THE INFLUENCE OF ANESTHESIA ON LACRIMATION AND INSTILLED FLUID DRAINAGE IN THE ALBINO RABBIT

- A POTENTIAL METHOD OF IMPROVING OPHTHALMIC DRUG BIOAVAILABILITY -

by

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

Drainage of an instilled drug solution away from the eye, as well as normal tear turnover, is responsible for a considerable loss of drug and hence affects the biological activity of drugs in the eye. A simple isotopic technique, using radioactive technetium adsorbed colloid, was developed and utilized in this study to evaluate the effects of both general and local anesthesia on lacrimation and instilled fluid drainage in the albino rabbit. Lacrimal fluid turnover rate in rabbits under general anesthesia is negligibly small or absent. Local anesthetics decrease tear formation according to their intrinsic activity and the number of drops instilled. Tetracaine HCl was able to produce as much as a 5-fold decrease in the normal lacrimal fluid turnover rate when compared to fully awake, unanesthetized rabbits. Drainage loss of a 25-µl. instilled solution was determined in rabbits under the influence of topically applied local anesthetics and in rabbits under general anesthesia. These results were compared with those obtained in unanesthetized rabbits. It is shown that the loss of instilled solution

via drainage is first order with respect to time. In addition, the apparent rate constant in rabbits under general anesthesia is independent of the volume instilled. General anesthesia was shown to cause a 3-fold decrease in drainage rate. This decrease was noted for animals in an upright position and will obviously vary depending upon the position of the animal during the course of the experiment. Topically applied local anesthetics decrease drainage according to their dosage, and range from no decrease to a 5-fold decrease depending upon the anesthetic used. A discussion of the possible implications of these results with respect to clinical practice is presented. The results reported here suggest that a possible reevaluation of some previously published ophthalmic animal data may be necessary. In addition, a potential method of screening topical anesthetics is suggested. The value of these findings as a general method for improving ophthalmic drug bioavailability is presented.

APPROVED Joseph R. Robinson

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INTRODUCTION

Ophthalmic drug delivery involves problems that are unique to the eye. In general, only a very small amount of an instilled dose remains in the eye to exert a local effect or to pass across the cornea and into the anterior chamber. Two major effects influencing this loss are the continuous replenishment of tears and their flow across the cornea, as well as instilled solution drainage. These factors can exert a very large influence on the biological activity of drugs in the eye. For example, since lacrimal fluid is turned over at an appreciable rate, reported to be about 16% per minute in humans (1), high concentrations of drug in lacrimal fluid are required for the first few minutes post instillation in order to maximize biological activity. Moreover, drugs instilled into the eye intended to exert either a local effect or to pass into the aqueous humor are subject to a large drainage loss, thus removing drug from the desired target area of the eye. Any attempt, then, to minimize drug loss by controlling tear production and/or instilled solution drainage should prove to be a useful step in improving drug bioavailability in the eye.

In the past, viscosity alterations of solutions, suspensions, and emulsions, as well as the use of solid delivery systems such as soft contact lenses (2, 3) have been attempts to avoid drainage rate problems and hence increase corneal contact time. These attempts have not been based on fundamental information related to tear production and movement

or instilled fluid dynamics, but rather on clinical observations using a trial and error approach of improving a particular biological response. This is due to the fact that a complete understanding of tear production and movement, as well as instilled solution drainage, has been unavailable in the literature. A recent publication from this laboratory (4) has discussed the influence of tears and instilled fluid dynamics on the bioavailability of drugs in the eye. It appears that a considerable amount of work concerning the influence of tears and instilled fluid drainage on drug bioavailability remains to be done in order to maximize topical drug therapy in the eye. However, it is known in a qualitative sense that these factors exert a large influence on drug activity.

Rather than using formulation factors, an alternative approach to increasing corneal contact time and hence drug bioavailability in the eye would be to decrease the extent of instilled solution drainage and tear production through the use of drugs or as part of the normal circadian rhythm. It has been reported that lacrimation is either significantly reduced (5) or absent (6-8) during normal sleep. If this is the case, then it is likely that anesthesia could also influence lacrimation, and in fact it has been reported that some general anesthetics do inhibit lacrimation (9). This information can be of considerable import in animal studies relating to drug penetration studies in the eye. In such experiments, largely for convenience purposes, anesthetized

animals are commonly used. The results of these studies could be expected to differ significantly from studies conducted in unanesthetized animals considering the difference in turnover rate of lacrimal fluid (10).

It has also been reported that tear flow can be considerably reduced by the retrobulbar injection of local anesthetics (11). In spite of these observations, little or no attention has been given to a complete study of the influence of anesthetics on drainage rate and tear production. This is surprising since clinically, topical anesthetics are used routinely in ophthalmic practice, including tonometry, cataract removal, removal of foreign bodies from the eye, suture removal, conjunctival scraping for diagnostic purposes, gonioscopic examination and similar procedures, as well as preceeding the Schirmer tests to determine normal lacrimal function. A knowledge of the influence of topical anesthesia on tear production could help to explain some unusual findings with regard to the Schirmer tests. For example, Norn reports (12) that many patients are symptom-free despite a much too low tear production as assessed by Schirmer's method. This decreased tear production could be due not to some pathological condition, but instead due to the fact that the Schirmer test was preceeded by topical application of a local anesthetic.

An added benefit of a thorough understanding of anesthetic influences on tear production and instilled solution drainage is the possible application of this information to

corneal drug delivery. For example, reducing lacrimation and instilled fluid drainage by the use of anesthetics would increase the concentration of drug at the site of action, thereby decreasing the loss of drug to the drainage apparatus. Side effects from potentially toxic substances could be minimized providing that the agent used to alter drainage loss is less toxic than the therapeutic agent itself.

The objectives of this study, then, were to examine the influence of anesthesia on lacrimation and instilled solution drainage. The implications of such a study are many. To the clinician the possibility of improved drug bioavailability and decreased side effects is an important consideration. In addition, some anomalous results such as those mentioned with the Schirmer test may be placed in proper perspective. To the surgeon who deals routinely with both general and local anesthesia, such information would be valuable. To the laboratory investigator, a re-evaluation of animal data obtained under general anesthesia may be necessary. Finally, the prospect of further insight into the function and mechanism of action of the lacrimal system is of great import.

EXPERIMENTAL

A. Materials

Water was doubly distilled from alkaline permanganate in an all glass distillation apparatus.

Technetium solutions were prepared using a package (ColloKit, Abbott Radio-Pharmaceuticals, North Chicago, Ill.) of solutions and equipment used to prepare technetium, ^{99m}Tc, suspensions.

Local anesthetics used were obtained from a commercial source.[1,2,3] All other chemicals were either reagent or analytical grade.

Adult, male, albino rabbits (Klubertanz, Edgerton, Wis.) were used throughout this study. The rabbits were fed a regular diet with no restrictions on the amount of food or water consumed. All rabbits used weighed between 1.80 and 2.40 Kg.

B. Solution Preparation

Technetium colloidal solutions were prepared by addition of technetium, ^{99m}Tc, to solutions provided in a colloidal sulfur technetium kit, from which the sulfur colloid is prepared in situ[4], Technetium ^{99m}Tc, was obtained by elution

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[1] Tetracaine HCl 0.5%, Cooper Laboratories, Inc., Wayne, N.J.
[2] Proparacaine HCl 0.5% (Ophthetic), Allergan Pharmaceuticals, Irvine, Calif.
[3] Lidocaine HCl 1% (Seracaine), Rachelle Laboratories, Inc., Long Beach, Calif.
[4] For details see the brochure "ColloKit for the Preparation of Technetium Sulfide, ^{99m}Tc, Injection", Abbott Labs, North Chicago, Ill., 1971, pp. 2-4.

off the generator column, in which it was prepared with normal saline. The technetium solution was added to a thiosulfate-mannitol solution, and the resulting solution was treated with hydrochloric acid and heated to produce a technetium sulfide colloid. The colloid was then added to a buffer solution and again heated to produce the final product. Throughout the study, the final technetium, ^{99m}Tc, suspension contained between 1 and 2 millicuries of activity per milliliter of solution.

Anesthetic solutions were composed of a mixture of sodium pentobarbital and sodium phenobarbital. Separate solutions of the two drugs were prepared in a vehicle containing 20% (v/v) propylene glycol and 10% (v/v) ethyl alcohol (95%). The pentobarbital solution contained 50 mg./ml. of drug and the phenobarbital solution contained 100 mg./ml. of drug. Both solutions were refrigerated between uses and were never kept for more than two weeks.

C. Anesthesia

A combination of sodium pentobarbital and sodium phenobarbital was used to induce and maintain the desired level of general anesthesia. A dose of 33 mg. of sodium pentobarbital and 100 mg. of sodium phenobarbital per kilogram of animal body weight was sufficient in most cases to anesthetize the rabbits for 8-12 hours. With this dose, the onset of anesthesia usually occurred in 15-30 minutes. Drugs were administered by intraperitoneal injection.

Local anesthesia was accomplished by instilling the anesthetic solutions[1,2,3] onto the cornea so that they collected in the lower cul-de-sac. All local anesthetics were placed in plastic dropper bottles before use so that the drop size delivered was approximately the same in all cases.

D. Technetium Studies: Sampling Method

Animal Preparation. Test animals for this study were either: (a) anesthetized where manual mixing of tracer solution with lacrimal fluid was employed, or (b) unanesthetized, where natural mixing occurred. The unanesthetized test animal was placed in a restraining box in its normal posture. The technetium suspension was instilled onto the cornea so that it collected in the lower cul-de-sac. To prevent loss of suspension during instillation, the lower eyelid was pulled slightly away from the globe to form a pocket. After instillation, the lid was returned to its former position. Thus normal movement of the eye was used to mix the colloidal suspension of technetium with the lacriaml fluid. After instillation of the tracer, the animal would close its eyelids, but would in all cases open the lids within 10-15 seconds after instillation. Closing of the lids was not due to irritation from the tracer substance, since instillation of normal saline evoked the same response.

Animals under general anesthesia were placed in restraining boxes and experiments were conducted with the animals in their normal posture. Technetium suspension was instilled

in the same manner as described for unanesthetized animals, except that after instillation of the suspension, the lower lid was lifted back and forth over the cornea to mix the tracer with lacrimal fluid. At no time was the lid ever massaged against the cornea, so only movement of the instilled suspension over the cornea occurred. To exert some degree of control in this method, the eyelid was lifted over the cornea exactly four times during about five seconds in all cases. To aid in reproducibility, all procedures were performed in precisely the same manner and with the same time intervals.

Procedures. Radiocative technetium colloidal suspensions were instilled into the rabbit eye from a syringe (The Hamilton Co., Whittier, Calif.) which had been prechecked for accuracy of delivery by weighing the delivered solutions.

After instillation of a suitable volume of technetium colloid, 15 seconds were allowed to elapse for either manual or natural mixing of the colloid with lacrimal fluid. A sample of tears was then obtained at appropriate time intervals by placing a 1-μl. capillary tube (Drummond Microcap, Scientific Glass Apparatus Co., Bloomfield, N.J.) in contact with the marginal tear strip. The capillary immediately filled with tears. All samples were withdrawn from the lower marginal tear strip, approximately in the middle of the strip. The samples were counted (Abbott Model 111 Well Counter, Abbott Laboratories, North Chicago, Ill.) for one minute.

Before and during the study, background readings were taken to determine the level of radioactivity in the test

room. Before instillation of the technetium colloid, three readings of the activity of isotope being introduced into the eye were made. In general, the number of counts per ul. of technetium colloid was in the range of 100,000 per minute. Suitable corrections for the normal decay of the technetium isotope were made whenever necessary.

Normal Lacrimal Turnover Rate. Various volumes, ranging from 1 to 50 µl. of technetium suspension, were instilled into the eye. Samples were withdrawn and assayed for activity at 5, 10, or 20 minute intervals. Sampling was continued until the isotopic activity had decreased to approximately background levels which varied from 30 minutes to 2 hours depending upon the volume instilled. Only one eye of each experimental animal was used, and no animal was used more than once. From a plot of the logarithm of technetium concentration versus time, it is possible to determine the turnover rate as shown in the Appendix.

Drainage Rate Determination. After instillation of various volumes of technetium colloid into the eye, samples were withdrawn and radioactivity measured at 5, 10, 15 or 20 second intervals. The sampling technique for drainage rate determination was not pursued because of the difficulty in obtaining frequent samples without irritating the eye. More importantly, this technique measures technetium concentration. As drug is drained out of the eye, concentration does not change appreciably whereas the total amount of drug is altered.

Technetium Studies: Non-Sampling Method

Animal Preparation. All procedures were the same as in the sampling method.

Procedures. The various volumes of colloidal technetium suspension were instilled into the eyes of both anesthetized and unanesthetized rabbits, and the decline in radioactivity was monitored with a thin-probe scintillation detector (Model 7498, Abbott Laboratories, North Chicago, Ill.) attached to a well counter. The thin-probe detector is a cylindrical vessel, approximately 22 cm. long and 5 cm. in diameter, containing a sodium iodide crystal capable of detecting β-emitters. Since the entire outer wall and bottom of the probe can respond to radioactivity, it was necessary to cover the entire probe except for a 1.5 cm. opening at the bottom with a 0.3 cm. (0.125 in.) thick lead shield. Thus the only radioactivity picked up by the probe would be that coming through the orifice at the bottom. This orifice was placed directly over the cornea of the test animal approximately 1 or 2 cm. away from the eye. Preliminary testing had shown that this distance from the eye and the size of the orifice in the lead shield at the bottom of the probe, were compromises to obtain maximum isotope counts from the eye, as well as to exclude extraneous radiation from the surroundings or from technetium that might have passed into the drainage apparatus.

With anesthetized animals, no special precautions were followed, while with unanesthetized animals it was necessary to precondition the animals to the testing procedure so that

head and eye movements were eliminated during the experiment. For preconditioning, the test animals were placed in the restraining box with the probe in position once a day for one hour on seven successive days prior to the experiments. After this conditioning, the animals showed no head or eye movement for the duration of the experiment, and if they did it was clear from the results and the experiment was terminated at that point.

In the case of the unanesthetized animals and those under general anesthesia, various volumes of technetium colloidal suspension were instilled into the rabbit eye as described in the Sampling Method section, the probe was positioned in front of the eye, and the experiments were begun. In the experiments where local anesthesia was used, a drop of the local anesthetic was instilled into the eye and a period of 10 minutes was allowed to elapse before technetium was instilled and counting commenced. The 10-minute interval was selected to allow complete drainage of the anesthetic solution (13). In those instances where multiple drops of local anesthetic were used, the drops were instilled at 1-minute intervals, and 10 minutes were allowed to elapse after the last drop before technetium was instilled.

In all cases, measurements of radioactivity in the eye were made every 10 seconds, and counts were determined for periods of 5 seconds. This measuring technique determines the amount of drug in the eye, and not concentration. Measurements were made until the technetium radioactivity had

decreased to approximately background level, which usually took 60 to 90 minutes, although shorter and longer times occurred depending upon the experiment.

Normal lacrimal turnover rate and instilled volume drainage rate constants were obtained by computation. Derivation of the appropriate equations is shown in the Appendix.

E. Determination of Onset and Duration of Action of Local Anesthetics

For each local anesthetic tested, 3 rabbits were positioned in restraining boxes and a drop of the appropriate local anesthetic instilled onto the cornea of one eye. A drop of saline was instilled into the second eye of each rabbit which served as a control. At 5-minute intervals, the effectiveness of the local anesthetics was measured by means of a corneal reflex. A thin smooth glass rod was touched to the center of the cornea and the blink response was observed. In this way a qualitative measure of the onset and duration of action of the local anesthetics was obtained. This was helpful in gaining an idea of which drugs might be useful in the isotope studies, as well as providing a means of observing the general response of the eye under the influence of local anesthetics.

F. Evaluation of the Technetium Dilution Technique for Lacrimal and Instilled Fluid Dynamic Studies

In order to use a tracer substance such as isotopic technetium as a test substance for instilled fluid dynamics

studies, it is necessary to satisfy several criteria: (a) the test substance must be lost from the eye solely via the drainage apparatus, i.e., no spillage; (b) no absorption/adsorption must occur or, if sorption does occur, its extent must be known; (c) the test substance must be non-irritating; and (d) if the substance is an insoluble solid, it must not interfere with loss of fluid into the tear drainage apparatus.

To show that neither loss nor retention of a significant portion of the technetium colloid occurred through binding to eye tissues and/or absorption, the amount of drug consumed in these possible routes was determined. Because of the differences between the two techniques (sampling and non-sampling) employed in the determination of the amount of ^{99m}Tc present, the amount shown to be present by each method could be different if any significant binding or absorption occurred. Since the ^{99m}Tc is a 142-kev. β-emitter, its presence can be detected by the probe through the conjunctiva and eyelids. Consequently, ^{99m}Tc bound to eye tissue or absorbed into the eye, with the exception of the portion that passed into the blood stream and was swept away, would still be seen by the probe, whereas ^{99m}Tc determined by the sampling technique would not show this retention. For this study, 25 µl. of technetium suspension was instilled into the eyes of several anesthetized animals. At various time intervals over 4 hours, representing the maximum duration of the experiments, the animals were sacrificed and isotope activity in aqueous humor, cornea, sclera, conjunctiva, and eyelids was determined. Less

than 0.1% of the instilled dose was absorbed or adsorbed into any portion of the eye tested during this time period. This small activity could have been due to contamination of the tissue samples rather than absorption/adsorption. Thus all loss appears to be through the drainage apparatus.

Since the technetium isotope is adsorbed on a colloid, there is the possibility of blockage or interference with drainage into the nasolacrimal duct. In preliminary studies using gelatin-stabilized technetium colloid, the drainage apparatus was apparently blocked by the colloid as judged by impaired or inhibited disappearance of the technetium isotope. This problem did not appear when mannitol-stabilized technetium was used, and repetitive application of the isotope solution at various times and under various conditions gave highly reproducible results.

Insofar as irritation is concerned, the isotope at very high concentrations does irritate the rabbit eye as judged by animal reaction and lacrimation. Concentrations of isotope used in this study did not cause observable irritation to the test animal.

RESULTS

A. General Anesthesia

Determination of Normal Lacrimal Fluid Turnover Rate in Rabbits Under General Anesthesia via the Sampling Technique

The turnover rate of lacrimal fluid can be determined by instilling a tracer substance of known concentration and activity and by monitoring the decline in isotope activity as a function of time. Known concentrations of technetium suspension were instilled into the eyes of rabbits under general anesthesia, and tear samples were obtained and assayed at various time intervals. The logarithm of the decline in technetium concentration was plotted against time, and the first order rate constant was determined from the slope of the line as shown in the Appendix. The results of this study are presented in Table I, together with standard deviations and resultant turnover rates based on a lacrimal fluid volume of 12.0-µl. for rabbits under general anesthesia (4).

As can be seen, the turnover rate was essentially zero for the 25- and 50-µl. runs and rather large for the 1- and 5-µl. instilled volumes. It was concluded that the turnover rate in rabbits under general anesthesia is essentially zero. The large rates observed for the 1- and 5-µl. runs are possibly due to irritation from repetitive sampling, since it was difficult to obtain more than two or three samples in each experiment. Thus, it was concluded that animals under general anesthesia have a turnover rate that is either

TABLE I - Turnover Rate of Lacrimal Fluid in Rabbits Under General Anesthesia by the Technetium Dilution Method Using the Sampling Technique

Instilled Volume (µl)	Sampling Time (minutes)	No. of Det'n	k min^{-1} Range	k min^{-1} Average	S.D.	Turnover[a] Rate (µl/min.)
1	10	3	0.063-0.071	0.0656	0.005	0.39
5	10	3	0.054-0.058	0.056	0.002	0.32
25	10	3	0.0077-0.0084	0.0081	0.0003	0.061[b]
50	10	3	0.0041-0.0074	0.0053	0.002	0.026[b]

[a]Obtained by multiplying the observed first order rate constant times the lacrimal volume, assumed to be 12.0 µl. and subtracting the sampling withdrawal rate constant.

[b]Subtracting the withdrawal rate gives a negative number to the turnover rate thus the sampling rate was neglected. This is not too unreasonable since the sample volume withdrawn is small compared to the instilled volume.

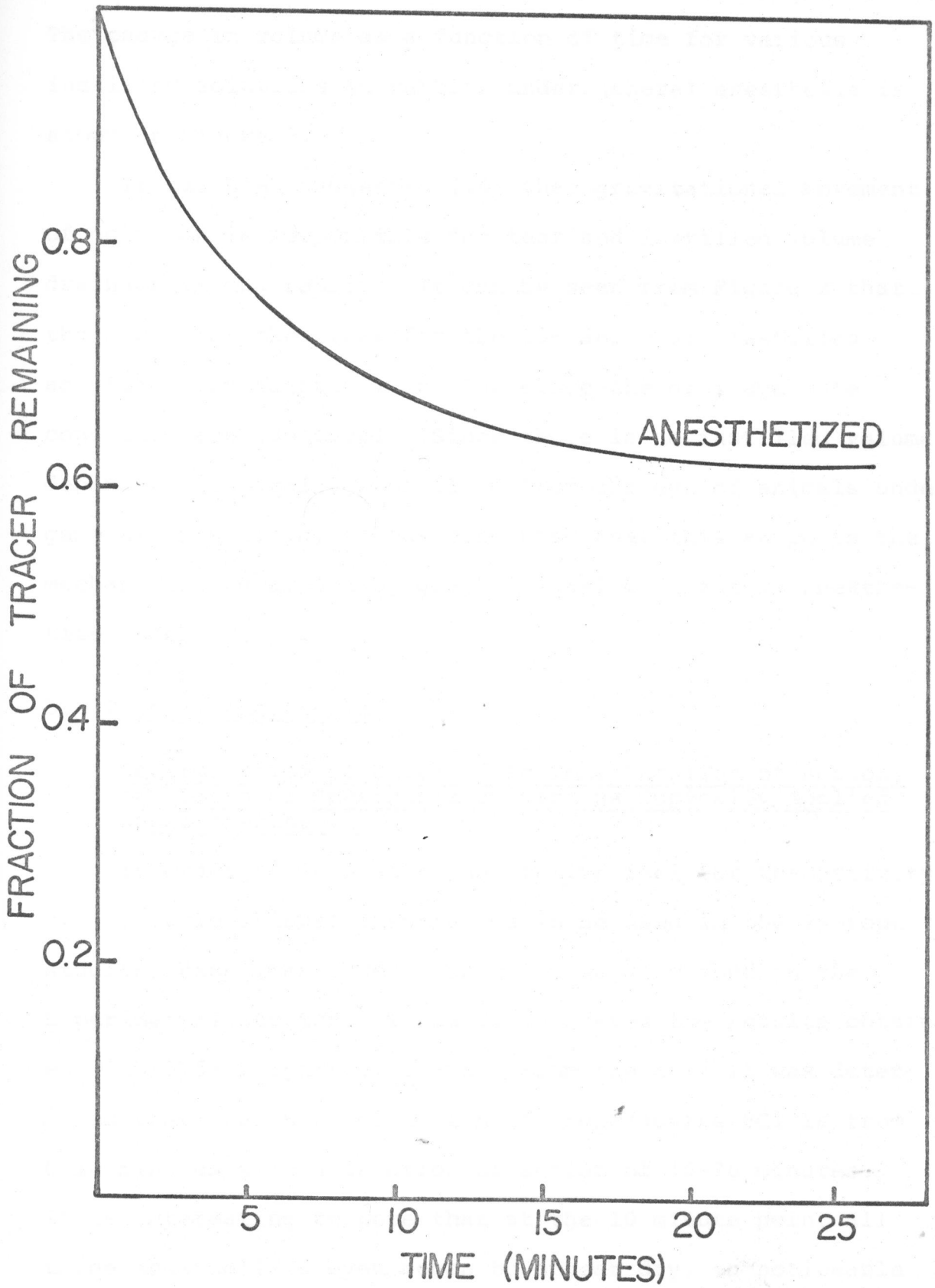
very small or nonexistent, which is in agreement with previously reported observations that humans produce either no tears (6-8) or a reduced amount of tears (5) during sleep.

Determination of Normal Lacrimal Fluid Turnover Rate and Instilled Volume Drainage Rate in Rabbits Under General Anesthesia via the Non-Sampling Technique

Loss in amount of tracer from the precorneal portion of the eye can be monitored without withdrawing samples, thus avoiding the potential error introduced by irritation. It is important to recognize that the non-sampling technique measures amount of tracer in the eye and not concentration. As shown in the Appendix, it is possible to obtain the necessary rate constants irrespective of which technique is used. A typical example of results obtained via the non-sampling approach is shown in Figure 1, using 5-µl. as the instilled volume in rabbits under general anesthesia. As expected, based on results from the sampling experiments, the terminal portion of the fraction of tracer remaining versus time plot reaches a plateau. The sampling studies of turnover of lacrimal fluid showed that no lacrimation occurred while the animal was under general anesthesia. Therefore, it was expected that the decline in drug amount would cease when drain-off was complete.

Utilizing equations presented in the Appendix, it is possible to calculate the volume remaining at various time intervals for different instilled volumes. From such a plot it is possible to determine the rate constant for drainage.

Figure 1. Fractional amount of drug remaining as a function of time for rabbits under general anesthesia using a 5-μl. instilled volume. The line represents the average of four separate determinations.


FRACTION OF TRACER REMAINING
0.8
0.6
0.4
0.2
ANESTHETIZED
5
10
15
20
25
TIME (MINUTES)

The change in volume as a function of time for various instilled solutions in rabbits under general anesthesia is shown in Figure 2.

It has been suggested (14) that gravitational movement of solution is responsible for tear and instilled volume drainage in the rabbit. It can be seen from Figure 2 that the slopes of the lines for the 50- and 5-µl. instilled solutions are identical and therefore the drainage rate constants are identical. Since there is no instilled volume dependency in drainage of fluid from the eye of animals under general anesthesia, it was concluded that this supports the mechanism of drainage by gravitational movement in anesthetized rabbits.

B. Topical Anesthesia

Determination of Onset of Action, Duration of Action, and Depth of Anesthesia of Various Topically Applied Local Anesthetics

In order to gain some qualitative feel for the activity of the various local anesthetics to be used in the isotope studies, experiments were conducted as described in the Experimental section. Table II indicates the results obtained with 0.5% proparacaine HCl. From the data it was determined that the onset of action of proparacaine HCl is from 0-10 minutes with a duration of action of 10-20 minutes. It is interesting to note that at the 10 minute point all three anesthetized eyes began to appear dry, in noticeable contrast to the control eyes. At the 25 minute point all

Figure 2. Change in volume of solution remaining from an instilled solution as a function of time in rabbits under general anesthesia. The top line is for an instilled volume of 50-µl. and the bottom line is for 5-µl. Both lines represent three different determinations, and the data points are mean values ± 5%.

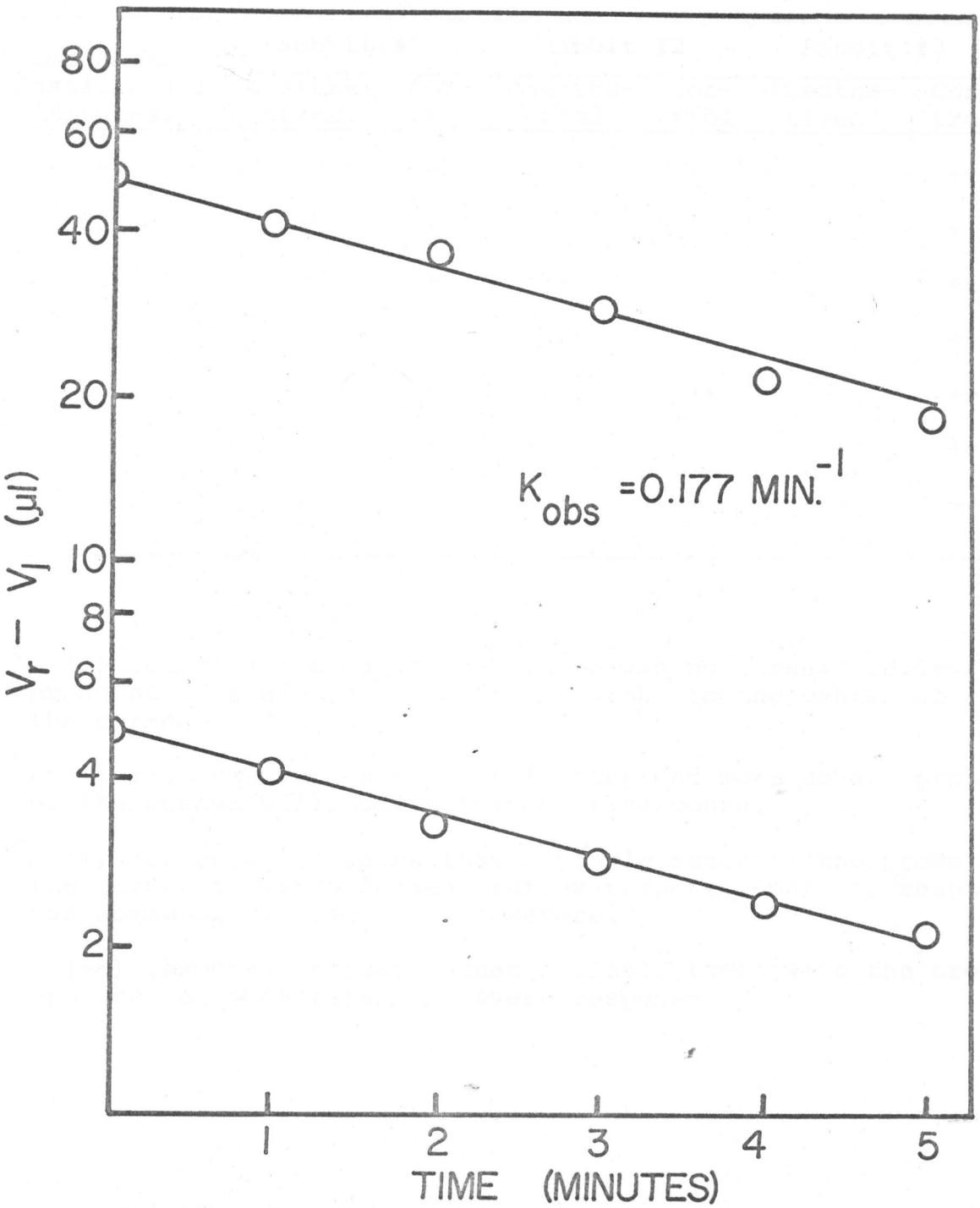

80
60
40
20
10
8
6
4
2
$V_r - V_l$ (µl)
K_{obs} =0.177 MIN.$^{-1}$
1
2
3
4
5
TIME (MINUTES)

TABLE II - Determination of Onset and Duration of Action of Topically Applied Proparacaine HCl 0.5% by the Corneal Reflex Technique

Time After Instillation (Minutes)	Rabbit #1		Rabbit #2		Rabbit #3	
	Anesthetized	Control	Anesthetized	Control	Anesthetized	Control
5	-[a]	+[c]	+	+	-	++
10	=[b]	++[d]	-	+	=	+
15	=	+	-	+	=	++
20	=	++	+	+	+	++
25	+	++	+	++	+	++
30	+	+	+	+	++	++
35	+	+	+	+	++	++

[a] A (-) response indicates that there was no corneal reflex produced by a single touch of the probe in the center of the cornea.

[b] A (=) response indicates that further and more severe probing of the cornea still did not evoke a response.

[c] A (+) response indicates that a single touch of the probe to the cornea evoked a corneal reflex response, but the response was somewhat delayed and not severe.

[d] A (++) response indicates that a single touch with the probe produced an immediate and severe response.

anesthetized eyes appeared to be regaining their moisture, and by 35 minutes the anesthetized eyes could not be distinguished from the control eyes on the basis of appearance of the tear film.

Table III reports the data obtained from identical experiments performed using 0.5% tetracaine HCl. The table indicates an onset of action of 0-25 minutes and a duration of action of 30-55 minutes. Again, as was the case with proparacaine HCl, drying of the eye and return to normal appearance seemed to closely approximate loss and resumption of the corneal reflex. In comparison with proparacaine HCl, tetracaine HCl appears to act somewhat slower but has a much longer duration of action. In addition, the incidence of the deeper (=) anesthesia appears to be sustained for longer periods of time. The implications of these observations will become clearer on examination of the results of the isotope studies to follow.

Similar experiments were performed using lidocaine HCl. Except for one out of the three rabbits tested, who experienced an extremely mild loss of the corneal reflex, 1% lidocaine HCl appeared to have virtually no effect in abolishing the corneal reflex. In addition to observing no loss of corneal reflex, there appeared to be little, if any, inhibition of lacrimation,i.e., the anesthetized eyes of all rabbits displayed no observable differences in appearance from the control eyes.

TABLE III - Determination of Onset and Duration of Action of Topically Applied Tetracaine HCl by the Corneal Reflex Technique

Time After Instillation (Minutes)	Rabbit #1		Rabbit #2		Rabbit #3	
	Anesthetized	Control	Anesthetized	Control	Anesthetized	Control
5	-[a]	++[d]	++	++	++	++
10	=[b]	+[c]	-	++	+	++
15	=	+	=	++	+	++
20	=	+	-	+	+	++
25	=	+	=	+	-	++
30	=	+	=	+	-	++
35	=	+	=	+	=	++
40	=	+	=	++	-	++
45	-	+	-	+	=	++
50	-	+	=	+	-	+
55	+	+	+	++	+	++
60	+	+	-	+	+	++
65	+	+	+	+	+	++
70	+	+	+	+	+	++
75	+	+	+	+	+	++

[a] A (-) response indicates that there was no corneal reflex produced by a single touch of the probe in the center of the cornea.

[b] A (=) response indicates that further and more severe probing of the cornea still did not evoke a response.

TABLE III cont'd

[c]A (+) response indicates that a single touch of the probe to the cornea evoked a corneal reflex response, but the response was somewhat delayed and not severe.

[d]A (++) response indicates that a single touch with the probe produced an immediate and severe response.

It should be noted that these experiments were in no way intended to be definitive quantitative studies, and in fact with somewhat more sophisticated equipment, more quantitative methods for measuring corneal sensitivity are available (11). However, the experiments performed here were reproducible when repeated on the same rabbit, and do give a basis for judging the effectiveness of various local anesthetics, as well as a means of making some overall observations as to changes in general characteristics of the eye while under the influence of these drugs.

Determination of Normal Lacrimal Fluid Turnover Rate and Instilled Volume Drainage Rate in Rabbits Under Topical Local Anesthesia via the Non-Sampling Technique

Varying numbers of drops of 0.5% tetracaine HCl were instilled into the eyes of rabbits at 1-minute intervals, and following a lapse of 10 minutes, 25 µl. of technetium suspension were instilled. As was done with the animals under general anesthesia, the logarithm of the decline in isotope activity was plotted versus time. Linear regression analysis was performed on the data, and the turnover rates obtained from the slopes of the lines are reported in Table IV together with data obtained using 1 drop of 0.5% proparacaine HCl. Analysis of the data reveals that tetracaine HCl, as well as proparacaine HCl, causes a significant decrease in the normal turnover rate of the lacrimal fluid indicating a decreased tear production. In addition, it is observed that tetracaine HCl has a more pronounced effect in reducing the turnover rate than proparacaine HCl at a given dosage.

TABLE IV - Turnover Rate of Lacrimal Fluid in Rabbits Following Various Doses of Local Anesthetics Using the Non-sampling Technique

No. of Drops of Local Anesthetic Instilled	Turnover[a] Rate (µl/min.)	S.D.	No. of Det'n
Tetracaine HCl 0.5%			
0[b]	0.66	0.06	3
1	0.20[c]	0.06	7
2	0.19[c]	0.12	11
3	0.13[c]	0.08	5
4	0.11[c]	0.03	4
5	0.10[c]	0.04	7
Proparacaine HCl 0.5%			
1	0.27[c]	0.07	4

[a]Obtained by multiplying the observed first order rate constant times the lacrimal volume, found to be 7.5 µl. in rabbits not under general anesthesia (4).

[b]Refers to data obtained in fully awake, unanesthetized animals (4).

[c]Statistical analysis of these values using the Student's t distribution (99% Level) shows them to be significantly different from the value for unanesthetized animals.

This observation is consistent with the results of the previous section that the duration of action and duration of deep anesthesia is greater with tetracaine HCl than proparacaine HCl.

Using a similar procedure as was used for rabbits under general anesthesia, the rate constants for drainage of a 25-µl. instilled dose of technetium suspension were determined following the instillation of various doses of tetracaine HCl and after the instillation of one drop of proparacaine HCl. The results of this study are shown in Table V. As was the case with turnover rates, tetracaine HCl is able to substantially reduce the drainage rate of an instilled solution. The higher the dosage, the more pronounced is the effect. Also, one drop of tetracaine HCl has a significantly greater effect in decreasing drainage than does a similar dose of proparacaine. In fact, a single drop of proparacaine HCl has no effect in decreasing drainage of a 25-µl. instilled dose of technetium suspension when compared with the data for fully awake, unanesthetized animals (4).

TABLE V - First Order Drainage Rate Constants For a 25-μl. Instilled Volume of Technetium Suspension Following Various Doses of Local Anesthetics Using the Non-Sampling Technique

No. of Drops of Local Anesthetic Instilled	First Order Drainage Rate Constant ($min.^{-1}$)	S.D.	No. of Det'n.
Tetracaine HCl 0.5%			
0[a]	0.54	b	3
1	0.39[d]	0.11	7
2	0.35[d]	0.19	9
3	0.42[d]	0.23	4
4	0.12[c]	0.08	3
5	0.06[c]	0.04	4
Proparacaine HCl 0.5%			
1	0.73[d]	0.17	3

[a]Refers to data obtained in fully awake, unanesthetized animals (4).

[b]Reported to be within ± 5% of the mean (4).

[c]Statistical analysis of these values using the Student's t Distribution (99% Level) shows them to be significantly different from the value for unanesthetized animals.

[d]Statistical analysis of these values using the Student's t Distribution (99%Level) shows no significant difference from the value for unanesthetized animals.

DISCUSSION

Lacrimal Fluid Turnover Rate

Complete absence of, or a severe reduction in turnover rate in animals under general anesthesia is shown by both the sampling and non-sampling results. Since these techniques measure both concentration and amount changes, reflecting inflow and outflow respectively, the conclusion seems valid. Support for this was obtained by observing an absence of tears when a rabbit under general anesthesia was placed on its back with its head hanging down. If tears were being produced under such conditions, one would expect to see an accumulation of tears along the upper lid and eventually spillage out of the eye since there would be no pathway for tear drainage. It is possible that tears are produced under general anesthesia and that tear removal by either absorption or evaporation maintains the constant volume. Neither of the techniques used in these studies would reveal such effects.

It has been shown (9) that not all general anesthetics have the same degree of inhibitory effect on tear production, but these differences appear to be related to depth of anesthesia rather than to differences in mechanism of action. It may well be that toxic doses of some general anesthetics would be required to obtain a depth of anesthesia which would result in a complete inhibition of lacrimation; nevertheless, the fact remains that general anesthesia does influence tear

production. The rate of tear production is, at least in part, responsible for removing an instilled drug from the precorneal area of the eye and, as such, has a controlling effect on the bioavailability of drugs in the eye.

Previous studies (4) have found the turnover rate, and hence the rate of tear production, in fully awake unanesthetized rabbits to be about 0.6600 µl./min. By varying the depth of local anesthesia through application of successively higher doses of the local anesthetic, it is shown that the rate of tear production can be decreased by at least a factor of 5. This again shows that the degree to which tear production can be suppressed by local anesthetics appears to be controlled by the depth of anesthesia, both for a given drug and between different drugs. It appears likely that the rate of tear production could be suppressed by tetracaine HCl to an even greater extent than was noted in these studies since the maximal effective concentration of tetracaine in humans has been reported (15) to be twice that which was used in these studies. In addition, the probable reason for the lack of success in abolishing the corneal reflex with lidocaine HCl is that the concentration used here was 4 times below the reported maximal effective concentration (15).

Instilled Volume Drainage Rate

The change in volume as a function of time in rabbits under general anesthesia is shown to be independent of the volume instilled. This is in sharp contrast to the volume-

dependent drainage reported in unanesthetized rabbits (4). The gravitational mechanism of drainage is supported in rabbits under general anesthesia while it is not for unanesthetized rabbits. For a 25-µl. instilled volume there exists a 3-fold difference in drainage rates between unanesthetized animals and those under general anesthesia. This drainage is a function of animal handling and position, and in all cases in these studies was determined with rabbits in a normal upright position. While it is not possible to elaborate on the exact mechanism of fluid drainage on the basis of the results of this study, it has already been demonstrated (10) that general anesthesia as well as position and handling of the animals can have a significant effect on the aqueous humor levels of topically applied drugs.

An even larger decrease in drainage rate can be obtained through the use of local anesthetics than was obtained in the case of general anesthesia. Again, any discussion of the mechanism by which this decrease occurs would be speculation. However, on the basis of these studies, it does seem reasonable that a possible enhancement of drug bioavailability as a result of decreased drainage is a distinct possibility.

Application of Results to Improved Drug Bioavailability and to Clinical Practice

Combining the results of this study with previously reported results on optimization of volume for drug instillation (4) could result in a significant improvement in drug bioavailability to the eye. These methods would allow the

maximum amount of drug to be in contact with the eye for the maximum period of time, with a minimum of drug loss.

An important benefit of improved drug bioavailability in the eye is the corresponding decrease in required dose, and a possible decrease in side effects from ophthalmic drugs. Improved utilization of an instilled volume of drug and hence a decreased loss to the drainage apparatus leaves less drug to be absorbed systemically and to exert toxic side effects.

Although the instillation of solutions preceeded by topical anesthetics seems to be a desirable procedure, this would not necessarily be true in the case of ophthalmic ointments or suspensions. It seems reasonable that the decrease of tears caused by topical anesthesia could actually decrease the availability of an applied dose of ophthalmic ointment or suspension by preventing the partitioning and/or dissolution of drugs from such dosage forms. It becomes apparent, then, that any procedure which involves concomitant administration of topical anesthetics and ophthalmic ointments or suspensions should be avoided.

It has been reported (15) that it has often been difficult to evaluate the efficacy of topical anesthetics in man because no really satisfactory method of study has been available. Since the methods described here indicate that the potency of a topical anesthetic roughly parallels its ability to inhibit tear production, a new method may be available to assess the potency of topical anesthetics for use in the eye.

In addition, it becomes apparent that a reevaluation of data obtained in ophthalmic investigations involving the use of anesthesia is in order. The extrapolation of data obtained in anesthetized animals to unanesthetized humans is invalid unless the fluid dynamics of the eye are understood and appropriate corrections are made. A recent study (10) has shown that the aqueous humor drug concentration-time profile for rabbits under general anesthesia is considerably different than for unanesthetized rabbits. Rabbits under general anesthesia show a shift in the time required to achieve a maximum aqueous humor concentration of Fluorometholone. This shift is ascribed to a change in the rate limiting step from absorption to dissolution control.

Several common ophthalmic procedures which are preceeded by topical anesthesia have been done routinely without a proper knowledge of the effects of local anesthetics on tear formation. The Schirmer test is a typical example of a potentially large source of error which has been introduced through the use of topical anesthetics. In order to obtain an accurate measure of tear production as assessed by Schirmer's method, the clinician must be aware that his choice of anesthetic, its concentration, and the dosage applied will all influence his results.

A problem which arises with the findings reported here and their application to clinical practice is that it has been reported that topical anesthetics used chronically may inhibit regeneration of the corneal epithelium (16, 17).

In severe cases, such use can even lead to permanent reduction in visual acuity (18). Moreover, it seems that clinically, the most effective topical anesthetics are those that also are the most toxic systemically (15). However, the choice of anesthetics as the specific agents to alter lacrimation and instilled fluid drainage is not as important as the concept underlying it, that is, the alteration of tear dynamics as a tool to increase ophthalmic drug bioavailability.

Further Studies

In order to give greater meaning to the results reported here, it would be beneficial to see if decreased drainage and tear production resulting from topical anesthesia really leads to improved aqueous humor levels of the drug in question. These studies are currently being conducted by the author and will be reported on in the future.

Certainly another area which ought to be explored is the implications of such studies in humans. Do anesthetics affect the lacrimal and drainage functions in humans to the same degree as in rabbits? Can such studies actually be applied to improve ophthalmic drug therapy in humans? To what degree can animal data be extrapolated to humans?

In addition, the further study of drainage mechanisms as well as movement of tears in the eye would be helpful. Since lacrimation and tear drainage have a potentially enormous influence on drug bioavailability in the eye, to

know in detail the function and mechanism of action of the lacrimal system would be a significant step in placing ophthalmic drug therapy on a rational basis.

Finally, since local anesthetics may not be the agents of choice for use on a chronic basis, a possible screening procedure could be instituted to determine the influence of other classes of compounds on tear production and instilled fluid drainage.

APPENDIX

Normal Lacrimal Fluid Turnover Rate Determination

Disappearance of an applied dose of drug or tracer substance after instillation into the eye can be described by the following equation:

$$-\frac{dC}{dt} = k(C) = \frac{F}{V_1}(C) \qquad \text{(Eq. A1)}$$

where k=first order rate constant for the decline in tracer concentration, F=turnover rate of lacrimal fluid, and V_1 is the lacrimal volume. This equation assumes that loss of applied drug is only through normal lacrimal fluid turnover. Integration of Eq. A1 and rearrangement yield:

$$\log C_o - \log C = - \frac{F}{2.303V_1} t \qquad \text{(Eq. A2)}$$

Therefore, a plot of the log change in tracer concentration versus time should be linear. The slope of the line yields the first order rate constant, k, for the decline in tracer concentration. When this rate constant is multiplied by the lacrimal volume, V_1, the result is the turnover rate, F. In the sampling technique, it should be remembered that if the sampling withdrawal rate constant is large with respect to the turnover rate constant, it must be subtracted from the turnover rate constant. For the non-sampling technique, activity (amount) can be substituted for concentration in Eq. A2.

Volume Remaining from an Instilled Dose

After a drop of solution is instilled, the volume at various times up to 6 minutes can be calculated. The assumption in this derivation is that one must neglect the volume of inflow of lacrimal fluid, a reasonable assumption, for the first 6 minutes post instillation of solution, since this volume is small compared to the volume of tracer solution instilled.

Upon instillation of a volume of tracer solution into the eye, the concentration will change upon dilution according to the equation:

$$C_o V_i = C(V_i + V_l) \qquad \text{(Eq. A3a)}$$

or

$$\frac{C_o}{C} = \frac{V_i + V_l}{V_i} \qquad \text{(Eq. A3b)}$$

where V_i=the volume instilled, C_o= the concentration (counts/ µl.) of technetium instilled into the eye, C=the concentration at some time, and V_l=the normal lacrimal fluid volume. The amount of tracer substance remaining at any time is given by:

$$A = C \times V_r \qquad \text{(Eq. A4)}$$

where A=amount of drug, and V_r=volume remaining. Substituting for C in Eq. A4 from Eq. A3 yields:

$$A = \frac{V_i C_o}{V_i + V_l} \times V_r \qquad \text{(Eq. A5)}$$

and since $V_i C_o$ is equal to A_o, one can substitute for $V_i C_o$ in Eq. A5 and after rearrangement obtain:

$$V_r = \frac{A}{A_o}(V_i + V_l) \qquad \text{(Eq. A6)}$$

and at time zero:

$$V_r = V_i + V_l = V_o \qquad \text{(Eq. A7)}$$

In this study, original and subsequent radioactivity was used to represent original amount of tracer, A_o, and amount of tracer remaining, A, respectively.

Drainage Rate Constant Determination via the Non-Sampling Technique

After instillation of a known quantity of isotope solution into the eye, there will be a decline in its activity corresponding to a decrease in the volume of solution in the eye. It is possible to obtain the rate constant describing the relationship between drainage rate and volume of solution present, as shown in the following derivation.

The amount of tracer present in the instilled solution can be calculated by:

$$A_o = V_i C_o \qquad \text{(Eq. A8)}$$

where C_o is the concentration of tracer substance in the instilled solution. The differential equation for the change in amount of tracer with respect to time is given by:

$$\frac{dA}{dt} = \frac{C dV_i}{dt} + \frac{V_i dC}{dt} \qquad \text{(Eq. A9)}$$

This equation is derived on the basis that the change in the amount of drug is due both to a continuous inflow of lacrimal fluid, which changes the concentration of tracer, and to an outflow or drainage which changes the amount of tracer present.

To simplify the treatment, it was assumed that the inflow rate of lacrimal fluid is sufficiently small to be neglected for the first 5 minutes post instillation. If there is no inflow of lacrimal fluid, then dc/dt is zero and Eq. A9 reduces to:

$$\frac{dA}{dt} = \frac{C dV_i}{dt} \qquad \text{(Eq. A10)}$$

Substituting for C produces:

$$\frac{dA}{dt} = \frac{dV_i}{dt}\frac{A}{V} \qquad \text{(Eq. A11)}$$

or:

$$\frac{dA}{A} = \frac{dV_i}{V} \qquad \text{(Eq. A12)}$$

which states that the fractional amount of tracer remaining is equal to the fraction of instilled volume remaining.

Thus it is now possible to examine the relationship between drainage rate and volume present. A first-order relationship between volume present and drainage rate was assumed:

$$\frac{dV_i}{dt} = -k_v V_i \qquad \text{(Eq. A13)}$$

to produce:

$$V_r - V_i = V_i \, e^{-k_v t} \qquad \text{(Eq. A14)}$$

which relates the change in volume to time.

REFERENCES

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

2. H. E. Kaufman, M.H. Votila, A.R. Gasset, T.D. Wood, and E.D. Ellison, Trans. Amer. Acad. Ophthalmol.Otol., 75, 361 (1971).

3. S.R. Waltman and T.C. Patrowicz, Invest. Ophthalmol., 9, 966 (1970).

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

5. L.T. Jones, Am. J. Ophthal., 62, 47 (1966).

6. "The American Encyclopedia of Ophthalmology", vol. XV, C.A. Wood, Ed., Cleveland Press, Chicago, Ill.

7. "System of Ophthalmology", vol. 4, S. Duke-Elder, Ed., C.V. Mosby, St. Louis, Mo., 1968.

8. D. Maurice, "The Precorneal Tear Film", International Ophthalmic Clinics, in press.

9. D.J. Cullen, E.I. Eger, W.C. Stevens, N.J. Smith, T.H. Cromwell, B.F. Cullen, G.A. Gregory, S.H. Bahlman, W.M. Dolan, R.K. Stoelting, and H.E. Fourcade, Anesthesiology, 36, 21 (1972).

10. J.W. Sieg and J.R. Robinson, "Corneal Absorption of Fluorometholone in Rabbits", submitted for publication in Arch. Ophthal.

11. J. Boberg-Ans, Brit. J. Ophthal., 39, 705 (1955).

12. M.S. Norn, Acta Ophthal., 43, 557 (1965).

13. S.S. Schrai, M. Makoid, S.P. Eriksen, and J.R. Robinson, "Drop Size and Initial Dosing Frequency Problems of Topically Applied Ophthalmic Drugs", submitted for publication in J. Pharm. Sci.

14. B. Rosengren and I. Kugelberg, Klin. Monatsbl. Augenheilk., 95, 764 (1935).

15. J. Adriani and R. Zepernick, J.A. M.A., 188, 93 (1964).

16. T. Gundersen and S.D. Leibman, Arch. Ophthal., 31, 29 (1944).

17. W.G. Marr, R. Wood, L. Senterfit, and S. Sigelman, Amer. J. Ophthal., 43, 606 (1957).

18. D.L. Epstein and D. Paton, New Engl. J. Med., 279,396 (1968).