Drop Size and Initial Dosing Frequency Problems of Topically Applied Ophthalmic Drugs

SUKHBIR S. CHRAI, MICHAEL C. MAKOID, STUART P. ERIKSEN*, and JOSEPH R. ROBINSON×

Abstract □ The amount and rate of drug loss through drainage, for a single drop of topically applied ophthalmic solution, increase with increasing volume of instilled solution. However, the concentration of drug in the precorneal tear film immediately after instillation of drug is higher with larger instilled volumes. Multiple drops administered at short time intervals have the advantage of increasing drug concentration in the precorneal film but the disadvantage of considerable drug loss through drainage, which for potent drugs can lead to systemic toxicity. When using radioactive technetium (^{99m}Tc) as the test substance in unanesthetized albino rabbits, it was shown that a 5-min interval between drops minimizes drainage loss of drug and the drug concentration buildup in the precorneal film as compared to the corresponding dosage regimen of drops administered at shorter time intervals. As the time interval between drops shortens to the point of one drop followed immediately by another drop, both the amount of drug lost through drainage and the tear film concentration of drug increase. Separate administration of two drugs presents a great problem as to dosage regimen. It was shown by radioactive technetium dilution and drainage studies, as well as by aqueous humor drug concentration and miosis studies, that the order of addition of drug to the eye, the volume of solution instilled, and the time of instillation of the first and second drugs all influence the ultimate activity of each drug. In general terms, the first drug administered always suffers a greater loss than the second drug and the degree of loss is proportional to the volume of each drug instilled and the time interval between drops. The implications of the present study to clinical practice are discussed. The multidrug data presented in this report constitute a strong argument for combination drug products whenever multidrug therapy is indicated.

Keyphrases □ Ophthalmic drugs, topically applied—drop size and initial dosing frequency problems, relevance to ophthalmic combination products discussed □ Drop size—considerations relative to optimum size and frequency discussed, topically applied ophthalmic drugs, albino rabbits □ Dosing frequency—considerations relative to topically applied ophthalmic drugs, drop size, relevance to ophthalmic combination products discussed

Dosing schedules for ophthalmic drugs should be based on such factors as intrinsic drug activity, pharmacokinetics of drug disposition, the disease being treated, and the type and method of drug delivery (1–5). The clinician can greatly influence the dosing schedule and, therefore, drug therapy through his or her choice of the drug delivery system and the method of drug delivery. However, understanding of both of these areas for ophthalmic drug delivery systems is minimal so that the clinician must rely on clinical experience to decide on a particular type and method of drug delivery. Unfortunately, this approach may not reveal an optimum drug treatment schedule since it is not possible to examine all variations, particularly when multiple-drug therapy is involved.

The purpose of the present report is to examine some aspects of the ophthalmic dosing regimen for topically applied, single- or multidrug systems. Specifically, it was of interest to investigate the influence of the volume of drug instilled and the time interval between drops on the resulting precorneal tear film concentration of drug as well as the extent of drug loss through solution drainage and tear turnover.

Chrai *et al.* (6) examined the kinetics of instilled solution drainage from the eye of rabbits. They showed that the rate of drainage of an instilled solution is linearly related to the volume instilled. Based on this work, it is reasonable to assume that instillation of multiple drops will result in an increased drainage rate due to a volume buildup. Therefore, excessive loss of instilled drug will occur unless the drops are instilled at time intervals that allow the volume in the eye to return to normal. Drugs instilled at this interval, 5–6 min in the rabbit (6), will reduce the drug loss but should also reduce the resultant tear film concentration of drug. Shorter dosing intervals should result in a reversal of this trend. The dosing picture should become considerably more complicated when two separate drugs are used. The concentration of each drug in the precorneal film as well as the rate of drainage is expected to be a function of the order of drug addition, the volume instilled for each drug, the time between drops, and the initial concentration of each drug.

EXPERIMENTAL

Materials—Water was doubly distilled from alkaline permanganate in an all-glass distillation apparatus. Radioactive technetium (^{99m}Tc) sulfur colloid suspensions were freshly prepared as previously described (6). Pilocarpine nitrate, epinephrine, ^{3}H-pilocarpine nitrate[1] (specific activity 0.44 mCi/mg), and ^{14}C-epinephrine[1] (specific activity 0.32 mCi/mg) were obtained from commercial sources. All other chemicals were reagent or analytical grade.

Methods—Unanesthetized, New Zealand, male, albino rabbits, weighing between 1.8 and 2.4 kg, were employed in all experiments. Experimental animals were placed in restraining boxes, with their heads in a natural position, during the experiments to minimize movement. All animals were conditioned to the experimental procedure by being kept in restraining boxes several hours each day for 7 days prior to an experiment and instilling normal saline into their eyes daily. Each restraining box was a simple wooden receptacle, just large enough to hold the rabbit, with an opening through which the animal's head protruded. All solutions were prepared fresh as outlined earlier (6, 7) and were discarded immediately after the experiment.

Drug or a tracer substance was instilled into the rabbit eye using a microliter syringe[2]. Solutions were instilled against the cornea and collected in the lower cul-de-sac. During instillation, the lower eyelid was extended slightly away from the globe of the eye to form a pouch in which to collect the instilled fluid. Immediately after instillation, the lower eyelid was returned to its normal position and natural movement of the eye and lid was used to mix the instilled solution with tear fluid. Experiments were conducted

[1] New England Nuclear, Boston, Mass.
[2] Hamilton Co., Whittier, Calif.

Table I—Concentration of Drug in the Tear Film after Dilution with Tears as a Function of Volume Instilled

Volume Instilled, μl	Concentration as Percent of Instilled Concentration[a]
5	40
10	57
25	77
50	87
75	91

[a] Assuming the same concentration in all cases.

with either eye of the test animal since earlier studies (6) had not shown any difference in drainage rates between right or left eyes.

Radioactive Technetium Dilution and Drainage Studies—After instillation of the radioactive technetium colloid, the concentration and/or amount of isotope remaining as a function of time were measured as described earlier (6). A minimum of four eyes was used for each experiment, and no animal was used more than once.

Miosis-Time Studies—Methods for the miosis-time studies were described earlier (7). However, in the present study, only unanesthetized animals were used. Twenty-five microliters of 2.5 × 10^{-2} *M* pilocarpine nitrate solution was instilled into the rabbit eye followed by 25 μl of normal saline at an appropriate time interval. A minimum of four eyes in four different experimental animals was used for each time interval. The second eye of each animal served as a control because no animal was used more than once.

Aqueous Humor Drug Concentration Studies—Separate solutions containing 1 × 10^{-2} *M* pilocarpine nitrate and epinephrine hydrochloride were prepared, both with and without the appropriate radioisotope, by addition of drug to a pH 5.5 Sørensen buffer. In addition, two combination drug solutions were prepared in which one drug was radioactive and the other was nonradioactive. For example, a solution containing tritiated pilocarpine nitrate, prepared as already described, was added to a nonradioactive epinephrine hydrochloride solution so that the concentration of each drug was 1 × 10^{-2} *M*. Similarly, a solution containing ^{14}C-epinephrine hydrochloride and nonradioactive pilocarpine nitrate was also prepared. Solution pH was checked and adjusted where necessary (6).

Fifty microliters of a radioisotopic drug solution was instilled into the eye followed in 1 min by 50 μl of the nonisotopic solution containing the other drug. The combination drug solutions were also instilled using 50 μl as the dose volume. By this approach, either tritiated pilocarpine nitrate or ^{14}C-epinephrine hydrochloride could be studied in the presence of the other drug.

Twenty-five minutes postinstillation, the test animal was anesthetized with a solution of sodium pentobarbital. Aqueous humor was withdrawn 30 min after the last drug was instilled. The aqueous humor samples were placed in vials containing Aquasol[1] and were stored for 48 hr to eliminate chemiluminescence. Drug concentrations in aqueous humor were determined by scintillation detection[3], and appropriate blank corrections were made.

Pilocarpine-nitrate-tritium exchange was determined not to be a problem in these experiments. Whether metabolized or intact drug was being measured in aqueous humor was not established.

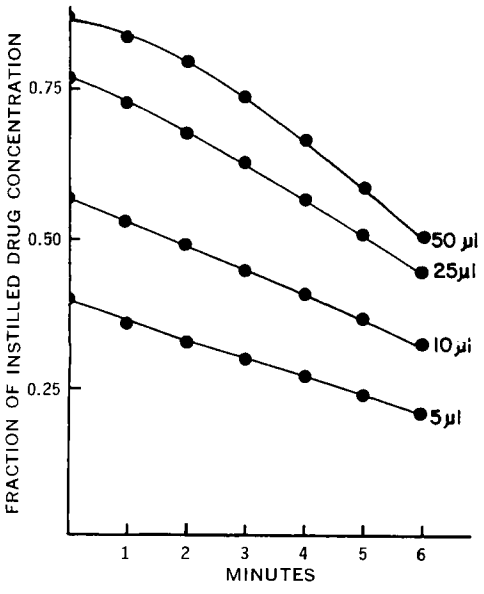

Figure 1—*Calculated concentration of drug in the precorneal film as a function of time for various instilled volumes. Absorption of drug into eye tissues is neglected in these calculations. For calculations, see Footnote a of Table II.*

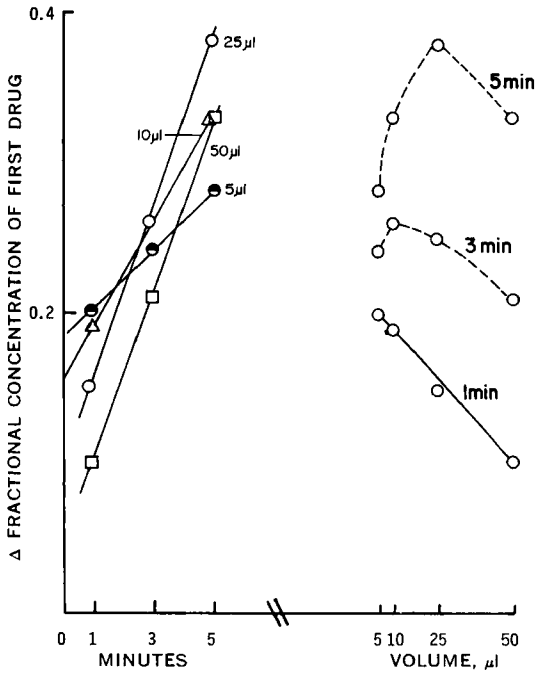

Figure 2—*Gain in fractional concentration of drug in the tear film after instillation of a second drop of drug as a function of time of instillation and volume of the drop instilled. Gain in fractional concentration of the drug is the difference between the fractional concentration before and after the instillation of the second drop.*

RESULTS AND DISCUSSION

Effect of Single and Multiple Drops on Concentration of Drug in Tear Film for a Single Drug—After instillation of a single drop of a drug into the eye, there will be a change in the concentration of the drug due to dilution of the instilled solution by the tears (Table I). The values in Table I are calculated values based on a tear volume of 7.5 μl (6) and neglect the small initial loss of unmixed drug which will occur with the larger instilled volumes (6) and which will cause a further lowering of drug concentration. As expected, the smaller the instilled volume, the greater is the dilution effect when the drug solution mixes with tears.

The tear-drug solution shown in Table I will undergo a further decrease in concentration as time progresses due to drug absorption into various eye tissues and normal tear turnover. Complicating the concentration change picture somewhat is the drainage of instilled solution that commences immediately after instillation of the drug solution into the eye. When assuming that absorption of drug into various eye tissues is negligible and that tear turnover in rabbits is 0.5 μl/min (6), the change in concentration of drug in the precorneal film can be calculated as a function of time. This change is depicted in Fig. 1 for the first 6 min postin-

[3] Packard model 2002 scintillation detector, Packard Instruments, Downers Grove, Ill.

Table II—Calculated Concentration of Drug in the Precorneal Film after Instillation of a Second Drop of Drug Administered at Various Times after the First Drop

Instilled Volume, μl	Time of Instillation of Second Drop, min	Fractional Concentration at Time of Instillation of Second Drop[a,b]	Volume Remaining Immediately after Instillation of Second Drop[c]	Final Fractional Concentration[d]	Percentage Improvement in Concentration
5	0.167[e]	0.40	17.5	0.57	—
	0.25[f]	0.394	17.2	0.57	—
	0.50[g]	0.387	16.9	0.57	—
	1	0.360	15.9	0.56	56
	3	0.30	14.5	0.54	90
	5	0.24	13.6	0.52	117
10	1	0.53	24.8	0.72	38
	3	0.45	21.0	0.71	57
	5	0.37	18.7	0.70	74
25	1	0.73	45.0	0.88	19
	3	0.63	36.9	0.88	39
	5	0.51	33.8	0.87	68
50	0.167[e]	0.87	107.5	0.93	—
	0.25[f]	0.87	101.2	0.93	—
	0.50[g]	0.86	91.7	0.94	—
	1	0.84	78.2	0.94	12
	3	0.74	62.4	0.95	29
	5	0.59	59.4	0.92	55

[a] $C_t = [(V_r - 0.5)C_{t-1}]/(V_r + 0.5)$, for $t \geq 1$, where C_t is the concentration at time t and V_r is the residual volume at time t. [b] Assuming lacrimal turnover rate to be equal to 0.5 μl/min (6). [c] Calculated assuming lacrimal fluid volume to be 7.5 μl (6). [d] $C_f = [(C_t \times V_r) + (C_0 \times V_i)]/(V_r + V_i)$. [e] $C_{0.167} \cong C_0$, where C_0 is the concentration at time zero. [f] $C_{0.25} = [(V_r - 0.125)C_0]/(V_r + 0.125)$. [g] $C_{0.5} = [(V_r - 0.125)C_{0.25}]/(V_r + 0.125)$.

Table III—Concentration of Two Drugs in the Precorneal Film from Administration of Either a Combined Solution or Separate Solutions at Various Times

Time of Instillation of the Second Drug, min	Volume of Drop Instilled, μl	Fractional Concentration of First Drug before Instillation of Second Drug[a]	Fractional Concentration of First Drug after Instillation of Second Drug[b]	Fractional Concentration of Second Drug[c]	Percentage Loss of Concentration of First Drug due to Instillation of Second Drug	Percentage Loss of Concentration of Second Drug due to Presence of First Drug
Both drugs together (same drop)	50	0.87	0.87	0.87	0	0
0		0.87	0.47	0.47	46.0	46.0
1		0.84	0.30	0.64	64.5	26.5
3		0.74	0.15	0.80	80.0	8.5
5		0.59	0.085	0.86	98.5	1.2
Both drugs together	5	0.40	0.40	0.40	0	0
0		0.40	0.29	0.29	27.5	27.5
1		0.36	0.25	0.32	30.6	20.0
3		0.30	0.20	0.35	33.4	12.5
5		0.24	0.15	0.37	37.5	7.5

[a] See Footnote *a* of Table II. [b] $C_f = (C_t \times V_r)/(V_r + V_i)$. [c] $C_f\ (C_0 \times V_i)/(V_r + V_i)$.

stillation. The calculated values were based in part on data reported elsewhere (6).

As can be seen in Fig. 1, the change in drug concentration as a function of time appears to be much larger in the case of larger instilled volumes than with smaller volumes, particularly in the 3–6-min period postinstillation. This observation is opposite to what is expected since tear turnover should have a larger influence on drug concentration in smaller volumes than in larger volumes. If the fraction of drug concentration at zero time for all of the instilled volumes were normalized to one, the influence of tear turnover would appear greater for the smaller instilled volumes. Thus, smaller volumes of drug solution are not only diluted more than larger volumes when placed in the eye but, as expected, they are influenced most by tear turnover.

The precorneal film drug concentration after instillation of two or more drops is a function of the drop size and the time interval between instillations. Calculated drug concentrations in the tear film after the administration of a second drop of drug solution at various times after the first drop are shown in Table II. The values are based on the various rate constants for instilled solution drainage (6), and drug absorption into various eye tissues is assumed negligible for calculation purposes.

Table I shows that there is a smaller dilution effect with larger instilled volumes as compared to smaller volumes. This appears to be the case also with administration of a second drop, as can be seen from the next to last column in Table II, since a higher concentration is generated for the 50-μl drop as compared to the 5-μl drop. However, the percentage improvement in drug concentration, by administering a second drop, is greater for smaller instilled volumes than for larger volumes, as shown in the last column of this table.

The time interval between the first and the second drops influences the final concentration of drug in the precorneal film. This point can be illustrated by plotting the data from Table II as shown in Fig. 2. The left side of this figure, where the improvement in drug concentration from the first to the second drop is plotted *versus* the time interval between drops, shows that the improvement is linearly related to the time interval and that the time of instillation of the second drop for the 5-μl case seems to have a relatively small influence on the final concentration

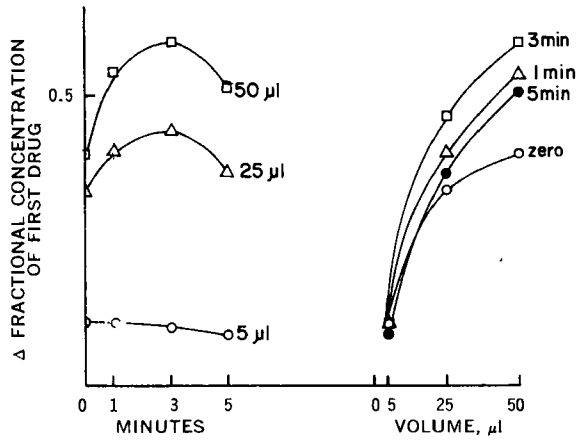

Figure 3—*Loss in fractional concentration of first drug in the precorneal tear film after instillation of a second drug as a function of time of instillation and volume of the drop instilled. Loss in fractional concentration is the difference between the fractional concentration of the first drug before and after instillation of the second drug.*

achieved as compared to the 50-μl drop. The increasing rate of drainage of instilled solution with larger volumes accounts for this change in sensitivity from small to large drops. The maximum improvement observed is a function of both the volume of instillation and the time at which the second drop is instilled, as shown in the right portion of Fig. 2 where improvement in drug concentration is plotted *versus* volume of drug instilled. The maximum improvement, when the second drop is instilled at 5 min, is with a 25-μl drop whereas the 10-μl drop is optimum for a 3-min interval.

It is apparent that two drops are preferable to one drop if elevated precorneal drug concentrations are desired and that large drops, even at a 5-min interval, will generate higher levels than will smaller drops at shorter time intervals. However, a more acceptable approach, based on amount of drug lost to drainage, is to administer smaller drops with a higher initial drug concentration rather than multiple large drops of lower concentration. A severe disadvantage to the multidrop approach is the variation in precorneal concentration that occurs with different time intervals between drops, a problem that exists when the drug solution is administered by the patient and that can lead to variation in efficacy for a particular drug.

Effect of Single and Multiple Drops on Concentration of Drugs in Tear Film for Two Drugs—When two drugs are to be administered in the form of topically applied drops, the concentration of each drug in the precorneal film will follow the results presented earlier for a single drug for the system where both drugs are in the same solution (combination product). The situation becomes considerably more complicated when the drugs are administered as separate solutions.

The concentration of drug in the precorneal film, after instillation of a second drop, was shown in the previous section to be related to the residual volume and concentration of the first drop. It would be expected, therefore, that the order of instillation of two drugs will affect the final concentration of each that is obtained. This can be seen in Table III for a hypothetical drug concentration, using 5- and 50-μl drop sizes and a variable time between instillation of the first and second drugs.

Clearly, the first drug administered will always show a larger decrease in concentration than the second drug. The extent of decrease is a function of the time interval between drops and the volume of solution instilled. In Fig. 3, the fractional concentration loss of the first drug due to instillation of the second drug is plotted as a function of instillation time and instillation volume. As can be seen from the right-hand portion of Fig. 3, the time of instillation of the second drug plays a significant role, particularly for larger instilled volumes. At first glance, it would seem that the extent of loss in fractional concentration is greatest with longer time periods between instillation. However, this is not the case since the line for the 5-min time period is between the 0- and 1-min time lines. The maximum loss in concentration is seen when the second drop is instilled at 3 min. This parabolic relationship is due to the fact that the rate of loss of instilled volume and rate of drug loss are not parallel. The loss in fractional concentration at zero time and 5 min is due principally to dilution, whereas the loss in fractional concentration at time periods between zero and 5 min is due to drainage and dilution.

It can be seen from Table III that smaller drops undergo less of a change in concentration upon instillation of the second drug than do larger drops, in concert with earlier observations on single drugs. Also apparent is the fact that, for any time interval between drops, the percentage loss of concentration of the first drug instilled is larger with larger drops than with smaller drops. The percentage loss of concentration of the second drug instilled is a function of the time interval between drops (Table III). Naturally, this situation does not exist when both drugs are administered together in the same solution.

To verify that changes in precorneal drug concentration can be calculated *a priori*, a study was initiated using radioactive technetium and normal saline to represent two different drugs. The change in radioactive technetium concentration was monitored for the system where 25 μl of technetium solution was instilled into the eye followed by 25 μl of normal saline at different time intervals (Table IV). Experimentally determined values are in good agreement with calculated values, showing that it is possible to calculate concentrations of drug in the precorneal film of unanesthetized rabbits with a good degree of confidence. Naturally, absorption of drug into various eye tissues adds a degree of com-

Table IV—Effect of Instillation of Second Drop of Different Drug on Precorneal Drug Concentration[a]

Time Interval between Drops, min	Fractional Concentration of Technetium (^{99m}Tc)[b]				
	Prior to Instillation of Normal Saline		Postinstillation of Normal Saline		
	Calculated[c]	Calculated[d]	Calculated[c]	Calculated[d]	Experimental[e]
0	0.77	0.66	0.44	0.37	—
1	0.73	0.61	0.32	0.29	0.26
2	0.68	0.55	0.26	0.21	0.22
3	0.63	0.51	0.20	0.18	—
4	0.57	0.47	0.16	0.16	0.18
5	0.51	0.43	0.13	0.15	0.15

[a] Twenty-five-microliter drops were used throughout. [b] Given as fraction of instilled concentration. [c] Calculated on basis of a lacrimal volume of 7.5 μl with tear turnover of 0.5 μl/min and drainage rate of 0.545 min^{-1} (6). [d] Calculated on basis of a lacrimal volume of 13.0 μl as consistent with instillation of 25 μl, as previously explained by Chrai *et al.* (6), with tear turnover of 0.5 μl/min and drainage rate of 0.545 min^{-1}. [e] Minimum of four separate determinations. Standard deviations of reported values were less than 7%. Student's *t* test failed to show a significant difference between calculated and experimental values at the 95% confidence level.

Table V—Calculated Amount of Drug Lost from the Precorneal Portion of the Eye for a Single Drop as a Function of Instilled Volume[a]

Instilled Volume, μl	Time Postinstillation, min	Initial Amount	Amount Lost, units	Percent of Drug Lost
5	1	5	0.625	12.5
	3	5	1.20	24.0
	5	5	1.55	31.0
10	1	10	1.50	15.0
	3	10	3.05	30.5
	5	10	3.95	39.5
25	1	25	12.25	49.0
	3	25	16.00	64.0
	5	25	18.25	73.0
50	1	50	27.0	55.0
	3	50	41.0	82.0
	5	50	43.75	87.5

[a] Assuming no absorption into eye tissues occurs, turnover rate of tears is 0.5 μl/min, and rate constants for drainage are as reported earlier (6).

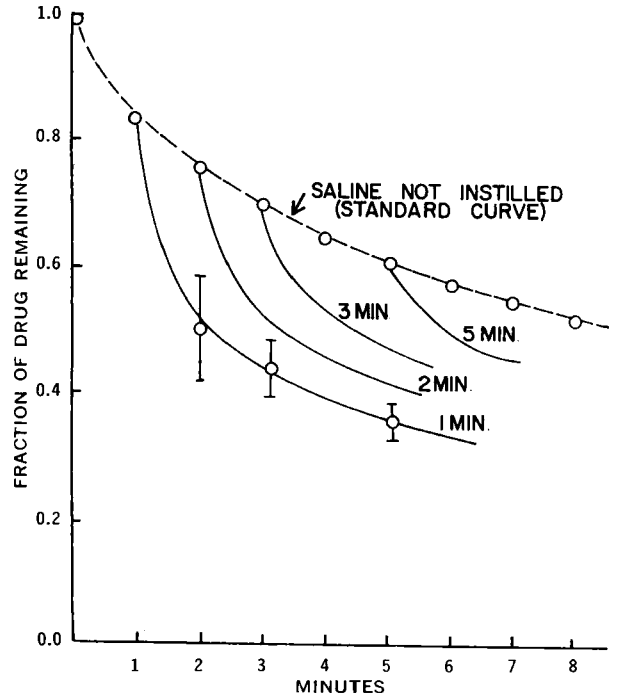

Figure 4—*Change in fractional amount of technetium in the precorneal tear film following instillation of a second drop of normal saline at various times. Volume of the technetium suspension and the normal saline instilled into the eye was 10 μl. The dotted line represents the change in amount of technetium when a second drop has not been administered, and the solid lines represent the change in amount when a drop of normal saline has been instilled. The solid lines are continuous tracings of the change in amount of technetium with time. Representative standard deviations are given at a few points for the 1-min run for the sake of clarity. A minimum of four experiments, in four separate animals, was conducted for each time interval.*

plexity to the calculation but can be accommodated if penetration rates are known.

Effect of Single and Multiple Drops on Amount of Drug in Precorneal Tear Film for Single and Multiple Drugs—As indicated earlier (6), the amount of drug lost from the eye due to drainage increases with increasing instilled volume. Table V gives a comparative loss of drug as a function of instilled volume. The considerable amount of drug lost for the larger instilled volumes can be an important factor for systemic side effects attendant with the use of some ophthalmic drugs (8, 9).

Instillation of a second drop of the same drug or of a different drug increases the volume present in the eye, which will result in a more rapid drainage of the instilled solution. The greater drainage results in a greater loss of drug from the precorneal area. Figure 4 shows the change in amount of technetium in the tear film following instillation of a drop of normal saline at various time intervals.

The rate of loss of drug from the rabbit eye is related to the volume of solution present in the eye at any time. For two drops, the volume present is the residual volume of the first drop plus the volume of the second drop instilled. The rate at which drug is lost from the eye is predictable, irrespective of the number of drops added as long as the volume of each drop and the time interval between drops is known. This is shown in Table VI for the technetium–normal saline system described in Fig. 3. The agreement between calculated and observed drainage rate constants is excellent (Table VI).

Earlier portions of this report described precorneal concentrations of drug from different dosing regimens and concluded that it was preferable to instill large drops rather than small drops to minimize the initial dilution effect and that two drops of a drug solution would give a larger precorneal concentration of drug than would one drop. However, based on the amount of drug lost, when

Table VI—Effect of Instillation of a Second Drop on Drainage Rate Constant[a]

Time	Extra Volume in Eye upon Instillation of Second Drop, μl[a]	Drainage Rate Constant, min^{-1}	
		Calculated[a,b]	Experimental[b,c]
0	20	0.486	0.486
15 sec	19.1	0.475	
30 sec	18.3	0.466	0.475
1 min	16.9	0.449	
2 min	14.8	0.424	0.425
3 min	13.3	0.406	
4 min	12.3	0.395	
5 min	11.6	0.386	0.378
∞ min	10.0	0.368	0.368

[a] Ten microliters of technetium colloid followed by 10 μl of normal saline at some appropriate time interval. [b] Calculated on the basis of a tear volume of 7.5 μl and a turnover rate of 0.5 μl/min (6). [c] Experimental values are based on at least four separate determinations and are given with a standard deviation of 7% or less. Student's *t* test failed to show a significant difference between calculated and experimental values at the 95% confidence level.

large volumes or multiple volumes are used, it would seem preferable to use a larger concentration of drug in the instilled solution as well as a small volume for instillation, particularly if systemic toxicity is a problem. For example, if the concentration of an ophthalmic solution was raised from 1 to approximately 2.2 units/μl and 5 μl was instilled into the eye, the resulting precorneal drug concentration would be the same as if 50 μl of the 1-unit/μl solution had been used. From a drug loss viewpoint, the amount of drug lost from the 5-μl drop over a 5-min period is 3.4 units whereas the 50-μl drop loses 43.75 units over this same time period.

Aqueous Humor Concentration of Two Drugs Administered Together and Separately as Topical Solution—The driving force for corneal penetration of drug is the concentration existing in the precorneal tear film. If two drugs are administered separately, with a time interval between drops, dilution limits the extent of absorption of both drugs as compared to a combined drug solution.

To verify this, a study was conducted whereby tritiated pilocarpine nitrate and ^{14}C-epinephrine hydrochloride were instilled into rabbit eyes and aqueous humor drug levels were determined

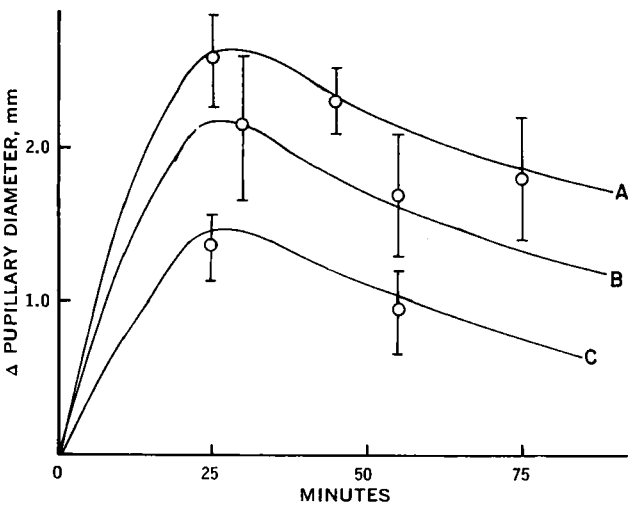

Figure 5—*Change in pupillary diameter as a function of time after instillation of 25 μl of 2.5 × 10^{-2} M pilocarpine nitrate followed by 25 μl of normal saline at various time intervals. The data represent the sum of five separate runs. Vertical lines indicate representative standard deviations at random points on the curve, and each curve was drawn on the basis of 35–40 experimental points. Key: A, drug alone; B, drug followed in 2 min by normal saline; and C, drug followed in 30 sec by normal saline.*

Table VII—Aqueous Humor Concentration of Pilocarpine Nitrate and Epinephrine Hydrochloride Determined 30 min Postinstillation of Combined or Separate Solutions

Dosing Regimen	Pilocarpine Concentration, μg/ml[a]	Relative Bioavailability	Epinephrine Concentration, μg/ml[a]	Relative Bioavailability
Monitoring first drug in series[b]	0.388	0.64	0.016	0.61
Monitoring second drug in series[b]	0.573	0.94	0.024	0.96
Monitoring drug in combination, 1 drop	0.707	1.16	0.026	1.02
Monitoring drug as single drop	0.611	1.00	0.025	1.00

[a] Minimum of three separate determinations with a standard deviation of 8% or less. [b] Fifty microliters of drug solution was instilled 1 min apart; see *Experimental* for details.

30 min postinstillation (Table VII). As expected, the combined product gives considerably higher levels of both drugs as compared to administration of separate solutions. The order of drug instillation, as described earlier and shown in Table III, is important since the first drug administered always suffers a greater loss than the second drug; this is verified in Table VII.

A 1-min time interval between drops was arbitrarily chosen in this study, and it is expected that shorter or longer time intervals would influence the aqueous humor drug levels obtained.

Not shown in Table VII, because only one determination was made, is the schedule where two drops of the combination drug solution were administered with a 1-min time interval between drops. As expected, the aqueous humor level of drug obtained from this treatment is higher than with the administration of one drop of the combination product. This expected behavior was described earlier and was shown in Table II, in which a higher precorneal concentration of drug was obtained by administration of two drops.

Miosis-Time Studies—To illustrate the effect of separate drops of two drugs on the bioavailability of drug, pilocarpine nitrate and normal saline were selected to represent the two drugs and the miosis-time effect of pilocarpine nitrate was monitored (10). Pilocarpine nitrate solution was instilled into rabbit eyes, followed at appropriate times by instillation of normal saline. Measurements of pupillary diameter as a function of time were made (Fig. 5).

As already discussed, concurrent administration of two solutions into the eye reduces the precorneal drug concentration of both drugs and, hence, reduces the apparent biological activity of the drugs. Curves B and C in Fig. 5 illustrate this effect. For time intervals between drops longer than 5 min, there was no modifying influence on miotic activity of pilocarpine nitrate. Since the resident volume from the first drop returns to normal within 5 min in the rabbit (6), this effect was expected.

All the work presented in this report dealt with albino rabbits as the experimental animal. Understanding of the physicochemical processes that occur in the precorneal portion of the rabbit eye is admittedly minimal, and thus extrapolation of the present data is done with caution. Nevertheless, it is presumed that tear and instilled fluid dynamics of the rabbit are proportional to humans, with humans having a larger tear turnover rate (11) and more rapid drainage rate of instilled solutions. This would mean that the various effects discussed in this report would be magnified when applied to humans.

With regard to qualitative application of these results to clinical therapy, several observations can be made. The use of a large as opposed to a small drop will result in a higher precorneal concentration of drug; however, toxicity can be a problem. It would appear more advisable to raise the concentration of the drug in the instilling solution and use a much smaller drop. At present, most commercial ophthalmic droppers deliver from 40 to 70 μl of drug solution and it is suggested that a 5- or 10-μl drop containing a larger concentration of drug would be more appropriate.

Two drops of a single drug will raise the precorneal concentration over that of a single drop. This effect, however, does not justify the use of two or more drops for the simple reason that the increase in precorneal concentration due to two or more drops will never exceed the precorneal concentration due to a single drop of a correspondingly higher concentration, while at the same time it will always exceed the single drop in amount of drug lost through drainage or spillage on the cheek. It would also appear that for large drops the use of three or more drops given consecutively or at short time intervals serves little purpose since most of the drug solution will either spill onto the cheek or be rapidly drained. The slight increase in precorneal drug concentration achieved by the third of fourth drop is minimal.

Spacing of drops by 5 min in rabbits and presumably a slightly shorter period in humans will reduce the drug loss problem but will also reduce the precorneal drug concentration over this time period. A more acceptable approach, as indicated earlier, is to reduce drop size and raise the concentration of drug in the drop to be instilled.

When two or more drugs are indicated for therapy, the results presented in this report constitute a strong argument for a single combination product rather than separate solutions.

REFERENCES

(1) *Clin. Trends*, **III** (5), 8(1972).

(2) G. L. Jenkins, G. J. Sperandio, and C. J. Latiolais, "Clinical Pharmacy," McGraw-Hill, New York, N.Y., 1966, p. 181.

(3) R. Praus, I. Brettschneider, L. Krejci, and D. Kalvadova, *Ophthalmologica*, **165**, 62(1972).

(4) C. H. Dohlman, *Ann. Ophthalmol.*, **4**, 823(1972).

(5) S. Lerman and M. D. Reininger, *Can. J. Ophthalmol.*, **6**, 14(1971).

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

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

(8) J. J. Gneco and C. D. Kelman, *Ann. Ophthalmol.*, **5**, 57 (1973).

(9) P. Ellis, "Side Effects of Drugs in Ophthalmology," *Int. Oph. Clinics*, Vol. 11, No. 2, Little, Brown, Boston, Mass.

(10) T. J. Mikkelson, Ph.D. thesis, University of Wisconsin, Madison, Wis., 1971.

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

ACKNOWLEDGMENTS AND ADDRESSES

Received May 4, 1973, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706*

Accepted for publication October 19, 1973.

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

The authors acknowledge the technical assistance of Mr. Randy Miller.

* Present address: Allergan Pharmaceuticals, Irvine, CA 92664

x To whom inquiries should be directed.