating a 1-μl sample. The glass capillary tubes then were treated in a manner similar to that described for the aqueous humor samples. At least 13 eyes were used for each data point, and the data were treated as described previously.

Multiple-Needle Puncture Hole—The study was identical to that described for the single-needle puncture except that multiple punctures were made in the conjunctival membrane. Three protocols were employed: (*a*) nine punctures, followed by dosing of 200 μl of the pilocarpine solution injected during the 10th puncture; (*b*) five punctures, followed by dosing on the sixth puncture, followed by four more punctures into the resulting bleb; and (*c*) injection of 200 μl, followed by nine more punctures into the bleb.

Immediately following the 10th puncture of the conjunctival membrane, 1-μl tear samples were collected over the same period and analyzed in the same way as described for the tear film study.

At the end of the tear sampling period, *i.e.*, 30 min, the animals were sacrificed; the aqueous humor, cornea, and blood samples were obtained. These samples were analyzed as already described.

At least 14 eyes were used for each data point, and the results were treated as described previously.

No Puncture Hole—The dose was injected into the subconjunctival space without puncturing the conjunctival membrane by piercing the upper eyelid and threading the needle to approximately the same spot in the subconjunctival space as from a subconjunctival injection.

Tear samples were collected and analyzed as described previously. At least 11 eyes were used for each data point.

Conjunctival Bleb Analysis—Rabbits were prepared and placed in restraining boxes. Following administration of the topical anesthetic, 200 μl of pilocarpine solution was administered to each eye either by subconjunctival or through-the-eyelid injection. At the end of 60 min, the animals were sacrificed. The upper quadrant conjunctival membrane was

[1] New England Nuclear, Boston, Mass.
[2] Klubertanz Rabbitry, Edgerton, Wis.
[3] Pontocaine, Winthrop Laboratories, New York, N.Y.
[4] Biopette, Schwartz-Mann, Orangeburg, N.Y.
[5] Combusti-Pads, Packard Instrument Co., Downers Grove, Ill.
[6] Combusto-Cone, Packard Instrument Co., Downers Grove, Ill.
[7] Model 2002, Packard Instrument Co., Downers Grove, Ill.
[8] Monophase 40, Packard Instrument Co., Downers Grove, Ill.
[9] The Vial, Research Products International Corp., Elk Grove Village, Ill.
[10] Microcap, Drummond Scientific Co., Bloomfield, N.J.

Table I—Cornea and Aqueous Humor Drug Concentration in the Dosed Eye *versus* Time for 1×10^{-2} *M* Pilocarpine in Various Volumes of pH 6.24 Isotonic Sorensen Buffer Injected Subconjunctivally through the Conjunctival Membrane

Minutes	Micrograms of Pilocarpine per Gram of Cornea (Wet Weight)				Micrograms of Pilocarpine per Milliliter of Aqueous Humor			
	50 μl	100 μl	200 μl	500 μl	50 μl	100 μl	200 μl	500 μl
5	0.304 (0.086)[a] [12][b]	0.200 (0.062)[a] [8][b]	4.203 (0.564)[a] [10][b]	14.802 (1.391)[a] [8][b]	0.047 (0.019)[a] [13][b]	0.089 (0.052)[a] [8][b]	0.386 (0.078)[a] [11][b]	0.704 (0.072)[a] [8][b]
10	0.231 (0.047) [13]	0.314 (0.083) [11]	4.226 (1.043) [11]	14.111 (2.586) [9]	0.127 (0.075) [11]	0.194 (0.063) [13]	0.703 (0.133) [14]	1.747 (0.258) [8]
20	0.059 (0.010) [14]	0.340 (0.069) [12]	6.434 (0.881) [10]	12.013 (0.753) [9]	0.180 (0.102) [11]	0.278 (0.086) [13]	1.442 (0.291) [14]	2.300 (0.140) [8]
30	0.081 (0.017) [8]	0.288 (0.049) [12]	6.482 (1.340) [10]	10.514 (2.278) [9]	0.195 (0.118) [9]	0.329 (0.103) [10]	1.817 (0.314) [13]	3.586 (0.487) [7]
45	0.333 (0.007) [7]	0.254 (0.111) [8]	5.713 (0.984) [12]	9.686 (2.653) [9]	0.136 (0.068) [8]	0.258 (0.110) [10]	1.385 (0.213) [13]	3.156 (0.691) [7]
60	0.030 (0.003) [11]	0.290 (0.052) [8]	1.802 (0.388) [13]	5.635 (2.223) [10]	0.049 (0.006) [8]	0.180 (0.032) [9]	0.505 (0.082) [13]	1.546 (0.498) [11]
90	0.026 (0.005) [7]	0.213 (0.031) [8]	NS[c]	4.798 (0.863) [9]	0.032 (0.012) [11]	0.115 (0.019) [8]	NS	1.370 (0.247) [10]
120	0.033 (0.014) [6]	0.081 (0.023) [8]	0.586 (0.144) [13]	1.894 (0.609) [8]	0.026 (0.008) [9]	0.039 (0.006) [10]	0.199 (0.035) [16]	0.694 (0.222) [8]
180	NS	NS	0.220 (0.051) [12]	NS	NS	NS	0.080 (0.012) [16]	NS
240	NS	NS	0.209 (0.034) [8]	NS	NS	NS	0.063 (0.009) [15]	NS

[a] Numbers in parentheses refer to the standard error of the mean. [b] Numbers in brackets refer to the number of determinations at that point. [c] Not sampled.

Table II—Cornea and Aqueous Humor Drug Concentration in the Contralateral Eye *versus* Time for 1×10^{-2} *M* Pilocarpine in Various Volumes of pH 6.24 Isotonic Sorensen Buffer Injected Subconjunctivally through the Conjunctival Membrane

Minutes	Micrograms of Pilocarpine per Gram of Cornea (Wet Weight)				Micrograms of Pilocarpine per Milliliter of Aqueous Humor			
	50 μl	100 μl	200 μl	500 μl	50 μl	100 μl	200 μl	500 μl
5	0.019 (0.002)[a] [10][b]	0.023 (0.006)[a] [10][b]	0.072 (0.010)[a] [14][b]	0.224 (0.036)[a] [10][b]	0.003 (0.0002)[a] [15][b]	0.004 (0.0002)[a] [10][b]	0.006 (0.001)[a] [14][b]	0.014 (0.002)[a] [11][b]
10	0.018 (0.002) [19]	0.027 (0.002) [12]	0.063 (0.011) [10]	0.406 (0.151) [10]	0.003 (0.0004) [15]	0.007 (0.0003) [14]	0.011 (0.002) [14]	0.037 (0.003) [11]
20	0.018 (0.002) [14]	0.026 (0.001) [14]	0.076 (0.015) [11]	0.408 (0.122) [8]	0.006 (0.0003) [14]	0.012 (0.0004) [15]	0.022 (0.002) [14]	0.053 (0.002) [12]
30	0.013 (0.001) [9]	0.025 (0.002) [13]	0.057 (0.008) [13]	0.205 (0.028) [10]	0.007 (0.001) [8]	0.015 (0.001) [15]	0.027 (0.002) [13]	0.081 (0.003) [11]
45	0.014 (0.001) [9]	0.025 (0.002) [8]	0.214 (0.053) [10]	0.537 (0.119) [9]	0.008 (0.0004) [7]	0.019 (0.002) [10]	0.038 (0.003) [14]	0.107 (0.005) [9]
60	0.015 (0.001) [11]	0.030 (0.002) [10]	0.114 (0.011) [16]	0.186 (0.049) [11]	0.007 (0.0003) [14]	0.020 (0.001) [10]	0.033 (0.001) [16]	0.111 (0.008) [12]
90	0.014 (0.002) [9]	0.027 (0.005) [10]	NS[c]	0.212 (0.031) [8]	0.007 (0.0003) [9]	0.020 (0.001) [10]	NS	0.083 (0.002) [11]
120	0.015 (0.001) [9]	0.025 (0.002) [10]	0.044 (0.008) [7]	0.135 (0.015) [11]	0.007 (0.0004) [10]	0.015 (0.0004) [10]	0.053 (0.004) [16]	0.084 (0.004) [10]
180	NS	NS	0.037 (0.006) [12]	NS	NS	NS	0.032 (0.001) [16]	NS
240	NS	NS	0.028 (0.003) [9]	NS	NS	NS	0.284 (0.001) [16]	NS

[a] Numbers in parentheses refer to the standard error of the mean. [b] Numbers in brackets refer to the number of determinations at that point. [c] Not sampled.

Table III—Circulating Blood Drug Concentration *versus* Time for 1×10^{-2} *M* Pilocarpine in Various Volumes of pH 6.24 Isotonic Sorensen Buffer Injected Subconjunctivally through the Conjunctival Membrane

Minutes	Micrograms of Pilocarpine per Milliliter of Blood			
	50 μl	100 μl	200 μl	500 μl
5	0.035 (0.001)[a] [9][b]	0.069 (0.002)[a] [8][b]	NS[c]	0.214 (0.021)[a] [9][b]
10	0.034 (0.002) [11]	0.058 (0.004) [10]	0.067 (0.009)[a] [9][b]	0.260 (0.029) [12]
20	0.041 (0.002) [12]	0.062 (0.003) [12]	0.108 (0.011) [10]	0.301 (0.014) [12]
30	0.033 (0.001) [11]	0.048 (0.003) [10]	0.048 (0.003) [10]	0.172 (0.014) [9]
45	0.027 (0.002) [12]	0.053 (0.002) [12]	0.132 (0.013) [10]	0.287 (0.020) [12]
60	0.023 (0.001) [10]	0.052 (0.002) [12]	0.101 (0.004) [10]	0.233 (0.015) [9]
90	0.016 (0.001) [10]	0.026 (0.001) [11]	NS	0.132 (0.010) [10]
120	0.014 (0.001) [12]	0.022 (0.001) [10]	0.022 (0.004) [7]	0.097 (0.006) [12]
180	NS	NS	0.007 (0.001) [9]	NS
240	NS	NS	0.007 (0.001) [10]	NS

[a] Numbers in parentheses refer to the standard error of the mean. [b] Numbers in brackets refer to the number of determinations at that point. [c] Not sampled.

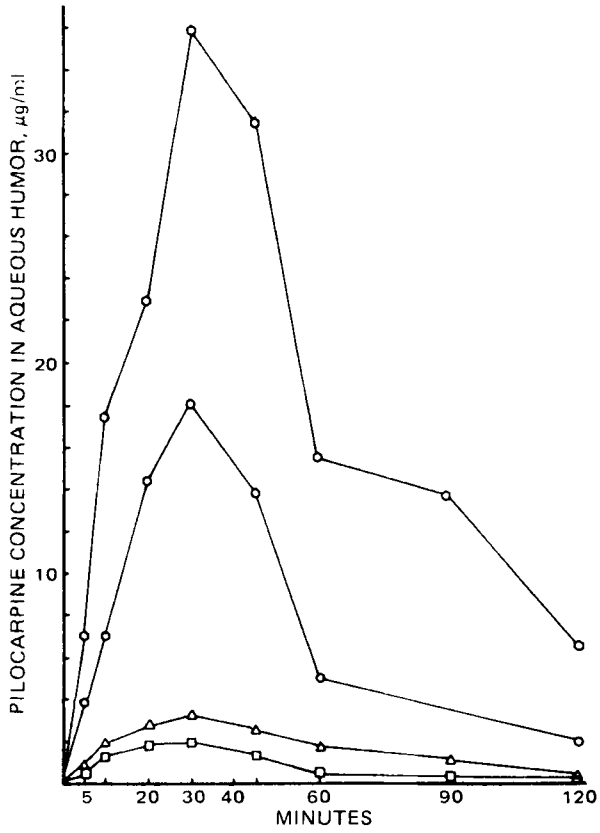


Figure 1—*Aqueous humor drug concentration in the dosed eye* versus *time profiles for 1 × 10^{-2} M pilocarpine in various volumes of pH 6.24 isotonic Sorensen buffer injected subconjunctivally through the conjunctival membrane. Key: □, 50 μl injected; △, 100 μl injected; ○, 200 μl injected; and ○, 500 μl injected. Each point represents a minimum of seven determinations. Error bars were eliminated for clarity.*

dissected and removed, including the bulbar and palpebral portions.

The obtained tissue was placed in a tared combustion cone[6] containing a combustion pad[5] to soak up any fluid and to aid in more even burning of the tissue in the combustion process. The wet weight of each conjunctiva then was determined. Subsequent analytical procedures were identical to those used in the corneal sample analysis.

At least 21 eyes were used for each point in the membrane puncture bleb analysis, and 18 eyes were used per point in the eyelid route bleb analysis. All data were subjected to the same analysis as described previously.

RESULTS

Subconjunctival Injection Delivered through Conjunctival Membrane—Drug concentration *versus* time profiles for each of the four dose volumes were obtained for the aqueous humor, cornea, and blood. Cornea and aqueous humor data were obtained for both the dosed and contralateral eyes.

The data are presented in Tables I–III for the four dose volumes studied. For visual comparison, the aqueous humor data are shown graphically in Fig. 1. An examination of Fig. 1 shows some expected trends. As the dosing volume increased, the amount of drug absorbed into the cornea or aqueous humor increased. The amount of drug absorbed was increased greatly by the increase in dosing volume from 100 to 200 μl. This result also occurred in the contralateral eye, although the drug levels were much smaller and the trend was not as clear.

Subconjunctival Injection Delivered through Upper Eyelid—Drug concentration *versus* time profiles for each of the two dose volumes were obtained for the aqueous humor, cornea, and blood. Cornea and aqueous humor data were obtained for both the dosed and contralateral eyes.

Tables IV–VI contain the pertinent data from this study, and Fig. 2 shows the representative aqueous humor data in graphical form. The drug levels in the cornea and aqueous humor were several orders of magnitude lower than those from the through-the-membrane injection. The levels in the contralateral eyes were about the same as those in the through-the-membrane injection. Finally, the circulating blood level data were comparable from both studies.

Lacrimal Fluid following Subconjunctival Injection—The tear film was monitored for drug as a function of time for three different experimental conditions: one puncture hole in the conjunctiva, multiple-puncture holes in the conjunctiva, and no puncture holes in the conjunctiva (through-the-eyelid injection). The data are shown in Table VII. Drug levels in the through-the-eyelid study were approximately three orders of magnitude less than those in the punctured conjunctival studies. These levels were so low that background radiation levels became important and should be considered when assessing the accuracy of the stated levels.

Data from the punctured conjunctiva are shown graphically in Fig. 3. The profiles were similar at all times except for the first 5 min. Previous reports from this laboratory described the procedures and problems of tear sampling (11, 20–22). Samples were collected for 30 min with emphasis on data obtained during the first 5 min. Therefore, considerable weight is given to the difference shown in Fig. 3 for the first 5 min post-injection.

After 30 min, the experimental animal was sacrificed; samples of the cornea, aqueous humor, and blood were taken (Table VIII). The aqueous humor drug levels for the dosed and contralateral eye in the through-the-eyelid study were not appreciably different. Cornea data from the same study showed the dosed eye levels to be about twice as large as those from the contralateral eye, although both were low relative to the injection study. The small, but measurable, difference in cornea levels is attributed to drug diffusing through the conjunctival membrane to the tear fluid and then absorbed into the cornea.

The aqueous humor and cornea data from the dosed eye in the punctured membrane case were two orders of magnitude greater than those from the through-the-eyelid study. Although the multiple-puncture

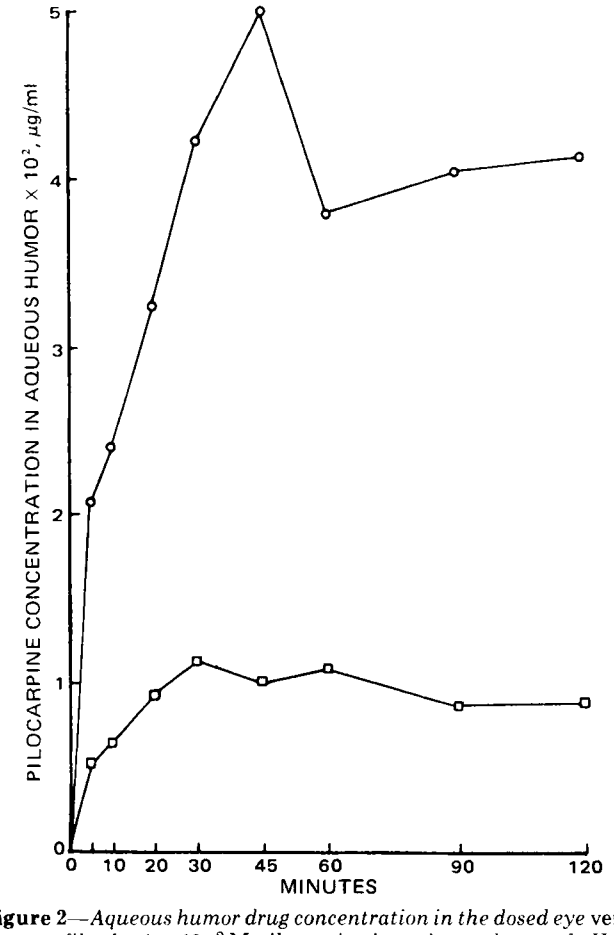


Figure 2—*Aqueous humor drug concentration in the dosed eye* versus *time profiles for 1 × 10^{-2} M pilocarpine in various volumes of pH 6.24 isotonic Sorensen buffer injected subconjunctivally through the upper eyelid. Key: □, 50 μl injected; and ○, 200 μl injected. Each point represents a minimum of seven determinations. Error bars were eliminated for clarity.*

Table IV—Cornea and Aqueous Humor Drug Concentration in the Dosed Eye *versus* Time for 1×10^{-2} *M* Pilocarpine in Various Volumes of pH 6.24 Isotonic Sorensen Buffer Injected Subconjunctivally through the Upper Eyelid

Minutes	Micrograms of Pilocarpine per Gram of Cornea (Wet Weight)		Micrograms of Pilocarpine per Milliliter of Aqueous Humor	
	50 μl	200 μl	50 μl	200 μl
5	0.062 (0.010)[a] [9][b]	0.0676 (0.129)[a] [8][b]	0.005 (0.0004)[a] [9][b]	0.021 (0.003)[a] [8][b]
10	0.064 (0.008) [10]	0.146 (0.22) [10]	0.006 (0.0006) [11]	0.024 (0.001) [10]
20	0.069 (0.009) [11]	0.130 (0.051) [12]	0.009 (0.0004) [12]	0.032 (0.001) [9]
30	0.065 (0.011) [12]	0.119 (0.034) [12]	0.011 (0.0006) [8]	0.042 (0.002) [10]
45	0.070 (0.009) [11]	0.092 (0.010) [9]	0.010 (0.0007) [12]	0.050 (0.003) [12]
60	0.111 (0.009) [7]	0.092 (0.009) [8]	0.011 (0.0007) [11]	0.038 (0.002) [10]
90	0.032 (0.005) [10]	0.104 (0.023) [10]	0.009 (0.0006) [9]	0.040 (0.002) [12]
120	0.039 (0.005) [10]	0.093 (0.13) [10]	0.009 (0.0004) [10]	0.040 (0.001) [7]

[a] Numbers in parentheses refer to the standard error of the mean. [b] Numbers in brackets refer to the number of determinations at that point.

Table V—Cornea and Aqueous Humor Drug Concentration in the Contralateral Eye *versus* Time for 1×10^{-2} *M* Pilocarpine in Various Volumes of pH 6.24 Isotonic Sorensen Buffer Injected Subconjunctivally through the Upper Eyelid

Minutes	Micrograms of Pilocarpine per Gram of Cornea (Wet Weight)		Micrograms of Pilocarpine per Milliliter of Aqueous Humor	
	50 μl	200 μl	50 μl	200 μl
5	0.033 (0.005)[a] [12][b]	0.208 (0.057)[a] [8][b]	0.004 (0.0005)[a] [12][b]	0.012 (0.0007)[a] [12][b]
10	0.053 (0.006) [12]	0.094 (0.020) [10]	.0.005 (0.0004) [12]	0.020 (0.001) [10]
20	0.052 (0.007) [10]	0.062 (0.006) [9]	0.008 (0.0004) [10]	0.032 (0.001) [10]
30	0.059 (0.009) [12]	0.058 (0.007) [12]	0.010 (0.001) [12]	0.036 (0.002) [10]
45	0.057 (0.008) [11]	0.057 (0.008) [10]	0.011 (0.0006) [9]	0.045 (0.002) [10]
60	0.108 (0.008) [8]	0.077 (0.011) [9]	0.011 (0.0007) [12]	0.034 (0.002) [12]
90	0.032 (0.006) [9]	0.081 (0.010) [11]	0.009 (0.001) [12]	0.040 (0.002) [12]
120	0.035 (0.005) [10]	0.088 (0.012) [9]	0.009 (0.0004) [12]	0.043 (0.001) [10]

[a] Numbers in parentheses refer to the standard error of the mean. [b] Numbers in brackets refer to the number of determinations at that point.

Table VI—Circulating Drug Concentration *versus* Time for 1×10^{-2} *M* Pilocarpine in Various Volumes of pH 6.24 Isotonic Sorensen Buffer Injected Subconjunctivally through the Upper Eyelid

Minutes	Micrograms of Pilocarpine per Milliliter of Blood	
	50 μl	200 μl
5	0.051 (0.004)[a] [12][b]	0.158 (0.007)[a] [12][b]
10	0.073 (0.003) [12]	0.172 (0.004) [10]
20	0.057 (0.003) [12]	0.157 (0.007) [10]
30	0.064 (0.004) [12]	0.148 (0.006) [12]
45	0.051 (0.003) [12]	0.112 (0.007) [8]
60	0.044 (0.002) [12]	0.091 (0.003) [10]
90	0.012 (0.002) [10]	0.053 (0.002) [12]
120	0.021 (0.001) [9]	0.061 (0.003) [10]

[a] Numbers in parentheses refer to the standard error of the mean. [b] Numbers in brackets refer to the number of determinations at that point.

aqueous humor and cornea drug levels were slightly higher than the corresponding drug levels from single puncture, they were not statistically different. A partial explanation for this lack of difference is that, upon dissection of the bleb, the area appears as a sponge with numerous aqueous compartments. Multiple punctures do not appear to have a significant effect because it is not like puncturing a balloon but rather like puncturing a sponge.

The drug level in lacrimal fluid at 30 min was as anticipated, with large values in the puncture studies and a low level in the nonpuncture case. The lower lacrimal level in the multiple-puncture case was not statistically different from the single-puncture case. One would expect, *a priori*, lower levels in tears at the 30-min point in the multiple-puncture case if more drug is absorbed into the cornea.

Conjunctival Bleb Injection through Membrane or through Eyelid—The bleb created by subconjunctival injection remains for hours postinjection. It was of interest to determine if the drug also remained for hours or if the bleb merely contained the aqueous injection solution or inflammatory fluid. Thus, at 1 hr postinjection, the bleb was removed surgically and analyzed for drug content. Simple aspiration of the bleb was not possible because of its sponge-like characteristics. The results are shown in Table IX.

After 60 min, there was a considerable amount of pilocarpine in the punctured membrane compared to the through-the-eyelid study. From the wet weights obtained, it is clear that localization of the drug solution in the through-the-eyelid case was much more difficult than in the punctured case. The bleb created by through-the-eyelid injection was distinctly smaller; it was difficult to place it near the bulbar conjunctiva. Undoubtedly, the difference in physical placement and localization of drug solution in the two studies contributed to the difference shown in Table IX. The fact that considerable pilocarpine was left at the site long after appreciable drug levels appeared in the cornea and aqueous humor is significant. This result supports the sustained-release concept of subconjunctival dosing. However, whether the drug is released locally or to the aqueous chamber for long periods depends on the specific drug properties and injection formulation.

DISCUSSION

Each of the four proposed mechanisms for drug movement into the eye interior following subconjunctival injections will be examined, and the

Table VII—Lacrimal Fluid Drug Concentration in the Dosed Eye *versus* Time for 1×10^{-2} *M* Pilocarpine in 200 μl of pH 6.24 Isotonic Sorensen Buffer following Subconjunctival Injection *via* Various Routes of Administration

Minutes	Micrograms of Pilocarpine per Milliliter of Lacrimal Fluid: Single-Puncture Injection through Membrane	Multiple-Puncture Injection through Membrane	Single-Puncture Injection through Eyelid
0.5	270.0 (86.0)[a] [13][b]	416.4 (99.9)[a] [14][b]	0.287 (0.184)[a] [11][b]
1.0	268.2 (93.5) [13]	398.4 (97.6) [14]	0.145 (0.075) [11]
2.0	275.1 (110.4) [13]	350.8 (74.7) [14]	0.082 (0.038) [11]
4.0	238.5 (100.6) [13]	259.3 (52.4) [14]	0.271 (0.143) [11]
5.0	283.5 (93.2) [13]	187.2 (39.7) [14]	0.243 (0.75) [11]
10.0	118.0 (31.7) [13]	95.4 (20.6) [14]	0.161 (0.070) [11]
15.0	75.0 (31.2) [13]	51.1 (11.3) [14]	0.195 (0.079) [11]
20.0	40.1 (12.4) [13]	44.4 (12.6) [14]	0.149 (0.070) [11]
30.0	18.8 (6.4) [13]	11.5 (5.1) [14]	0.437 (0.279) [11]

[a] Numbers in parentheses refer to the standard error of the mean. [b] Numbers in brackets refer to the number of determinations at that point.

results from the present study will be interpreted in light of these proposals. Each mechanism contributes to the overall movement of drug, and it is the magnitude of the contributions that is of concern.

The magnitude and shape of the cornea or aqueous humor pilocarpine–time profile from subconjunctival injection did not differ greatly from those achieved after topical dosing (14, 20, 23), despite the greater amount of drug administered by injection. For example, a 25-μl topical dose of 1×10^{-2} *M* pilocarpine (0.2% solution) produced a maximum level of ~1 μg/ml in ~30 min. A 200-μl injection of the same concentration produced a maximum level of ~1.8 μg/ml in ~30 min, less than a twofold difference despite an eightfold difference in the amount of drug instilled or injected. Moreover, the duration of drug levels was almost identical. Thus, with pilocarpine, no apparent sustaining effect was evident, unless it was at a low level, and only a modest increase in the peak level occurred. It was possible to produce higher aqueous humor levels of drug with the same drug concentration merely by injecting larger volumes.

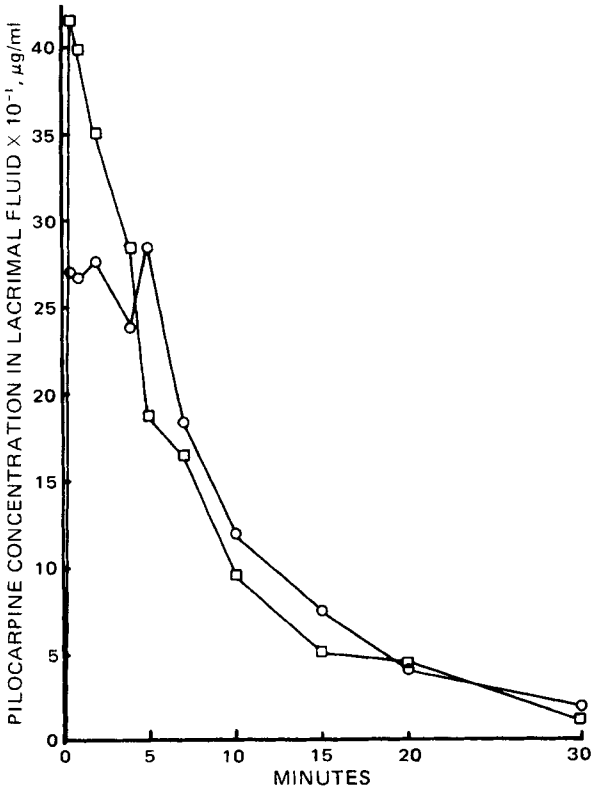


Figure 3—*Lacrimal fluid drug concentration* versus *time profiles for* 1×10^{-2} M *pilocarpine in 200 μl of pH 6.24 isotonic Sorensen buffer following subconjunctival injection* via *various routes in the dosed eye. Key:* ○, *single-puncture injection through the membrane; and* □, *multiple-puncture injection through the membrane. Each point represents a minimum of 13 determinations. Error bars were eliminated for clarity.*

To aid in assessing the experimental changes, the areas under the curve (*AUC*) were measured using a polar planimeter[11] (Table X). In addition, the peak levels and the time to achieve peak levels are important for mechanistic discussion purposes. An assessment of drug bioavailability from this administration route forms the basis for a separate report[12].

The data in Table X are depicted graphically in Figs. 4 and 5 to show the relationship between the dose volume and *AUC* for the cornea and aqueous humor. The insufficient number of points does not fully characterize the lines, but the sigmoidal shape is evident, particularly in the corneal data in Fig. 4. These two graphs may be contrasted with the data for the contralateral eye shown in Fig. 6. A linear relationship was evident over the dose volume range of 50–500 μl. With the contralateral eye, only one mechanism apparently was operative over the dose volume range studied; in the dosed eye, more than one mechanism probably was involved.

Mechanism 1: Drug Passes from Injection Site to Systemic Circulation and Back to Ocular Tissues and Fluids—For this mechanism

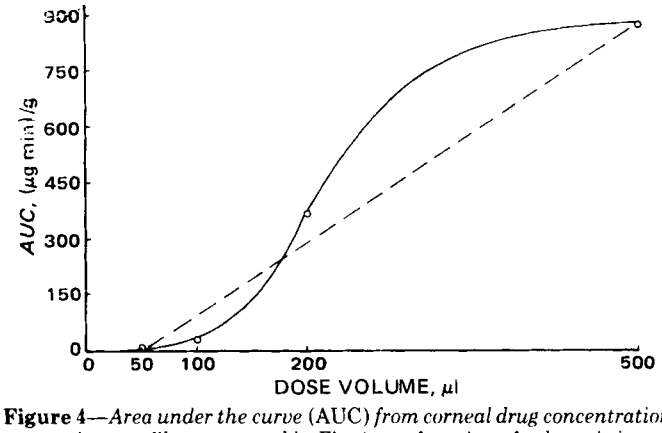


Figure 4—*Area under the curve* (AUC) *from corneal drug concentration* versus *time profiles presented in Fig. 1 as a function of volume injected subconjunctivally through the conjunctival membrane. The dashed line represents the best-fit line through the points, whereas the solid line represents the interpretation of the correct shape of the curve.*

[11] Model 1810-N-10, Dietzgen Corp., Chicago, Ill.
[12] J. Conrad and J. R. Robinson, manuscript in preparation.

Table VIII—Comparison of Fluid or Tissue Drug Concentrations at 30 min following Subconjunctival Dosing with 200 μl of 1×10^{-2} *M* Pilocarpine in pH 6.24 Isotonic Sorensen Buffer *via* Various Routes of Administration

Tissue or Fluid	Single-Puncture Injection through Membrane	Multiple-Puncture Injection through Membrane	Single-Puncture Injection through Eyelid
Aqueous humor[a], dosed eye	1.817 (0.314)[b] [12][c]	2.250 (0.399)[b] [14][c]	0.042 (0.002)[b] [10][c]
Aqueous humor[a], contralateral eye	0.027 (0.002) [13]	0.036 (0.002) [14]	0.036 (0.002) [10]
Cornea, dosed[d] eye	6.482 (1.340) [10]	7.384 (1.285) [13]	0.119 (0.034) [12]
Cornea, contralateral[d] eye	0.057 (0.008) [13]	0.082 (0.006) [13]	0.058 (0.007) [12]
Blood[e]	0.172 (0.014) [10]	0.134 (0.003) [25]	0.148 (0.006) [12]
Lacrimal fluid[f]	18.8 (6.4) [13]	11.5 (5.1) [14]	0.437 (0.279) [11]

[a] Micrograms of pilocarpine per milliliter of aqueous humor. [b] Numbers in parentheses refer to the standard error of the mean. [c] Numbers in brackets refer to the number of determinations at that point. [d] Micrograms of pilocarpine per gram (wet weight) of cornea. [e] Micrograms of pilocarpine per milliliter of blood. [f] Micrograms of pilocarpine per milliliter of lacrimal fluid (tears).

Table IX—Concentration of Drug in Excised Conjunctival Bleb at 60 min following Subconjunctival Dosing with 200 μl of 1×10^{-2} *M* Pilocarpine in pH 6.24 Isotonic Sorensen Buffer *via* Injection through the Conjunctival Membrane or through the Upper Eyelid

	Micrograms of Pilocarpine per Gram of Conjunctiva (Wet Weight)
Through membrane	25.28 (3.11)[a] [21][b,c]
Through eyelid	3.267 (0.966)[a] [18][b,c]

[a] Numbers in parentheses refer to the standard error of the mean. [b] Numbers in brackets refer to the number of determinations at that point. [c] The wet weight of the conjunctival bleb was 240.6 (11.7) μg for the through-the-membrane tissue and 128.2 (7.8) μg for the through-the-eyelid case.

Table X—Comparison of *AUC* Values Obtained after Each Route of Administration for 1×10^{-2} *M* Pilocarpine in pH 6.24 Isotonic Sorensen Buffer Injected Subconjunctivally or Intravenously

Tissue or Fluid	Volume Injected, μl	Through Membrane, Single Puncture, (μg min)/g or ml	Through Upper Eyelid, (μg min)/g or ml
Cornea, dosed[a] eye	50	7.329	7.326
	100	28.363	
	200	367.970	15.136
	500	874.680	
Cornea[a], contralateral eye	50	1.753	4.268
	100	3.140	
	200	11.127	8.498
	500	31.441	
Aqueous humor[a], dosed eye	50	9.742	1.094
	100	20.606	
	200	89.602	4.574
	500	216.598	
Aqueous humor[a], contralateral eye	50	0.759	1.075
	100	1.952	
	200	4.078	4.260
	500	9.701	
Circulating blood[a]	50	2.867	4.603
	100	5.085	
	200	10.279	11.744
	500	24.257	
Lacrimal fluid[b]	200	3265.250	6.699

[a] Measured over first 120 min. [b] Measured over first 30 min. The value was 3137.250 (μg min)/ml in the multiple-puncture through-the-membrane study.

to be important, equivalent, or at least close to equivalent, drug concentration–time profiles should occur in both the dosed and the contralateral eyes. This clearly is not the case, and earlier studies that measured drug in both the dosed and contralateral eye (24, 25) agree with this finding. The time to achieve peak levels in the aqueous humor of the dosed eye was ~30 min, whereas it was ~60 min or longer in the contralateral eye. Moreover, the maximum concentrations achieved in the dosed and contralateral eyes differed by orders of magnitude, as did the *AUC*.

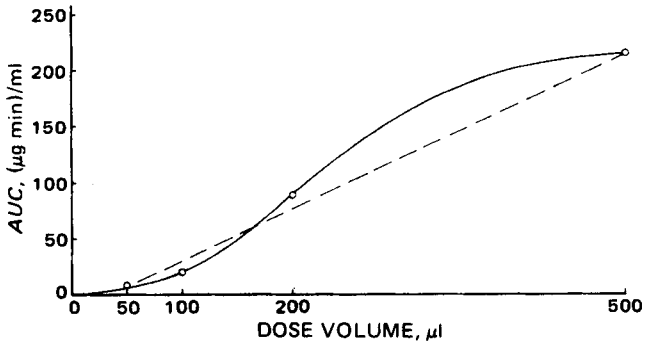


Figure 5—*Area under the curve* (AUC) *from aqueous humor drug concentration* versus *time profiles presented in Fig. 2 as a function of volume injected subconjunctivally through the conjunctival membrane. The dashed line represents the best-fit line through the points, whereas the solid line represents the interpretation of the correct shape of the curve.*

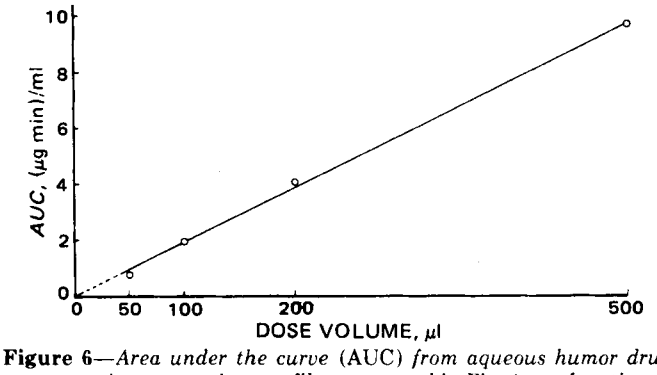


Figure 6—*Area under the curve* (AUC) *from aqueous humor drug concentration* versus *time profiles presented in Fig. 4 as a function of volume injected subconjunctivally through the conjunctival membrane.*

AUC is related directly to the dose volume injected (Fig. 7). Most of the injected drug will be absorbed into the systemic circulation, so a linear relationship is expected.

When the *AUC* for the circulating blood levels at each volume is plotted *versus* the aqueous humor *AUC* for the dosed and contralateral eyes, the relationships shown in Figs. 8 and 9 result. The intercept in the case of the contralateral eye is zero, suggesting that all of the drug in that eye came from drug in the blood. However, for the dosed eye, the intercept is not zero but intercepts on the blood *AUC* axis, suggesting a drug source other than the blood.

Another aspect of the blood level studies related to mechanistic in-

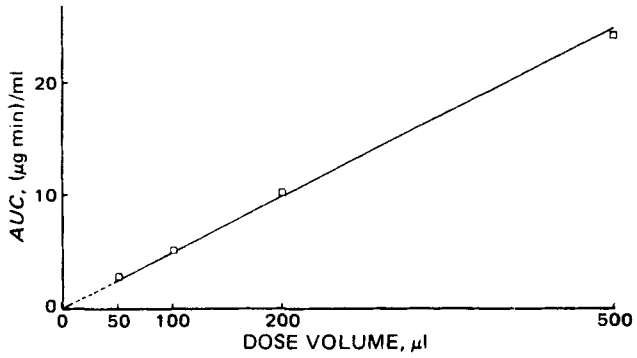


Figure 7—*Area under the curve* (AUC) *from circulating blood drug concentration* versus *time profiles presented in Table III as a function of volume injected subconjunctivally through the conjunctival membrane.*

terpretation is the time–concentration profile resulting from through-the-eyelid injection in contrast to through-the-membrane injection (Table XI). Aside from some minor differences in the peak time, the data are very similar. Moreover, the *AUC*s in these studies are very similar as seen in Table XI.

The present work was conducted in rabbits with an estimated blood volume of about 1 liter. The human eye is similar in size to that of the rabbit, but the circulating blood is 7–8 liters, so the dilution effect is expected to be even more dramatic. It seems reasonable to conclude that a minor amount of drug in the aqueous chamber of the dosed eye comes from the circulating blood.

Mechanism 2: Drug Penetrates Eye from Subconjunctival Injection Site by Simple Diffusion through Tissues Underlying Site—Earlier autoradiographic studies (5) showed that hydrocortisone penetrates the sclera, choroid, and other layers of the globe of the eye. In addition, it is expected that drug moves across limbal vessels to enter the iris and cornea. However, with pilocarpine, this mechanism does not appear to be major for drug gaining access to the anterior segment of the eye.

If movement of drug through the layers of the globe is the principal mechanism, then the method of placement, *i.e.*, puncture or no puncture, should have no effect on the drug levels. Clearly, the through-the-eyelid injection produced drug levels in the cornea and aqueous humor that were orders of magnitude lower (Table XII).

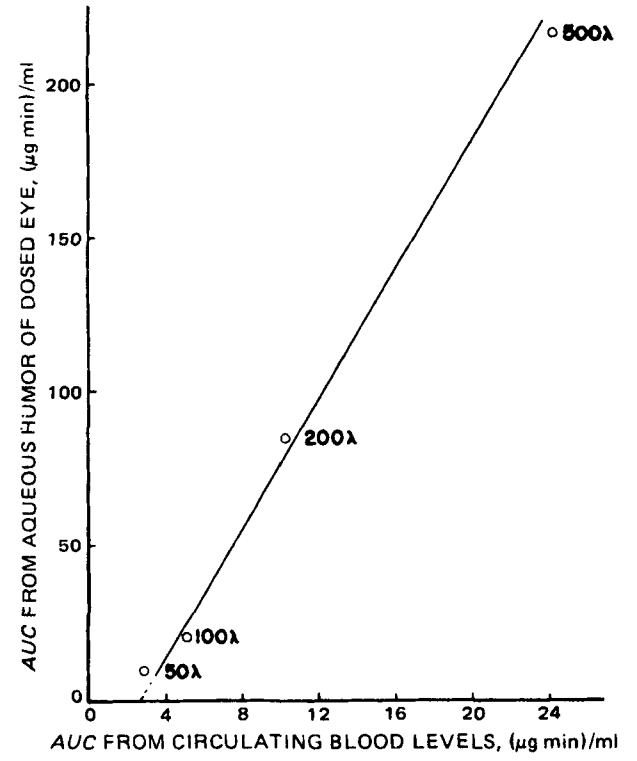


Figure 8—*Comparison of the area under the curve* (AUC) *from aqueous humor drug concentration* versus *time profiles presented in Fig. 2 to the area under the curve* (AUC) *from circulating blood drug concentration* versus *time profiles presented in Fig. 5 at each volume injected subconjunctivally through the conjunctival membrane.*

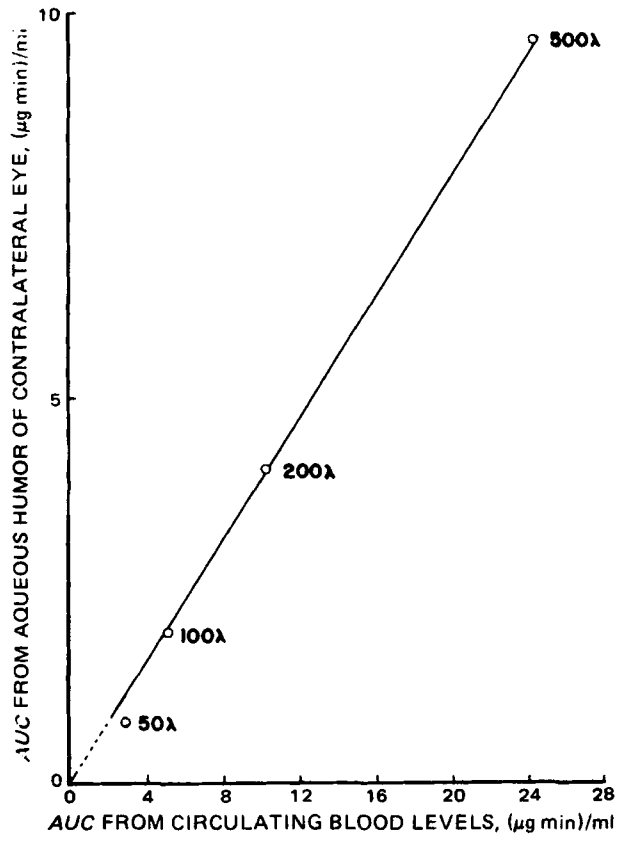


Figure 9—*Comparison of the area under the curve* (AUC) *from aqueous humor drug concentration* versus *time profiles presented in Fig. 4 to the area under the curve* (AUC) *from circulating blood drug concentration* versus *time profiles presented in Fig. 5 at each volume injected subconjunctivally through the conjunctival membrane.*

Additional support for the argument that the majority of drug does not come from penetration of the globe can be seen by comparing the drug level in the cornea with that in the aqueous humor (Fig. 10). The linearity of this plot, particularly at high dose volumes, suggests that almost all of the drug in the aqueous humor is being provided by the cornea. Earlier studies in this laboratory (11, 14, 20, 21, 23, 26) showed that back-diffusion from the aqueous humor to the cornea is not significant, so the high levels in the cornea and the linear relationship with aqueous humor levels suggest that the drug is provided by the cornea and not the globe of the eye.

Thus, for pilocarpine, direct penetration of the globe is not a major factor in getting the drug to the aqueous chamber. For more oil-soluble

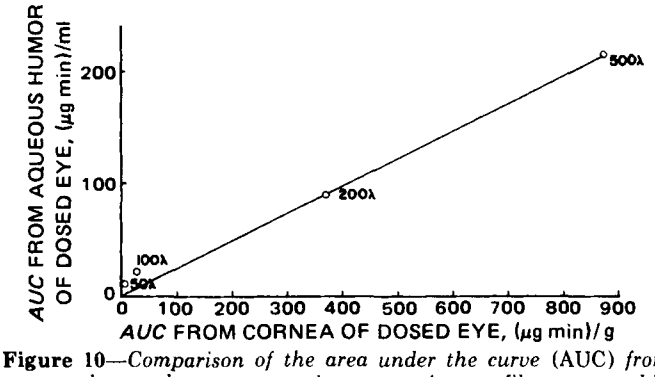


Figure 10—*Comparison of the area under the curve* (AUC) *from aqueous humor drug concentration* versus *time profiles presented in Fig. 1 to the area under the curve* (AUC) *from corneal drug concentration* versus *time profiles presented in Table I at each volume injected subconjunctivally through the conjunctival membrane.*

Table XI—Comparison of Circulating Blood Level Drug Concentration *versus* Time following 50- and 200-μl Subconjunctival Injections of 1×10^{-2} *M* Pilocarpine in pH 6.24 Isotonic Sorensen Buffer [a]

Minutes	50-μl Dose		200-μl Dose	
	Through Membrane, Single Puncture	Through Upper Eyelid	Through Membrane, Single Puncture	Through Upper Eyelid
5	0.035 (0.001)[b] [9][c]	0.051 (0.004)[b] [12][c]	NS[d]	0.158 (0.007)[b] [12][c]
10	0.034 (0.002) [11]	0.073 (0.003) [12]	0.067 (0.009)[b] [9][c]	0.172 (0.004) [10]
20	0.041 (0.002) [12]	0.057 (0.003) [12]	0.108 (0.011) [10]	0.157 (0.007) [10]
30	0.033 (0.001) [11]	0.064 (0.004) [12]	0.172 (0.014) [10]	0.148 (0.006) [12]
45	0.027 (0.002) [12]	0.051 (0.003) [12]	0.132 (0.013) [10]	0.112 (0.005) [8]
60	0.023 (0.001) [10]	0.044 (0.002) [12]	0.101 (0.004) [10]	0.091 (0.003) [10]
90	0.016 (0.001) [10]	0.012 (0.002) [10]	NS[d]	0.053 (0.002) [12]
120	0.014 (0.001) [12]	0.021 (0.001) [9]	0.022 (0.004) [7]	0.061 (0.003) [10]

[a] Values are the micrograms of pilocarpine per milliliter of blood. [b] Numbers in parentheses refer to the standard error of the mean. [c] Numbers in brackets refer to the number of determinations at that point. [d] Not sampled.

drugs, such as some corticosteroids, this route may be important. Additional studies are needed to confirm or reject this possibility.

Mechanism 3: Drug Gains Entrance to Eye by Refluxing out of Injection Site followed by Corneal Absorption—The linearity of the data in Fig. 10 suggests that transcorneal absorption of drug is the primary mechanism for providing pilocarpine to the aqueous humor from a subconjunctival injection. In Fig. 10, the 50- and 100-μl dose volumes fall slightly off the line, suggesting that multiple mechanisms may account for the lower volumes, *i.e.*, Mechanisms 1–3; with larger volumes, a transcorneal mechanism appears predominant. Figures 4 and 5 indicated that multiple mechanisms may have occurred based on the relationship between the *AUC* and dose volume; as the injection volume increases, a back-pressure at the injection site may force leakage out of the puncture hole.

The case for leakage seems evident after comparing aqueous humor drug levels in the dosed eye from through-the-membrane and through-the-eyelid studies. Supportive data come from the lacrimal fluid studies. At the 200-μl injection volume, it is clear from the profiles given in Table VII and in Figs. 3 and 4, as well as from the *AUC* values found in Table XI, that leakage must have occurred in the membrane puncture studies. Injection of drug through the eyelid shows a small quantity of drug in the tear film, presumably from diffusion across the conjunctiva and into the tear film, but the three orders of magnitude difference in tear film concentration shown in Table VII apparently is due to leakage.

It is anticipated that at larger injected volumes, such as 500–1000 μl, the leakage mechanism would remain predominant. On the other hand, smaller volumes are not expected to be as dramatic and, for the 25–100-μl doses, far less drug is expected in the tear film since other mechanisms appear important.

A recent study on the mechanism of the release of fluorescein from subconjunctival injection in the rabbit was reported by Maurice and Ota (6). In this study, small injection volumes (*i.e.*, 1 μl) were employed to avoid a bleb and the drug was administered by membrane puncture and by eyelid puncture. These investigators visually observed the leakage of fluorescein out of the injection site, which is in agreement with the present findings.

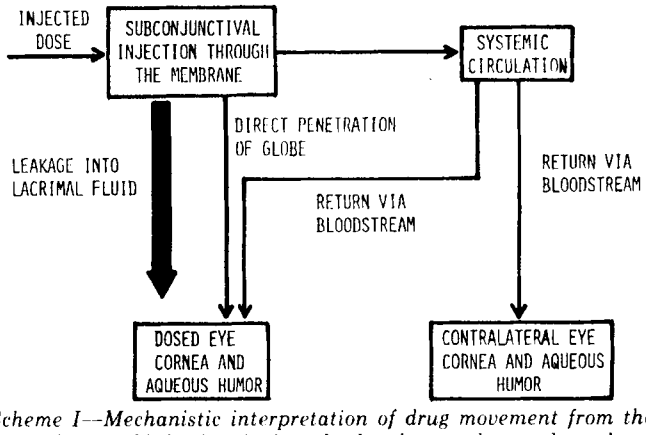
Relationship of This Study to Other Drugs and to Humans—The present study was conducted with the water-soluble alkaloid pilocarpine. It is not possible to extrapolate from this single drug entity to other drugs as to their mechanisms of absorption from subconjunctival injection. However, since the principal mechanism for pilocarpine absorption involves leakage, a process independent of drug structure, it is reasonable to assume that a pulse of drug will exit from the injection site and enter the tear film in the early minutes after dosing. Whether this initial dose can establish a high level of drug in the cornea and aqueous humor is dependent primarily on the initial concentration of the drug injected and the injected volume. Presumably, increasing the viscosity of the injected solution or injection of a drug suspension would minimize the reflux process and thus diminish the peak drug level.

It seems unlikely that drugs with a more favorable partition characteristic for absorption will be absorbed so rapidly into the eye, either by direct scleral absorption or absorption across the conjunctiva followed by corneal absorption, so as to overwhelm the contribution from leakage during the early stages following injection. At later times, all drugs probably will show a local sustaining effect since pilocarpine was shown to be present in the bleb a long time after injection. Whether this localized drug can penetrate the globe of the eye over a long time to give a sustaining effect should be a function of its partitioning characteristics. The

Table XII—Comparison of Aqueous Humor Drug Concentration *versus* Time following 50- and 200-μl Subconjunctival Injections of 1×10^{-2} *M* Pilocarpine in pH 6.24 Isotonic Sorensen Buffer [a]

Minutes	50-μl Dose		200-μl Dose	
	Through Membrane, Single-Puncture	Through Upper Eyelid	Through Membrane, Single Puncture	Through Upper Eyelid
5	0.047 (0.019)[b] [13][c]	0.005 (0.0004)[b] [9][c]	0.386 (0.078)[b] [11][c]	0.021 (0.003)[b] [8][c]
10	0.127 (0.075) [11]	0.006 (0.0006) [11]	0.703 (0.133) [12]	0.024 (0.001) [10]
20	0.180 (0.102) [11]	0.009 (0.0004) [12]	1.442 (0.291) [14]	0.032 (0.001) [9]
30	0.195 (0.118) [9]	0.011 (0.0006) [8]	1.817 (0.314) [12]	0.042 (0.002) [10]
45	0.136 (0.068) [8]	0.010 (0.0007) [12]	1.385 (0.213) [13]	0.050 (0.003) [12]
60	0.049 (0.006) [8]	0.011 (0.0007) [11]	0.505 (0.082) [13]	0.038 (0.002) [10]
90	0.032 (0.012) [11]	0.009 (0.0006) [9]	NS[d]	0.040 (0.002) [12]
120	0.026 (0.008) [9]	0.009 (0.0004) [10]	0.199 (0.035) [16]	0.041 (0.001) [7]

[a] Values are the micrograms of pilocarpine per milliliter of aqueous humor. [b] Numbers in parentheses refer to the standard error of the mean. [c] Numbers in brackets refer to the number of determinations at that point. [d] Not sampled.



Scheme I—Mechanistic interpretation of drug movement from the subconjunctival injection site into the dosed eye and contralateral eye. At large injection volumes, leakage is the predominant mechanism, illustrated by the thick arrow. At low injection volumes, all mechanisms contribute to the process.

water-soluble drug pilocarpine did not penetrate, but apparently the more oil-soluble hydrocortisone did (5). Additional studies with other drug entities and formulation changes will clarify this picture.

The relationship of the results in rabbits to expected behavior in humans is not as clear. Maurice and Ota (6) injected small volumes of fluorescein subconjunctivally into rabbits and humans. They observed leakage of the dye in the rabbit but not in humans; however, higher levels of fluorescein in the human aqueous humor compared to the rabbit were obtained. Based on these findings, the question of the suitability of the rabbit as a model for subconjunctival injections was raised.

There has been controversy over the behavior of fluorescein in these two species (27), and the differences may be due to the dye itself. Moreover, while the small volume interpretation of these investigators may be accurate, it probably bears little relationship to what occurs at higher volumes. It is probable, based on the work of Maurice and Ota (6), that human and rabbit eyes differ in the importance of one mechanism over another, especially at low volumes. Intuitively, however, since leakage depends on pressure and large volumes are used clinically, it is not unreasonable to assume that humans and rabbits will behave similarly insofar as major mechanisms of drug availability are concerned.

CONCLUSIONS

The study was initiated to provide mechanistic insight into the movement of drug from the site of subconjunctival injection to the aqueous chamber. Several mechanisms apparently are involved in pilocarpine disposition in albino rabbits, depending on the volume of solution injected. At low volumes, it appears that transconjunctival permeation followed by corneal absorption, systemic absorption followed by return *via* the vascular bed, reflux out of the injection site, and, finally, direct penetration of the globe of the eye all contribute to aqueous humor drug levels. However, at larger volumes, leakage from the injection site is the overwhelming mechanism for drug availability (Scheme I).

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