it did so diametrically along a sharp line parallel to the direction of the applied force. It appears then that the film exerted its primary influence along this line under the test conditions employed. To verify this observation, cuts were carefully made with a scalpel through the coating along the diameter while other samples had cuts made around the circumference. The tablets with the cuts along the diameter were placed in the hardness tester such that the cut was either parallel or perpendicular to the applied force. Hardness measurements were made on these tablets (Table IV).

In cases where the cut was perpendicular to the applied force or the cut was around the outside edge, only minimal changes in hardness from intact coated tablets were observed. However, when the cut was aligned parallel to the applied force where normal tensile failure occurs, the film was unable to absorb any tensile stress and hardness values fell close to those for uncoated cores. These data support the use of the term A_t as the proportionality factor introduced in Eq. 2.

REFERENCES

(1) C. J. Endicott, W. Lowenthal, and H. M. Gross, *J. Pharm. Sci.*, **50**, 343(1961).

(2) H. J. Fairchield and F. Michel, *ibid.*, **50**, 966(1961).

(3) J. A. Seitz and G. M. Flessland, *ibid.*, **54**, 1353(1965).

(4) D. B. Brook and K. Marshall, *ibid.*, **57**, 481(1968).

(5) J. T. Fell and J. M. Newton, *J. Pharm. Pharmacol.*, **20**, 657(1968).

(6) J. T. Fell and J. M. Newton, *J. Pharm. Sci.*, **59**; 688(1970).

(7) E. N. Hiestand and C. B. Peot, *ibid.*, **63**, 605(1974).

ACKNOWLEDGMENTS AND ADDRESSES

Received August 18, 1975, from *Pharmaceutical Chemistry, Smith Kline & French Laboratories, Philadelphia, PA 19101*

Accepted for publication November 10, 1975.

The author thanks Mr. Robert Dolan for technical assistance.

Quantitative Precorneal Disposition of Topically Applied Pilocarpine Nitrate in Rabbit Eyes

THOMAS F. PATTON * and JOSEPH R. ROBINSON ˣ

Abstract □ The present study was designed to quantitate the influence of several precorneal factors on the disposition of topically applied ophthalmic drugs. With tritiated pilocarpine nitrate, methodology was developed for *in vivo* assessment of the relative contribution of tear turnover, instilled solution drainage, and nonproductive absorption to the loss of drug from the precorneal area. Studies were conducted in both awake and anesthetized rabbits whose drainage ducts were either unobstructed or plugged, and the loss of drug was monitored directly from the precorneal area or as appearance in the aqueous humor. By selective variation in experimental conditions, the influence of tear turnover, instilled solution drainage, and nonproductive absorption on ocular drug bioavailability was separately studied and quantitated. Instilled solution drainage was by far the largest contributing factor in the loss of drug from the precorneal area of the eye and, in the range of instilled volumes normally employed, tear turnover played a relatively minor role in drug loss. Compared to the cornea, precorneal tissue other than the cornea has a considerably greater surface area and thus is a potentially significant route for drug loss. However, under normal circumstances, loss by this route was minimal as compared to loss *via* instilled solution drainage.

Keyphrases □ Pilocarpine nitrate—topically applied, precorneal disposition, effect of tear turnover, instilled solution drainage, and nonproductive absorption □ Precorneal disposition—pilocarpine nitrate, topically applied, effect of tear turnover, instilled solution drainage, and nonproductive absorption □ Bioavailability—pilocarpine nitrate, topically applied, effect of tear turnover, instilled solution drainage, and nonproductive absorption □ Topically applied drugs—pilocarpine nitrate, precorneal disposition □ Ophthalmic drugs—pilocarpine nitrate, topically applied, precorneal disposition

Topical application of drugs to the eye is the most frequently employed route of administration for the treatment of various eye disorders. Unfortunately, the disposition of drugs administered by this route is not well understood, although it is generally agreed that the bioavailability of topically applied drugs is extremely poor. The present study was designed to provide a quantitative accounting of the precorneal distribution of pilocarpine nitrate and to generate some mechanistic insight into its relatively poor bioavailability.

Many factors can affect the bioavailability of topically applied ophthalmic drugs. The presence of tears in the cul-de-sac dilutes any instilled drug, and the continual addition and removal of tears can cause a significant loss of applied drug. In addition, the efficient drainage apparatus, used for removal of tears, also serves as a conduit through which instilled drug solutions can be lost from the precorneal area. Moreover, substances normally present in tear fluid can bind and/or degrade instilled drugs. Finally, topically applied drugs may be absorbed into a variety of ocular tissues, most notably the cornea and conjunctiva. To maximize therapy with topically applied ocular drugs, it is necessary to know the amounts and rates at which drugs are lost to these various precorneal routes and the relative contribution of each to the bioavailability question.

Numerous studies over the years were directed toward an understanding of tear properties, production, and drainage (1–20). However, only recently has attention been focused on the role of tear turnover and instilled solution drainage on ocular drug bioavailability (21). Studies describing some interactions of drugs with the components of tears also were reported (22, 23).

Topically applied drugs in the eye that are not lost to the drainage apparatus nor absorbed by the cornea are potentially available to be absorbed by the conjunctiva and, ultimately, the sclera or to be absorbed onto the lids. These routes are referred to as nonproductive, since it is unlikely that much or any drug from these areas will penetrate the interior portions of the eye due to rapid removal by local circulation (24–29).

The present study attempted to evaluate individually these routes of drug loss and to quantitate drug move-

ment and distribution in the precorneal portion of the eye.

EXPERIMENTAL

Materials—Pilocarpine nitrate USP was obtained commercially[1] and used without further purification. Tritiated pilocarpine[2] (specific activity 6.95 Ci/mmole) was purified by vacuum evaporation as previously described (30, 31). All other chemicals were either reagent or analytical grade and were used as received.

Male albino rabbits[3], 1.8–2.4 kg, were maintained on a normal diet, and no restrictions were placed on the amount of food or water consumed.

Methods—*Solution Preparation*—Pilocarpine nitrate solutions were prepared in isotonic Sorensen's phosphate buffer, pH 6.24 (32). In all studies, the concentration of pilocarpine nitrate employed was 1×10^{-2} *M*. No attempt was made to maintain the sterility of the solutions, but they were filtered for clarity. Solutions were prepared fresh and kept for a maximum of 48 hr. For each milliliter of 1×10^{-2} *M* pilocarpine nitrate to be prepared, 0.25 mCi of tritiated pilocarpine provided a final activity of 125,000–150,000 counts/min/μl. The small amount of isotopic material present did not affect the molarity of the final solution.

Anesthesia—Procedures for inducing and maintaining the desired level of general anesthesia were reported previously (21).

Blockage of Lacrimal and Instilled Fluid Drainage—In certain experiments, it was necessary to prevent instilled drug solution drainage through blockage of the drainage duct. While humans possess two puncta for drainage, rabbits have only one punctum, located deep in the inner surface of the lower lid near the medial canthus (33). Access to this punctum is difficult, and several methods of sealing it were attempted before the following procedure was adopted.

Polyethylene tubing[4] (PE 50), 0.61 mm i.d. and 0.96 mm o.d., was cut into sections approximately 5 mm in length. One end was cut into a bevel, and the other end was heat sealed. The beveled end was inserted into the punctum and threaded into the duct. The small cap which results from heat sealing serves to prevent the plug from slipping down into the duct and rests just at the surface of the punctum.

Care must be taken to ensure that the plug is inserted far enough so that the head does not protrude and scratch the cornea or conjunctiva. It was determined by the use of dye solutions that the plugs were effective in completely preventing any instilled solution drainage from the eye. It was also established that no drug was absorbed into the plugs.

Pilocarpine Nitrate Aqueous Humor Concentration–Time Profiles—In this series of experiments, four different categories of test animals were used: (*a*) normal unanesthetized rabbits, (*b*) unanesthetized rabbits whose drainage ducts had been plugged, (*c*) normal anesthetized rabbits, and (*d*) anesthetized rabbits whose drainage ducts had been plugged.

During the experiments, all test animals were kept in restraining boxes in the normal upright position. Twenty-five microliters of drug solution was instilled[5] onto the cornea of the test animal and collected in the lower cul-de-sac. In animals whose drainage ducts had been plugged, drug instillation was made 1 min after insertion of the plug. During instillation, the lower lid was pulled slightly away from the globe but was returned to its normal position immediately after instillation.

At various times postinstillation, rabbits were sacrificed by rapid injection of an overdose of pentobarbital sodium into a marginal ear vein. Eyes were immediately rinsed with distilled water and blotted with tissues to remove any residual radioactivity in the precorneal area. A single puncture then was made at the corneo-scleral junction, and 100–200 μl of aqueous humor was aspirated from the anterior chamber. One hundred microliters of aqueous humor was transferred[6] to a scintillation counting vial[7] containing 5 ml of liquid scintillation cocktail[8]. Vials containing the cocktail were refrigerated for 24 hr prior to addition of the samples; after addition of the samples, they were stored in the dark at room temperature for at least 24 hr prior to counting to minimize chemiluminescence.

Two consecutive 1-min counts were made[9] on each sample. Since quenching of radioactivity by aqueous humor was relatively constant from animal to animal, blank corrections were made on the basis of an aqueous humor sample from a single animal in each experiment. After suitable corrections, the counts were converted to micrograms per milliliter of aqueous humor.

The data were fit to a one-compartment open model, and graphical estimates of the parameters were obtained. The estimates were then subjected to computer analysis[10] to obtain final parameter values.

Loss of Pilocarpine Nitrate from Precorneal Area—Loss of drug directly from the precorneal area was determined by periodic sampling of the tear film following instillation of 25 μl of pilocarpine solution. Methodology for monitoring the loss of drug from the precorneal area of the eye had been developed previously (21), but such experiments had not been carried out for a drug that is absorbed into tissues.

Both awake and anesthetized animals were prepared in exactly the same manner as just described. In this series of experiments, however, only animals with drainage ducts plugged were employed. One minute after insertion of the plugs, pilocarpine solution was instilled. Fifteen seconds postinstillation, a 1-μl sample was withdrawn[11] from the tear film and assayed for drug content. The 15-sec time point was chosen to allow for mixing of the drug solution with the resident tear fluid and was designated as time zero. Additional samples were withdrawn at 5, 15, 30, 45, 60, and 90 min postinstillation. All samples were withdrawn from the center of the marginal tear strip.

Glass capillaries containing the 1-μl samples were dropped into 5 ml of scintillation cocktail and counted after 24 hr of storage. Procedures and preparation for liquid scintillation counting were identical to those previously described.

Data were converted to micrograms per milliliter of lacrimal fluid and analyzed *via* linear regression analysis. In so doing, it was possible to obtain first-order rate constants for the disappearance of drug from the precorneal area of the eye in both anesthetized and unanesthetized rabbits.

RESULTS

Aqueous Humor Concentration–Time Profiles—The concentrations of pilocarpine found in aqueous humor at various times postinstillation are compiled in Table I for the various categories of test animals. Examination of these data reveals that both anesthesia and the absence of drainage appear to result in more or less of an increase in the bioavailability of instilled pilocarpine solutions over that seen in unanesthetized animals with normal drainage function. Anesthesia significantly reduces or eliminates tear production in rabbits (21), so this experiment is a direct measure of the influence of lacrimation on ocular drug bioavailability. The purpose of the blocked drainage duct experiment is self-evident.

Analysis of the data in Table I produced the pharmacokinetic values shown in Table II. Two points are immediately evident when these data are inspected, specifically with regard to k_{abs}, FD/V, area, and peak time. Data for the duct open case show significant differences from those with the duct plugged, and, within a given category, either duct open or duct plugged, there is only a marginal, if any, difference in the parameter values. Areas under the aqueous humor drug concentration–time profiles reflect the differences in pilocarpine bioavailability for these experimental variations.

Measurement of Pilocarpine Nitrate Loss from Precorneal Pocket—An alternative approach to measuring drug appearance in aqueous humor is to measure its disappearance from the precorneal area of the eye. Since aqueous humor sampling presumably only measures drug that has penetrated the cornea, sampling of drug in the precorneal pocket should provide a measure of the loss of drug to other precorneal routes as well. This sampling procedure has the inherent disadvantage that, since the normal resident volume in the precorneal area is small, removal of even the smallest usable sample appreciably disturbs the system unless it is replaced. However, replacing the withdrawn volume can change the fluid dynamics in this area and is therefore undesirable. In addition, since the volume is so

[1] Merck and Co., Rahway, N.J.
[2] New England Nuclear, Boston, Mass.
[3] Klubertanz, Edgerton, Wis.
[4] Intramedic, Clay Adams Division of Becton Dickinson Co., Parsippany, N.J.
[5] Hamilton Co., Reno, Nev.
[6] Biopette, Schwarz/Mann, Orangeburg, N.Y.
[7] Mini vials, ICN, Irvine, Calif.
[8] Aquasol, New England Nuclear, Boston, Mass.
[9] Packard model 2002, Packard Instruments Co., Downers Grove, Ill.
[10] Madison Academic Computing Center Subroutine NREG.
[11] Microcap, Drummond Scientific Co., Bloomfield, N.J.

Table I—Aqueous Humor Concentration–Time Profiles following Instillation of 25 μl of a 1 × 10^{-2} *M* Solution of Pilocarpine Nitrate

Minutes	Micrograms of Pilocarpine per Milliliter of Aqueous Humor			
	Unanesthetized Duct Open	Unanesthetized Duct Plugged	Anesthetized Duct Open	Anesthetized Duct Plugged
5	0.34 (0.02)[a] [18][b]	0.38 (0.06) [8]	0.36 (0.05) [6]	0.70 (0.07) [6]
10	0.72 (0.09) [15]	0.82 (0.07) [8]	1.15 (0.22) [8]	1.53 (0.09) [7]
15	0.73 (0.06) [17]	1.33 (0.12) [8]	0.97 (0.10) [7]	1.81 (0.15) [6]
20	1.03 (0.08) [19]	1.66 (0.14) [7]	1.05 (0.14) [8]	1.79 (0.11) [7]
30	0.72 (0.07) [19]	2.16 (0.12) [7]	1.45 (0.30) [6]	2.40 (0.29) [6]
45		1.88 (0.18) [8]	0.70 (0.17) [7]	2.04 (0.33) [7]
60	0.62 (0.06) [15]	2.58 (0.38) [11]	0.51 (0.13) [5]	1.57 (0.24) [5]
75		1.70 (0.21) [6]		
90	0.26 (0.02) [14]	1.22 (0.22) [11]	0.65 (0.13) [10]	1.10 (0.17) [8]
105		1.00 (0.24) [6]		
120	0.12 (0.01) [7]	0.77 (0.23) [8]	0.19 (0.02) [6]	1.01 (0.22) [5]

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

small and diffuse, withdrawing samples is quite difficult and can result in substantial irritation to the eye with subsequent lacrimation.

Due to these considerations, samples were withdrawn following the instillation of pilocarpine nitrate solutions only in rabbits whose drainage ducts had been plugged. Blockage of the drainage duct causes the instilled solution, after mixing with the resident tear fluid, to form a "pool" on the lower marginal tear strip. This makes withdrawal of 1-μl samples a relatively easy procedure and does not result in irritation to the eye. Also, the removal of several 1-μl samples over 90 min does not appreciably change the volume present, especially in awake animals whose normal tear production more than compensates for the withdrawal rate.

Withdrawal of 1-μl fluid samples from the precorneal area of rabbits with occluded drainage ducts at various times postinstillation revealed a first-order decline in drug concentration. Drug concentrations were plotted semilogarithmically *versus* time and subjected to linear regression analysis, and first-order rate constants were determined. Tables III and IV show the results of these studies. The data show good reproducibility both within and among rabbits and no significant variations due to either the eye used or the weight of the animal.

The first-order loss of drug from the precorneal area in the absence of drainage accounts for the loss of drug to all routes including conjunctival and corneal except drainage. It is necessary, however, to account for the difference in the observed rate constants between awake and anesthetized rabbits. Rabbits under systemic anesthesia of the type employed here do not produce tears (21). It is suggested that the larger rate constant for the decline in precorneal drug concentration in awake rabbits can be attributed to the dilution effect of incoming tears.

The rate of tear production in normal unanesthetized rabbits was determined (21) to be 0.66 μl/min. Using this rate enables one to calculate the dilution effect of tears for a given initial concentration of drug in the precorneal area in the absence of drainage. Table V illustrates the calculated dilution effect of tears on precorneal drug concentration following instillation of 25 μl of drug solution in rabbits whose drainage apparatus has been blocked.

This first-order decline in drug concentration has an associated rate constant of 1.16 × 10^{-2} min^{-1}. If this rate constant is added to that obtained for the decline in drug concentration in anesthetized rabbits with drainage ducts plugged (2.17 × 10^{-2} min^{-1}), the sum is 3.33 × 10^{-2} min^{-1}. This number is virtually identical to that obtained in awake rabbits where both absorption and dilution by tears are operable. Similarly, if one calculates the theoretical decline in precorneal drug concentration as a result of both absorption and tear production,

Table II—Pharmacokinetic Parameters for Topically Applied Pilocarpine Nitrate Solution[a]

Parameter	Unanesthetized Duct Open	Unanesthetized Duct Plugged	Anesthetized Duct Open	Anesthetized Duct Plugged
k_{abs}, min^{-1} [b]	7.80 × 10^{-2} (4.75 × 10^{-2}–1.08 × 10^{-1})	2.83 × 10^{-2} (2.00 × 10^{-2}–3.66 × 10^{-2})	8.31 × 10^{-2} (3.41 × 10^{-2}–1.32 × 10^{-1})	4.44 × 10^{-2} (3.38 × 10^{-2}–5.50 × 10^{-2})
K_{el}, min^{-1}	2.00 × 10^{-2}	2.00 × 10^{-2}	2.00 × 10^{-2}	2.00 × 10^{-2}
$\frac{FD}{V}$ [b]	1.39 (1.15–1.62)	4.81 (4.10–5.52)	1.81 (1.38–2.24)	4.23 (3.74–4.73)
Area[c]	69.5	240.5	90.5	211.5
Peak time[d], min	20–25	35–40	20–25	35
r-squared for function[e]	0.978	0.973	0.943	0.988
Correlation coefficient for function	0.963	0.952	0.889	0.968

[a] Obtained by fitting the data to the one-compartment open model *via* the computer program NREG. In all cases, the program was modified by fixing K_{el} prior to computer analysis. This procedure was necessary due to the closeness of k_{abs} to K_{el} in cases where drainage ducts were plugged. In these cases, the program was not able to distinguish between k_{abs} and K_{el} and continually set them equal. This approach was justified since extensive prior experience had confirmed that the value for K_{el} was about 2 × 10^{-2} min^{-1}. [b] Numbers in parentheses refer to the 95% confidence limits of the computer-generated values. [c] Area = FD/VK_{el}. [d] Approximate values read from the graphical printout of the computer-generated fit of the model. [e] r^2 = (Σ squared observations − Σ squared derivations)/Σ squared observations.

Table III—First-Order Rate Constants for the Decline in Drug Concentration in the Precorneal Area of Awake Rabbits with Drainage Ducts Plugged

Rabbit	Weight[a], kg	Eye	k, min^{-1}[b]	Correlation Coefficient for Line
1	1.9	Right	2.64×10^{-2}	0.968
	2.0	Right	3.61×10^{-2}	0.985
	1.9	Left	5.36×10^{-2}	0.957
	2.0	Left	2.94×10^{-2}	0.934
2	1.7	Right	2.66×10^{-2}	0.869
	2.1	Right	3.09×10^{-2}	0.914
	1.7	Left	4.82×10^{-2}	0.946
	2.1	Left	3.26×10^{-2}	0.942
3	2.1	Right	2.30×10^{-2}	0.974
	2.1	Right	2.64×10^{-2}	0.886
	2.1	Left	3.10×10^{-2}	0.978
	2.1	Left	3.42×10^{-2}	0.975
Mean k(min^{-1}) = 3.32×10^{-2} (2.64×10^{-3})[c]				

[a] Weights may vary for the same animal due to the time interval between reuse. [b] Calculated from the slope of the line determined *via* linear regression analysis. [c] Number in parentheses refers to the standard error of the mean.

the resulting first-order rate constant is in exact agreement with that found experimentally in Table III. This result is illustrated in Table VI. It is, therefore, confirmed that the larger rate constant for the decline in precorneal drug concentration in unanesthetized rabbits can be attributed to tear production.

In interpreting the results of the tear sampling studies, two other problems deserve comment. The first problem involves separating the observed drug loss into its component parts. In other words, absorption can occur *via* the cornea and into the anterior chamber, *i.e.*, productive absorption, or *via* the conjunctiva from where drug may enter the general circulation, *i.e.*, nonproductive absorption. The tear sampling studies demonstrated the overall loss of drug due to absorption from the precorneal area. To gain a complete quantitative picture, it is necessary to discuss these absorptive routes separately.

The second problem is that of extrapolating the results of the precorneal drug concentration decline in animals with plugged drainage ducts to the precorneal drug loss in normal rabbits. An independent measurement of precorneal drug concentrations in animals with normally functioning lacrimal drainage systems was not possible for reasons mentioned previously; therefore, the extension of these results to normal rabbits is of concern. However, both problems can be resolved with available data.

The first problem appears easily resolved by simply considering the amount of drug reaching the aqueous humor *via* corneal absorption. Table VII shows a summary of calculations for the amount of drug absorbed into the anterior chamber based on the parameters of Table II. Even in the largest case, only 1.44 μg or 2.12% of the instilled dose reaches the anterior chamber *via* corneal absorption. If one refers to the tear sampling studies and specifically to Table VI, it can be seen that absorption can account for a substantial loss of drug from the precorneal area. In fact, the amount of drug absorbed by the corneal (productive) route is insignificant when compared with the overall absorptive loss of drug. This finding indicates that, for all practical

Table IV—First-Order Rate Constants for the Decline in Drug Concentration in the Precorneal Area of Anesthetized Rabbits with Drainage Ducts Plugged

Rabbit	Weight, kg	Eye	k, min^{-1}[a]	Correlation Coefficient for Line
4	2.4	Right	2.19×10^{-2}	0.988
		Left	1.87×10^{-2}	0.973
5	2.0	Right	2.24×10^{-2}	0.913
		Left	2.12×10^{-2}	0.903
6	2.2	Right	2.96×10^{-2}	0.922
		Left	1.64×10^{-2}	0.882
Mean k(min^{-1}) = 2.17×10^{-2} (1.83×10^{-3})[b]				

[a] Calculated from the slope of the line determined *via* linear regression analysis. [b] Number in parentheses refers to the standard error of the mean.

Table V—Calculated Dilution Effect of Tears on Precorneal Drug Concentration following Instillation of 25 μl of a 1×10^{-2} *M* Pilocarpine Nitrate Solution into Eyes of Rabbits Whose Drainage Apparatus Has Been Blocked

Minutes	Amount of Drug Present, μg[a]	Volume of Fluid Present, ml[b,c]	Concentration of Drug, μg/ml
0	67.82	0.0325	2087
5	67.82	0.0358	1894
15	67.82	0.0424	1600
30	67.82	0.0523	1297
45	67.82	0.0622	1090
60	67.82	0.0721	941
90	67.82	0.0919	738
k(min^{-1})[d] = 1.16×10^{-2}			

[a] No drug is lost *via* drainage, and it is assumed that no absorption takes place in this case. [b] Initial volume is based on the normal resident volume of the rabbit eye, 7.5 μl, plus the instilled volume of 25 μl. Subsequent volumes are calculated on a rate of tear production of 0.66 μl/min. [c] It is assumed that no volume is lost due to evaporation. [d] Calculated from the slope of the line obtained by linear regression analysis.

purposes, the tear sampling studies measured only the loss of drug to the nonproductive route with an absorption rate constant of 2.17×10^{-2} min^{-1}. An implicit assumption is that drug penetrates to the anterior chamber only by corneal absorption.

Extrapolation of the results of the tear sampling studies to rabbits with unobstructed drainage ducts can be accomplished if the kinetics of the absorptive process are considered. If normal instilled solution drainage had been a factor in the tear sampling studies, large amounts of instilled drug would be lost to the drainage apparatus, leaving much less drug available for absorption. However, this would not affect the rate constant for nonproductive absorption—only the amount absorbed.

In addition to decreasing the amount of drug available for absorption, drainage reduces the volume present in the eye. Therefore, incoming tears have a more substantial effect on the concentration of drug in the precorneal area. The effect of the decreased concentration caused by the dilution by tears is a decreased rate of nonproductive absorption but an unchanged rate constant. Thus, the rate constant of 2.17×10^{-2} min^{-1} for nonproductive absorption is applicable to normal rabbits as well as to those with their drainage ducts plugged provided that the mechanism of pilocarpine absorption does not change.

DISCUSSION

Precorneal Drug Distribution in Normal Unanesthetized Rabbits—Instilled drug solution may be lost from the precorneal area

Table VI—Calculated Concentrations of Pilocarpine Nitrate in the Precorneal Area of the Eye following Instillation of 25 μl of a 1×10^{-2} *M* Solution which is Both Absorbed and Diluted by Tears in Rabbits Whose Drainage Apparatus Has Been Blocked

Minutes	Amount of Drug Present, μg[a]	Volume of Fluid Present, ml[b,c]	Concentration of Drug, μg/ml
0	67.82	0.0325	2087
5	60.85	0.0358	1700
15	48.98	0.0424	1155
30	35.37	0.0523	676
45	25.54	0.0622	411
60	18.45	0.0721	256
90	9.62	0.0919	105
k(min^{-1})[d] = 3.32×10^{-2}			

[a] No drug is lost *via* drainage, but loss occurs by a first-order absorption process with a rate constant of 2.17×10^{-2} min^{-1}. [b] Initial volume is based on the normal resident volume of the rabbit eye, 7.5 μl, plus the instilled volume of 25 μl. Subsequent volumes are calculated on a rate of tear production of 0.66 μl/min. [c] It is assumed that no volume is lost due to evaporation. [d] Calculated from the slope of the line obtained by linear regression analysis.

Table VII—Total Amount of Drug Appearing in the Aqueous Humor of Rabbits following Instillation of 25 μl of a 1×10^{-2} *M* Pilocarpine Nitrate Solution

Parameter	Unanesthetized Duct Open	Unanesthetized Duct Plugged	Anesthetized Duct Open	Anesthetized Duct Plugged
$\frac{FD}{V}$	1.39	4.81	1.81	4.23
F^a	6.15×10^{-3}	2.13×10^{-2}	8.01×10^{-3}	1.87×10^{-2}
Amount of drug absorbed[b], μg	0.42	1.44	0.54	1.27
Percent of instilled dose absorbed	0.62	2.12	0.80	1.87

[a] Calculated based on an instilled dose (D) of 67.82 μg and an aqueous humor volume (V) of 0.30 ml. [b] Amount = FD.

of the eye by any of several routes, *e.g.*, instilled solution drainage, tear turnover, corneal absorption, and nonproductive absorption. Following instillation, fluid is lost by drainage until the volume returns to the normal resident volume of 7.5 μl (21). Instilled solution drainage does not affect the concentration of drug present—only the amount—whereas absorption affects both the concentration and amount.

Instilled solution drainage is only operative until the volume in the eye returns to its normal resident volume. After this time, the volume remaining in the eye is constant and drug is lost *via* absorption and by the removal of 0.66 μl of solution/min. Drug concentration will be affected by absorption and by the dilution effect of incoming tears, with the net result that both the amount and concentration of pilocarpine nitrate in the precorneal area will decline in a biphasic fashion. Initially, the amount of drug decreases very rapidly due to drainage, while concentration declines more slowly, influenced only by absorption and tear turnover. As the volume in the eye returns to its normal level, the amount of drug present declines more slowly since drainage is no longer a factor. On the other hand, concentration now declines more rapidly due to the dilution effect of incoming tears.

When using these arguments, it is possible to comment on the effects of changing the volume or the concentration of the instilled drug solution. Varying the concentration of instilled drug solution changes the absolute values of both the concentration and amount of drug in the precorneal area at any time but probably does not change the pattern in which they decline. The more important consideration is the variation of instilled solution drainage with the variation in the instilled volume. This information is available (21), and it is known that the drainage rate varies linearly with the instilled volume over a wide range of instilled volumes. Thus, the initial decline in the amount of drug in the precorneal area is greater than the larger instilled volume. Once the volume returns to normal, however, the decline should be identical, regardless of the instilled volume.

Precorneal Drug Distribution in Unanesthetized Rabbits with Drainage Ducts Plugged—Blockage of the drainage canal eliminates instilled solution drainage as a source of drug loss from the precorneal area. Thus, solution volume in the eye, rather than decreasing to the normal resident tear volume, increases at a constant rate of 0.66 μl/min. Also, in contrast to unanesthetized normal rabbits, both the amount and concentration of drug decrease in a monophasic rather than biphasic fashion. In this case, the decline in concentration occurs at a faster rate than does the decline in amount due to the dilution effect of incoming tears.

Changing either the concentration or the volume instilled has no effect on the rate constant for nonproductive absorption. Likewise, simply changing the concentration of the instilled drug solution changes the absolute values of the concentration at any time but not the rate constant for the decline in concentration. However, the rate constant for the decline in concentration becomes larger as the instilled volume is decreased due to the greater dilution effect of the incoming tears on smaller volumes. Theoretically, if the instilled volume were large enough, limited, of course, by the capacity of the precorneal area to hold a given dose, incoming tears would have no effect on the concentration of drug, and the rate constant for the decline in concentration would be identical to that for the decline in amount, namely, $k_n = 2.17 \times 10^{-2}$ min^{-1}.

Precorneal Drug Distribution in Anesthetized Rabbits with Drainage Ducts Obstructed—There exist two distinct differences between rabbits under general anesthesia and awake rabbits with regard to the movement and distribution of instilled drug solutions. First, anesthetized rabbits do not produce tears (21); second, although instilled solution drainage does occur, it occurs at a considerably reduced rate.

In addition to the fact that the drainage of an instilled solution is considerably different in anesthetized and unanesthetized rabbits, it is important to note that there is no instilled volume dependency in drainage of fluid from the eye of anesthetized animals (21). Thus, the drainage rate constant, $k_d = 1.77 \times 10^{-1}$ min^{-1}, for anesthetized animals remains constant regardless of instilled volume. Moreover, the normal lacrimal volume is considerably larger in anesthetized than in unanesthetized rabbits. This higher value is probably due to relaxation of the eye muscles under anesthesia to accommodate a larger volume (21).

The volume remaining, amount remaining, and concentration at any time postinstillation in anesthetized rabbits should be considered. As was the case with unanesthetized rabbits, the amount decreases in a biphasic fashion. The initial decline in amount is considerably slower than that observed in unanesthetized rabbits but still greater than the initial decline in concentration. Concentration can only be changed by absorption of drug and, therefore, declines at a single rate governed by k_n. As such, the terminal decline in amount has the same slope as that for concentration. The rate constant for the decline in concentration is unaffected by changes in either the concentration or the volume instilled. Since k_d remains invariant with the volume instilled, the decline in amount also is unaffected by changing the volume; only the magnitude of the amounts varies by altering the concentration of the instilled solution.

Precorneal Drug Distribution in Anesthetized Rabbits with Drainage Ducts Plugged—The final category of rabbits employed in these studies consisted of rabbits under general anesthesia whose drainage ducts had been blocked. Neither tear production nor instilled solution drainage is operative in this case; therefore, they do not contribute to precorneal drug loss.

As noted previously with awake animals whose drainage ducts had been plugged, since there is no instilled solution drainage, both concentration and amount decline in first-order fashion and do not exhibit the biphasic decline observed when drainage is operative. Since, in addition to no drainage, there is also no tear production, the volume in the eye remains constant throughout. The result is that both concentration and amount decline with the same rate constant. This rate constant is simply the rate constant for absorption, $k_n = 2.17 \times 10^{-2}$ min^{-1}. Finally, the rate constants for the decline in both concentration and amount are unaffected by changing either the concentration or the volume of the instilled solution.

Precorneal Effects on Ocular Drug Bioavailability—If one examines the areas under the aqueous humor concentration–time profiles shown in Table II for the four categories of rabbits studied, some general comments can be made on the relative influence of precorneal factors on ocular drug bioavailability. Drainage apparently is the overwhelming factor in causing bioavailability differences for instilled drug solutions. In both anesthetized and unanesthetized rabbits, blocking the drainage duct results in considerable increases in bioavailability over cases in which instilled solution drainage is operative.

In animals whose drainage ducts were plugged, the unanesthetized animals gave bioavailabilities slightly higher than the anesthetized rabbits. At first this may seem surprising because no tears are being produced in the anesthetized rabbits. If the dilution effect of tears were a controlling factor in determining bioavailability, one would expect more drug to reach the aqueous humor of anesthetized rabbits. This is not the case, however, which seems to minimize the influence of tear production on ocular drug bioavailability. The difference in

the amount of drug reaching the anterior chamber can be explained by the fact that the initial drug concentration is smaller in anesthetized rabbits due to their larger resident tear volume.

Consider also that anesthetized rabbits show only a slight increase in bioavailability over unanesthetized rabbits in spite of a large difference in the drainage rate constant, k_d, and the fact that tear production is absent. It is already known that for a constant initial concentration and a normal rate of tear production (compare unanesthetized rabbits with drainage duct open and plugged), elimination of drainage significantly increases bioavailability. The small magnitude of the bioavailability increase observed in anesthetized rabbits over unanesthetized rabbits can be attributed to the decreased initial concentration in anesthetized animals. What appears to be happening is that the increase expected from decreasing drainage is being minimized by the lower initial concentration. This finding illustrates the important role played by the initial concentration of drug in the precorneal tear film in determining ocular drug bioavailability.

It is also confirmed that tear production appears to have a minimal influence on ocular drug bioavailability. Tear production would probably become a controlling influence only if its rate were significantly increased, by irritating the eye, for example, or if the instilled volume were very small. In the absence of these special circumstances, instilled solution drainage and the initial concentration of drug in the precorneal tear film appear to be the most important precorneal influences on ocular drug bioavailability.

In addition to these observations, some correlations can be made which, within limits, have predictive capabilities for determining the amount of an instilled dose of pilocarpine expected to reach the aqueous humor under various conditions. These correlations were reported elsewhere.

Several complicating factors make a mechanistic understanding of pilocarpine transport in the eye much more difficult than it initially appears. For example, in normal, unanesthetized rabbits, drainage accounts for the loss of as much as 75% of the instilled drug within 5 min after instillation of a 25-μl dose. One would intuitively expect that inhibiting this drainage loss would dramatically improve aqueous humor levels of drug simply by virtue of the improved contact time of the drug with the corneal membrane. That this is not the case can be illustrated as follows.

In normal, unanesthetized rabbits, instillation of 25 μl of 1×10^{-2} M pilocarpine, 67.82 μg, results in a total of 0.42 μg of drug being recovered in the aqueous humor. If drainage is inhibited, only 1.44 μg of drug reaches the aqueous humor. This unexpectedly small bioavailability increase was observed repeatedly in cases where viscous solutions were used to improve contact time of drug in the precorneal area (31, 34–36). At least as far as pilocarpine is concerned, for a given concentration, ocular contact time has only a very limited role in influencing the amount of drug reaching the anterior chamber unless the drug can be restricted to essentially the corneal area such as might occur with contact lenses impregnated with drug. This finding emphasizes the limited utility of viscous polymer solutions, which is common practice in ophthalmic formulations. Although the use of such formulations has some effect on ocular drug bioavailability, the ultimate solution to the problem is much more complicated than simply delaying drainage.

A second unexpected result that indicates a more complicated picture than would be expected is the variation in the absorption rate constants determined from the aqueous humor concentration–time profiles. As discussed previously, this rate constant is taken to represent the rate constant for the penetration of drug into the aqueous humor from the cornea. In both unanesthetized and anesthetized rabbits where the drainage ducts are plugged, the value of this rate constant is approximately one-half of that observed in those two cases where drainage is not obstructed.

When assuming that the rate constant remains first order, which it does, there are only two kinetic explanations for this shift in peak time and the resultant decrease in the rate constant. The first is that the larger rate constant is not a single rate constant but a sum of rate constants, indicating that drug is entering the aqueous humor by one or more additional routes in animals with open drainage ducts. This would have to be accounted for by drug reaching the anterior chamber from systemic circulation. Unpublished results from this laboratory showed that with the concentration and amounts of drug employed here, no drug that reaches the systemic circulation *via* drainage loss can be detected in the aqueous humor, at least within the time course of these studies.

The alternative explanation for the decrease in the rate constant is a change in the mechanism of the absorptive process, either by some alteration of the membrane or by another unknown process. It is this latter case that must be responsible for the shift in the peak aqueous humor concentration to later times in rabbits whose drainage ducts are plugged.

Some seemingly anomalous results of these studies can now be accounted for in light of some current work being conducted in this laboratory. Studies involving rinsing of the drug solution from the precorneal area of the eye at various times postinstillation have been conducted. Preliminary results indicate that if 25 μl of 1×10^{-2} M pilocarpine is instilled into the eyes of normal rabbits and then rinsed from the eye at anywhere from 2 to 5 min postinstillation, the aqueous humor concentration–time profile remains virtually identical to that reported here for unanesthetized, normal rabbits. There are two important implications of these findings for discussion purposes:

1. In rabbits with normal drainage function, the first few minutes postinstillation represent the critical period for drug uptake by the cornea.
2. A very rapid binding or equilibrium of pilocarpine with either the cornea or possibly the protein-rich mucin layer, which forms a protective film over the cornea, is indicated.

There is also preliminary evidence to indicate that a kinetic explanation for the observed peak shift can be made by considering a rapid parallel elimination step from the absorption depot.

Quantitation of corneal drug uptake is under study and will be reported later. Further speculation on the mechanism of the uptake and transport of pilocarpine by the cornea would be premature.

The observations reported here apply only to one drug, pilocarpine. In some respects, precorneal movement, for example, instilled solution drainage is not necessarily a function of the drug involved. However, with other factors such as absorption rate constants and mechanisms of transport, the specific drug obviously will be critical.

SUMMARY

Instilled solution drainage, tear production, and absorption have all been shown to be contributing factors in the movement and distribution of drugs in the precorneal area of the eye. The relative magnitude of each of these effects has been isolated and quantitated for the drug pilocarpine nitrate, and *in vivo* methodology has been established so that other drugs may also be investigated. In examining the effects of these precorneal factors on ocular drug movement and distribution, it is necessary to be mindful of the way in which both the amount and concentration of drug in the precorneal area are affected.

Despite the fact that loss of an instilled drug solution from the precorneal area is rapid and of considerable magnitude, the contact time of pilocarpine nitrate in this area has only modest effects on the amount of drug reaching the anterior chamber. Complete elimination of drainage of an instilled pilocarpine nitrate solution resulted in less than a 3.5-fold increase of drug reaching the aqueous humor. However, the initial concentration of drug in the tear film can have a tremendous effect on the amount of drug reaching the anterior chamber.

From studies in which the initial concentration, the drainage rate, and the rate of tear production were varied, either independently or simultaneously, it was possible to make some correlations as to the relative influence of their effects. Within limits, these correlations appear to have predictive capabilities for determining the relative bioavailability of pilocarpine nitrate in different concentrations and volumes of solutions.

By considering that instilled solution drainage is volume dependent, it becomes obvious that tremendous quantities of drug are wasted when large volumes, as are used commercially, are instilled into the eye. In fact, these large volumes are not only wasted but are potentially harmful, since drug lost to the drainage apparatus is potentially available to act systemically. On this basis, a much more rational approach to topical ophthalmic drug therapy would be a smaller instilled volume and a somewhat larger drug concentration. For example, 5 μl of 1.92×10^{-2} M pilocarpine nitrate provides the same initial concentration of drug in the tear film as 25 μl of 1×10^{-2} M. The 5-μl dose, however, contains more than 2.5 times less drug. Since the drainage of the 5-μl dose is considerably less than the 25-μl dose, the bioavailability is expected to be somewhat greater, while the amount of drug available to act systemically is dramatically reduced.

REFERENCES

(1) S. Mishima, A. Gasset, S. D. Klyce, Jr., and J. L. Baum, *Invest. Ophthalmol.*, **5**, 264(1966).

(2) O. Schirmer, *Graefes Arch. Ophthalmol.*, **56**, 197(1903).

(3) N. Ehlers, S. V. Kessing, and M. S. Norn, *Acta Ophthalmol.*, **50**, 210(1972).

(4) J. W. Henderson and W. A. Prough, *Arch. Ophthalmol.*, **43**, 224(1950).

(5) A. de Roetth, Sr., *ibid.*, **49**, 185(1953).

(6) J. C. Wright and G. E. Meger, *ibid.*, **67**, 564(1962).

(7) M. S. Norn, *Acta Ophthalmol.*, **43**, 567(1965).

(8) J. A. Brienen and C. A. R. D. Snell, *Ophthalmology*, **159**, 223(1969).

(9) L. T. Jones and M. L. Linn, *Am. J. Ophthalmol.*, **67**, 75(1969).

(10) S. Mishima, Z. Kubota, and R. L. Farris, *Excerpta Med. Found. Int. Congr., Ser. n222*, **1970**, 1801.

(11) D. M. Maurice, *Exp. Eye Res.*, **11**, 30(1971).

(12) J. Francois and A. Neetens, *Am. J. Ophthalmol.*, **76**, 351(1973).

(13) W. W. O'Nan, J. D. Wirtschafter, and D. F. Preston, *Arch. Ophthalmol.*, **91**, 187(1974).

(14) G. F. Rochat and C. E. Benjamins, *Graefes Arch. Ophthalmol.*, **91**, 66(1916).

(15) L. T. Jones, *Arch. Ophthalmol.*, **66**, 137(1961).

(16) M. S. Norn, *Acta Ophthalmol.*, **43**, 557(1965).

(17) L. T. Jones, M. M. Marquis, and N. J. Vincent, *Am. J. Ophthalmol.*, **73**, 658(1972).

(18) B. Rosengren, *Ophthalmology*, **164**, 409(1972).

(19) B. Rosengren, *Inaug. Diss.*, **1917**, 71.

(20) R. M. Rossomondo, W. H. Carlton, J. H. Trueblood, and R. P. Thomas, *Arch. Ophthalmol.*, **88**, 523(1972).

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

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

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

(24) L. Bellarminoff, *Arch. Ophthalmol.*, **39**, 38(1893).

(25) E. Ubry and M. Frezals, *Arch. Ophthalmol.*, **19**, 159(1899).

(26) T. Leber, "Graefe Saemische: Handbuch d. ges Augenhk.," 2:2.2 Aufl., Leipzig, Germany, 1903.

(27) J. Ursing, "On the Disappearance of Radiosodium Ions from Conjunctival and Subconjunctival Deposits in the Rabbit," Berlinska Boktryckeriet Lund, Malmö, Sweden, 1967.

(28) A. Sorsby and J. Ungar, *Br. Med. J.*, **2**, 723(1946).

(29) R. G. Janes and J. F. Stiles, *Arch. Ophthalmol.*, **62**, 69(1959).

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

(31) S. S. Chrai, Ph.D. thesis, University of Wisconsin, Madison, Wis., 1973.

(32) D. L. Deardorff, in "Remington's Pharmaceutical Sciences," 13th ed., Mack Publishing Co., Easton, Pa., 1965, p. 468.

(33) "The Rabbit in Eye Research," J. H. Prince, Ed., Charles C Thomas, Springfield, Ill., 1964.

(34) S. S. Chrai and J. R. Robinson, *J. Pharm. Sci.*, **63**, 1218(1974).

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

(36) *Ibid.*, **64**, 1312(1975).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 8, 1975, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706*

Accepted for publication November 10, 1975.

Abstracted in part from a thesis submitted by T. F. Patton to the Graduate School, University of Wisconsin, Madison, Wis., in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported by Allergan Pharmaceuticals, Irvine, CA 92664, and by a grant from the Graduate School, University of Wisconsin, Madison, WI 53706.

* Present address: Department of Pharmaceutical Chemistry, School of Pharmacy, University of Kansas, Lawrence, KS 66044

ˣ To whom inquiries should be directed.

Substituted 5-Nitro-1,3-dioxanes: Correlation of Chemical Structure and Antimicrobial Activity

LEWIS C. LAPPAS ˣ, CLARENCE A. HIRSCH, and CARL L. WINELY

Abstract □ Various derivatives of 5-nitro-1,3-dioxane were synthesized to determine the relative effect of chemical substitution in the 2- and 5-positions on broad spectrum antimicrobial activity. Each compound was evaluated quantitatively by calculation of a microbiocidal index, which measured the time to kill several different microorganisms. This test system indicated that 5-bromo-5-nitro substitution was essential for significant activity. Optimal activity was effected by 2-methyl substitution in the alkyl series and 2-hydroxyphenyl substitution in the aryl series. The antimicrobial activity of the substituted dioxanes was not related directly to water solubility or hydrolysis to microbiocidal diols or aldehydes.

Keyphrases □ Dioxanes, substituted—series synthesized, effect of substituents on antimicrobial activity □ Antimicrobial activity—series of substituted dioxanes synthesized, effect of substituents evaluated □ Structure–activity relationships—substituted dioxanes synthesized, effect of substituents on antimicrobial activity

Chemical agents that prevent microbial growth are essential ingredients for various topical products (1–3). The recent increased awareness of the potential health hazard of microbiologically contaminated pharmaceutical and cosmetic preparations (4–6) initiated a trend toward greater regulatory requirements for these products (7, 8). This turn of events emphasized the urgent need for more effective preservatives.

Several reviews described the significant factors contributing to the optimal efficacy of antimicrobial agents (9–11). Ideally, a preservative for topical preparations should be effective over a wide pH range (pH 5–9) and be physically and chemically compatible with the formulation ingredients. Furthermore, this agent should not be irritating, sensitizing, or absorbed to toxic levels after application.

To satisfy these requirements, chemical modification of the antimicrobial agent is often necessary. The design of the microbial test system to reflect significant differences in the activity of these modified compounds becomes a critical factor in their evaluation (12–14).