Lacrimal and Instilled Fluid Dynamics in Rabbit Eyes

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Abstract ☐ Drainage of an instilled drug solution away from the eye is responsible for a considerable loss of drug and, hence, affects the biological activity of drugs in the eye. The rate of this drainage is related to the volume of drug solution instilled and increases with increasing volume. A demonstration of the influence of this solution drainage on drug activity is presented using miosis studies in albino rabbits. It is shown that over a range of 5–50 μl. of instilled volumes, using various concentrations of pilocarpine nitrate so that the total amount of drug instilled at each volume is the same, there is a fourfold change in the area under the pupillary diameter–time curves. A simple isotopic technique, using radioactive technetium-adsorbed colloid, was developed and utilized in this study to determine lacrimal volume and turnover rate of lacrimal fluid as well as to evaluate drainage of instilled solutions in albino rabbits. By using both a sampling and a nonsampling technique with the technetium colloid, it is shown that unanesthetized rabbits have a lacrimal volume of 7.5 ± 2.5 μl. whereas anesthetized rabbits have a slightly larger volume of 12.0 ± 2.5 μl. In addition, lacrimal fluid turnover rate in unanesthetized rabbits is approximately 0.53 μl./min., with a range of 0.47–0.66 μl./min., while in anesthetized animals it is negligibly small or absent. Drainage loss of instilled solutions was also determined and analyzed. It is shown that the loss of instilled solution via drainage is first order with respect to time. The apparent first-order rate constant in unanesthetized rabbits contains a volume-dependent and a volume-independent term, whereas in anesthetized rabbits the apparent rate constant is independent of volume instilled. A discussion of the meaning of these results as related to the mechanism of fluid drainage from the eye is presented. A comparison of the present studies in rabbits to expected behavior in humans is made. It is suggested that drainage rates of instilled solutions in humans should be larger than those of rabbits; therefore, to maximize activity of drugs in humans, the drop size of ophthalmic delivery systems ought to be reduced from its present 50–75-μl. size to at most a 5- or 10-μl. drop.

Keyphrases ☐ Ophthalmic solutions—determination of lacrimal and instilled fluid dynamics, relationship to drug activity, rabbits ☐ Miosis—used to study lacrimal and instilled fluid dynamics in the rabbit eye, relationship to drug activity, rabbits ☐ Fluid dynamics, ophthalmic—lacrimal fluid turnover and instilled volume drainage, rabbits ☐ Technetium, 99mTc, colloid—used to study lacrimal volume, lacrimal fluid turnover rate, and drainage of instilled solutions, rabbits ☐ Lacrimal volume and fluid turnover—determined for albino rabbits

A severe problem accompanying the instillation of a drug into the eye is the loss that occurs via drainage into and through the drainage apparatus. This loss increases in severity as the instilled volume increases and can have a large influence on the biological activity of a drug, since it removes drug from the area of the eye, making it less available to exert a local effect or to be absorbed into the eye. The present report demonstrates that the volume of instilled dose in the eye influences the activity of drugs through drainage loss. In addition, an attempt was made to describe quantitatively the relationship between volume present and rate of drainage in albino rabbits.

Since lacrimal fluid is turned over at an appreciable rate, reported to be some 16%/min. in humans (1), high concentrations of the drug in lacrimal fluid are required for the first few minutes after instillation to maximize biological activity. Viscosity alterations of solutions, suspensions, and emulsions, as well as the use of solid delivery systems such as soft contact lenses (2, 3), are popular methods attempting to reduce or avoid drainage rate problems and, hence, to increase corneal contact time. Clearly, the volume of instilled dose must have a sizable influence on the quantity and rate of drug solution loss to the drainage apparatus.

Mishima et al. (1) reported that the normal lacrimal volume in humans is approximately 7 μl. and that the human eye can hold about 30 μl. without overflow or spillage at the lacrimal lake or outer angle, provided that great care is exercised and the subject does not blink. If blinking is allowed, then the eye can hold approximately 10 μl. (4). The normal dropper used in commercial ophthalmic preparations delivers approximately 50–75 μl. Apparently, when this volume of solution is instilled into the cul-de-sac, the reflex action of blinking causes a substantial portion of the drop to be sucked into the drainage apparatus while another portion may be spilled onto the cheek. Removal of the instilled solution continues until the total tear volume is back to the normal lacrimal volume of 7 μl.

Thus, as the volume of instilled dose is increased, the volume of solution lost by spillage on the cheek and drainage increases. This suggests that the optimum volume of drug solution to instill into the eye is no volume, since increasing the instilled volume increases the volume lost and the corresponding percentage of drug that is lost. However, an additional factor that complicates the picture is the effect of turnover rate of lacrimal fluid on the concentration and amount of drug remaining in the eye. If one assumes a constant turnover rate of lacrimal fluid of 16%/min., it seems clear that as the precorneal volume of fluid in the eye (lacrimal plus instilled) gets smaller, the turnover rate of lacrimal fluid will have a greater influence on the residual drug concentration. Thus, the rate of turnover of lacrimal fluid favors a larger instilled volume to maximize the bioavailability of ophthalmic drugs.

Selection of an "ideal" volume for instillation of drugs into the eye requires information on the magnitudes of the rate constants for lacrimal fluid turnover and for drainage of instilled solutions. Lacrimal fluid turnover studies have been conducted in humans (4–10), but instilled volume drainage studies have apparently not been reported. In addition, although rabbits are the main experimental animals in ophthalmic research, neither lacrimal fluid turnover nor instilled volume drainage has been studied in this animal. Thus, the problem of lacrimal and instilled fluid dynamics as it relates to drug activity seems to have been largely ignored in the ophthalmic field. The paucity of reported work in this area is somewhat surprising, since these fluid dynamics have great bearing on studies of release rates and dissolution of dosage forms and drugs as well

as the transport and general movement of drugs into the eye.

EXPERIMENTAL

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

Pilocarpine nitrate USP was obtained from a commercial source[1] and used without further purification. Technetium solutions were prepared using a package[2] of solutions and equipment used to prepare technetium, ^{99m}Tc, suspensions. All other chemicals were either reagent or analytical grade.

Adult, male, albino rabbits[3] 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.

Solution Preparation—Pilocarpine nitrate solutions were prepared fresh and were discarded immediately after the experiment. The solutions were prepared by addition of pilocarpine nitrate to Sorensen's pH 7.38, 0.067 M, phosphate buffer. Solution pH was checked and adjusted where necessary. All pH measurements and adjustments were made on a digital pH meter[4], using a wide range glass electrode[5]. The pH meter and electrode system were standardized against phosphate buffer as described by Bates (11). No attempt was made to sterilize the solutions. The highest drug concentration used yielded a slightly hypertonic solution; the tonicities of all drug solutions were adjusted to this value.

Technetium colloidal solutions were prepared by addition of technetium, ^{99m}Tc, to solutions provided in a colloidal sulfur technetium kit[2], from which the sulfur colloid is prepared in situ[6]. Technetium, ^{99m}Tc, was obtained by elution 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 approximately 1 mc. of activity/ml. of solution.

Anesthetic solutions were composed of a mixture of sodium pentobarbital and sodium phenobarbital. Separate solutions of sodium pentobarbital and sodium phenobarbital 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 2 weeks.

Anesthesia—A combination of sodium pentobarbital and sodium phenobarbital was used to induce and maintain the desired level of anesthesia. A dose of 33 mg. of sodium pentobarbital and 100 mg. of sodium phenobarbital/kg. of animal body weight was sufficient in most cases to anesthetize the rabbits for 8–12 hr. With this dose level of drugs, the onset of anesthesia usually occurred in 15–30 min. Drugs were administered by intraperitoneal injection.

Miosis–Time Studies—Anesthetized animals were used in these experiments to minimize possible lacrimation from any stimulus, since this would influence the results. After onset of anesthesia, the test animals were placed in a restraining box with the head elevated in such a manner that the lid line, upon closure, was approximately horizontal. This position, which is not the natural posture for the rabbit, was used to facilitate pupillary diameter measurements. The unnatural posture should have the effect of decreasing the rate of lacrimal drainage, thus modifying the magnitude of the effect for which the experiment was designed. Both upper and lower eyelids were then taped open to enable accurate measurements of pupillary diameter. The lower tape was loosened at time of instillation to allow for manual mixing of drug with lacrimal fluid, after which it was replaced. Keeping the eyelids open hastens evapo-

ration of water from the eye and again modifies the effect expected from this experiment. Since the interest was in a reproducible system giving at least a qualitative indication that volume of instilled dose affects drug activity, the experiment was conducted under these conditions. No desiccation of the cornea was observed.

Lighting in the test room was accomplished by use of one 200-w., half-silvered overhead light and remained constant throughout the study. Temperature in the room was maintained at $25 \pm 1.0°$. The entire room was as isolated as conditions would permit to keep audiovisual stimuli to the test animal at a minimum.

The observer was hidden from the test animal by a cardboard shield; this shield contained a vertical slit through which measurements of pupillary diameter could be made. The observer, once inside the room, was not allowed movement in or out of the room until the experiment was complete. All of these precautions were necessary to obtain reproducible results.

Measurement of pupillary diameter was made by use of a cathetometer[7], positioned approximately 50 cm. from the test animal. These measurements could be made with an accuracy of ± 0.1 mm.

After positioning the animal in the restraining box, in line with the cathetometer and prior to drug instillation, pupillary diameter readings were made at 100-sec. intervals. This procedure was continued until the test animal had adapted to its environment, as judged by the constancy of pupillary diameter (± 0.1 mm. for 10 min.). The adaptation period varied from 15 to 30 min., and the initial diameter ranged from 6.0 to 7.8 mm.

Pipets[8] with blowout delivery were used to instill the drug solution and were checked for accuracy of delivery prior to use by weighing the delivered solutions.

Drug solutions were instilled onto the cornea, so that they collected in the lower cul-de-sac. To prevent loss of solution during instillation, the lower eyelid was pulled slightly away from the globe of the eye to form a pocket. This particular form of solution instillation was followed at all times.

After instillation of drug solution, the lower lid was lifted back and forth over the cornea to mix the drug solution with lacrimal fluid. At no time was the lid ever massaged against the cornea, so only movement of the drug solution 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 5 sec., in all cases. To aid in reproducibility, all procedures were performed in precisely the same manner and with the same time intervals.

After instillation of the drug solution, the lower eyelid was taped open and pupillary diameter was measured as a function of time. The other eye of the test animal served as a control. One hundred-second intervals were used for the first 50 min. of the experiment, 300-sec. intervals were used from 50 to 80 min., and 600-sec. intervals were used thereafter. Measurements were terminated when the measured diameter reached a constant value close to the initial value.

No experimental animal was used more than once, and each experiment was repeated at least four times.

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 eyelids were not taped open and, after instillation of the technetium suspension, the lid was returned to its former position. Thus, normal movement of the eye was used to mix the colloidal suspension of technetium with lacrimal fluid. After instillation of the tracer, the animal would close its eyelids but would, in all cases, open the lids within 10–15 sec. after instillation. Closing of the lids was not due to irritation from the tracer substance, since instillation of normal saline produced the same response.

Anesthetized animals were placed in restraining boxes, and experiments were conducted with the animals in their normal posture. The eyelids were not taped open. Technetium suspension was instilled in the same manner as in the pilocarpine studies.

Procedures—Radioactive technetium colloidal suspensions were instilled into the rabbit eye from a syringe[9] which had been prechecked for accuracy of delivery by weighing the delivered solutions. Volumes as small as 0.1 μl. could be accurately delivered from the

[1] Mallinckrodt Chemical Works, St. Louis, Mo.
[2] Collokit, Abbott Radio-Pharmaceuticals, North Chicago, Ill.
[3] Klubertanz, Edgerton, Wis.
[4] Orion model 801.
[5] Beckman type E3.
[6] For details, see the brochure "Collokit for the Preparation of Technetium Sulfide, ^{99m}Tc, Injection," Abbott Laboratories, North Chicago, Ill., 1971, pp. 2–4.

[7] Central Scientific Co., Chicago, Ill.
[8] Scientific Products, Evanston, Ill.
[9] The Hamilton Co., Whittier, Calif.

various size syringes, although it was necessary to shake the syringe gently to discharge the drop in the case of the 0.5- and 0.1-μl. volumes.

After instillation of a suitable volume of technetium colloid, 15 sec. was 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[10] in contact with the marginal tear strip. The capillary tube would immediately fill with tears. All samples were withdrawn from the lower marginal tear strip, approximately in the middle of the strip. The samples were counted[11] for 1 min.

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 also were made. In general, the number of counts per microliter of technetium colloid was in the range of 100,000/min. Suitable corrections for the normal decay of technetium isotope were employed wherever necessary.

Lacrimal Volume Determination—Various volumes, ranging from 0.1 to 50 μl. of technetium suspension, were instilled into the eye, 15 sec. was allowed to elapse, and then a sample was withdrawn and its radioactivity was determined. Both eyes of the animal were used, and no animal was used more than once. Knowing the initial radioactivity of the technetium colloid instilled into the eye, as well as the radioactivity after dilution, allows calculation of the lacrimal volume present as described in the *Appendix*.

Normal Lacrimal Volume Turnover Rate—Various volumes of technetium suspension, ranging from 1.0 to 50 μl., were instilled into the eye; samples were withdrawn and assayed for activity at 5-, 10-, or 20-min. intervals. No initial sample was taken after 15 sec. in this study. Sampling was continued until the isotopic activity had decreased to approximately background level, which, depending upon the volume of isotope instilled, varied from 30 min. to 2 hr. 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 was measured at 5-, 10-, 15-, or 20-sec. 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. Thus, as drug is drained out of the eye, concentration does not change appreciably whereas the total *amount* of drug is altered.

Technetium Studies: Nonsampling Method—*Animal Preparation*—All procedures were the same as in the sampling method.

Procedures—The various volumes of colloidal technetium solution were instilled into the eyes of both anesthetized and unanesthetized animals, and the decline in radioactivity was monitored with a thin-probe scintillation detector[12] attached to a well counter[11]. The thin-probe detector is a cylindrical vessel, approximately 22 cm. long and 5 cm. in diameter, containing a sodium iodide crystal and 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, and 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, as well as the size of the orifice in the lead shield at the bottom of the probe, was a compromise 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. 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 over their eyes once a day for 1-hr. duration on 7 successive days prior to the experiment. After this conditioning, the animals showed no head or eye movement for the duration of the experiment; if they did, it was clear from the results and the experiment was terminated at that point.

Various volumes of technetium colloidal suspension were instilled into the rabbit eye, as described in the *Sampling Method* section, and the probe was placed in front of the eye. Measurements of radioactivity in the eye were made every 5 sec., and counts were determined for periods of 5 sec. 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 about 60–90 min., although shorter and longer times occurred depending upon the type of experiment. Only one of the rabbit's eyes was used, and no rabbit was used more than once.

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

Evaluation of Technetium Dilution Technique for Lacrimal and Instilled Fluid Dynamic Studies—To use a tracer substance such as isotopic technetium as a test substance for tear and instilled fluid dynamic studies, it is necessary to satisfy several criteria. These criteria are: (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 occurs, its extent must be known; (c) the test substance must be nonirritating; 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 tissue and/or absorption, the amount of drug consumed in these possible routes was determined. Because of the differences between the two techniques (sampling and nonsampling) 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 eyelid. Consequently, ^{99m}Tc bound to eye tissue or absorbed into the eye, with the exception of the portion that passed into the bloodstream 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 solution was instilled into the eyes of several anethetized animals. At various time intervals over 4-hr., 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 colloid 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

Miosis–Time Study to Demonstrate Effect of Dose Volume on Drug Activity—To determine the influence of dose volume on drug activity, several solutions of varying concentration of pilocarpine nitrate were prepared and instilled into the eyes of anesthetized rabbits. The volumes of solution instilled were adjusted so that the amount of drug delivered to all rabbit eyes was the same; *i.e.*, the amount of drug delivered from the 75-μl. dose was the same as that

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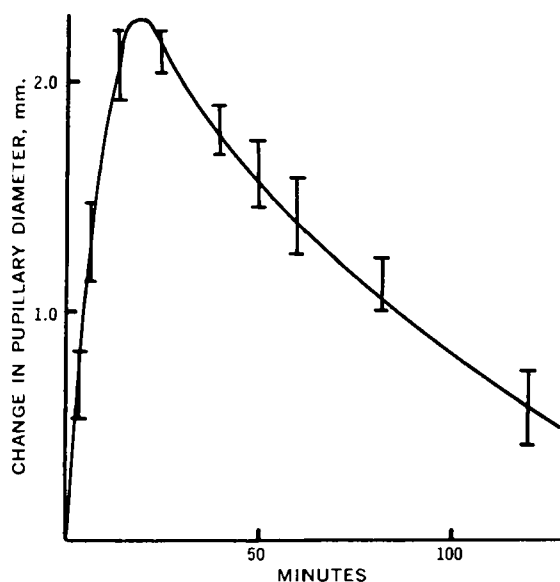**Figure 1**—*Change in pupillary diameter as a function of time after instillation of 50 μl. of a 1 × 10⁻² M pilocarpine nitrate solution. The data represent the sum of five separate runs, and the vertical lines indicate standard deviations.*

from the 5-μl. dose. If there is *no* loss of drug solution from the eye, it would be expected that the areas under the pupillary diameter–time curves would be the same, although the shape of curves for various concentrations of drug would be expected to be different.

Previous extensive work on the miotic effect of pilocarpine nitrate (12) had determined the dose–response curve.

A typical pupillary diameter–time relationship is shown in Fig. 1, along with standard deviations which are presented as vertical bars. The results shown in Fig. 1 were obtained from five animals in five different experiments and are typical of all the experiments.

Table I shows the area under the pupillary diameter–time curves for various instilled volumes. These areas are proportional to the concentration of drug reaching the target and are presumably proportional to the *amount* of drug present in the tear fluid. Since all of these solutions had identical amounts of drug, it would be expected that all would show the same area under the curve if no drug solution was lost from the application site. The ratio of productive to nonproductive drug loss by adsorption/absorption will be the same whether low volume (high concentration) or high volume (low concentration) is used. Thus, fluid loss by drainage would determine differences in area under the curve. Clearly, the increased area under the curve that is observed as the instilled volume decreases demonstrates that a substantial portion of the instilled dose is unavailable for absorption to the target area, due apparently to the drainage loss.

The range of volumes employed was restricted at the lower end by drug irritancy and at the upper end by the maximum volume the eye can hold without overflowing onto the lid and cheek. In the case of the largest dose volume, 75 μl., great care was needed in mixing the drug solution with lacrimal fluid to prevent overflow onto the cheeks.

Determination of Lacrimal Volume and Normal Lacrimal Fluid Turnover Rate by Sampling Technique—*Lacrimal Volume Determination*—Instillation of a known volume and activity of a technetium colloidal suspension, followed by sampling of the tear fluid for activity, allows determination of the volume of fluid originally present in the eye as described in the *Appendix*. This procedure assumes that good mixing takes place and that no tear production occurs from time of instillation until sampling time, which was 15 sec. in the present study.

Both anesthetized and unanesthetized animals were used, and each involved a different method of mixing the technetium suspension with lacrimal fluid as described in the *Experimental* section.

The results of the study are shown in Table II together with the range of values, mean value, and standard deviation for various instilled volumes. A broad range of volumes was used to determine if adequate mixing or loss of unmixed solution was occurring. As can be seen from Table II, the range of median values for both un-

Table I—Effect of Instilled Volume on Activity of Pilocarpine Nitrate in the Rabbit Eye

In-stilled Volume, μl.	Concentration[a], M	Number of Animals	Peak Height, mm.[b]	Area under Curve, cm.[2][c]	Activity[d], %
75	6.7 × 10⁻³	5	2.05 ± 0.10	63.7	27.7
50	1 × 10⁻²	5	2.40 ± 0.17	72.6	31.5
25	2 × 10⁻²	5	2.53 ± 0.21	83.9	36.5
10	5 × 10⁻²	4	3.53 ± 0.15	131.3	57.0
5	1 × 10⁻¹	5	4.17 ± 0.40	230.2	100.0

[a] The amount instilled is 0.136 mg. [b] Units refer to average peak height plus or minus standard deviation. [c] Units are millimeters-minute. On the vertical axis, 1 cm. = 0.25 mm.; on the horizontal axis, 1 cm. = 10 min. [d] For comparison purposes only, it was assumed that the 5-μl. instilled volume case gives 100% activity.

anesthetized and anesthetized animals is not excessively large, considering that a 500-fold range of instilled solution was used. The standard deviation increases in the unanesthetized animals as the instilled volume increases, presumably due to incomplete mixing or loss of unmixed solution. In addition, all standard deviations are larger than desirable, pointing to the disadvantage of the sampling procedure as described by Mishima *et al.* (1).

In unanesthetized animals, the calculated lacrimal volumes show a range of values depending on the instilled volume. For the lower instilled volumes of 0.1, 0.5, and 1.0 μl., the mean lacrimal fluid volume value of approximately 7.5 ± 2.5 μl. is essentially constant, and this value is interpreted to be the true lacrimal volume in the unanesthetized rabbit. With larger instilled volumes of 5.0, 25, and 50 μl., the calculated values for lacrimal fluid volume are considerably higher than for the smaller instilled volumes. These higher values are interpreted as being due to a loss of unmixed technetium solution or perhaps to better mixing with lacrimal fluid under the lids. The larger standard deviations with larger instilled volumes, as well as the broader range of values, suggest that loss of unmixed solution is the more likely explanation.

Anesthetized animals show a relatively constant lacrimal volume for instilled volumes ranging from 1 to 50 μl. This relatively constant value of approximately 11–12 μl. is considerably larger than the values for the lower instilled volumes in the unanesthetized animals. These higher values are probably due to relaxation of the eye musculature under anesthesia to accommodate a larger volume. Alternative explanations of loss of unmixed solutions or of irritation on mixing were considered and discarded. It is conceivable, however, that manual mixing is more efficient than natural mixing and that the true lacrimal fluid volume in both anesthetized and unanesthetized rabbits is approximately 12 μl.

Normal Lacrimal Turnover Rate Determination—Different volumes of technetium solution were instilled into the eyes of both anesthetized and unanesthetized rabbits, and samples were with-

Table II—Lacrimal Volume in Rabbits as Determined by Diluting Technetium with Tears and Sampling the Resultant Solution for Activity

| Instilled Volume, μl.	Number of Determinations	Range of Calculated Lacrimal Volume, μl.	Mean, μl.	SD, μl.
Unanesthetized without Manual Mixing				
0.1	5	4.55–10.42	7.55	2.09
0.5	5	6.89–11.10	7.61	2.96
1.0	11	4.19–15.50	7.37	3.54
5.0	5	5.16–13.84	9.32	4.15
25.0	5	9.10–16.99	13.09	3.59
50.0	4	6.28–17.84	12.84	4.80
Anesthetized with Manual Mixing				
1.0	5	8.46–14.50	11.55	2.62
5.0	4	6.59–18.94	11.96	5.62
25.0	5	10.53–14.33	12.30	1.57
50.0	4	9.86–14.45	12.21	1.98

Table III—Turnover Rate of Lacrimal Fluid in the Rabbit by the Technetium Dilution Method Using the Sampling Technique

Instilled Volume, μl.	Sampling Time, min.	Number of Determina- tions	k, min.$^{-1}$, Range	k, min.$^{-1}$, Average	SD	Turnover[a] Rate, μl./min.
			Unanesthetized without Manual Mixing			
1	10	3	0.074–0.080	0.076	0.002	0.47
5	10	3	0.074–0.084	0.080	0.005	0.50
25	5	3	0.080–0.083	0.081	0.002	0.46
25	10	3	0.075–0.087	0.082	0.006	0.52
25	20	3	0.073–0.076	0.075	0.002	0.50
50	10	3	0.075–0.079	0.077	0.002	0.48
						Average = 0.49
			Anesthetized with Manual Mixing			
1	10	3	0.063–0.071	0.0656	0.005	0.69
5	10	3	0.054–0.058	0.056	0.002	0.57
25	10	3	0.0077–0.0084	0.0081	0.0003	0.00[b]
50	10	3	0.0041–0.0074	0.0053	0.0002	0.00[b]

[a] Obtained by multiplying the observed first-order rate constant times the lacrimal volume, 7.5 μl. for unanesthetized and 12.0 μl. for anesthetized rabbits, and subtracting the sampling withdrawal rate constant. [b] Subtracting the withdrawal rate gives a negative number to the turnover rate.

drawn 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 III, together with standard deviations and resultant turnover rates based on a lacrimal fluid volume of 7.5 μl. for unanesthetized rabbits and of 12.0 μl. for anesthetized rabbits.

In the case of unanesthetized rabbits, good linearity is observed when the logarithm of decline in isotope activity is plotted against time (Fig. 2). The range of observed rate constants is fairly narrow for all volumes studied (Table III). The relatively consistent results of the turnover rate under conditions of varying instilled volume as well as different sampling schedules can be seen from the last column on the right of Table III. Various sampling times were
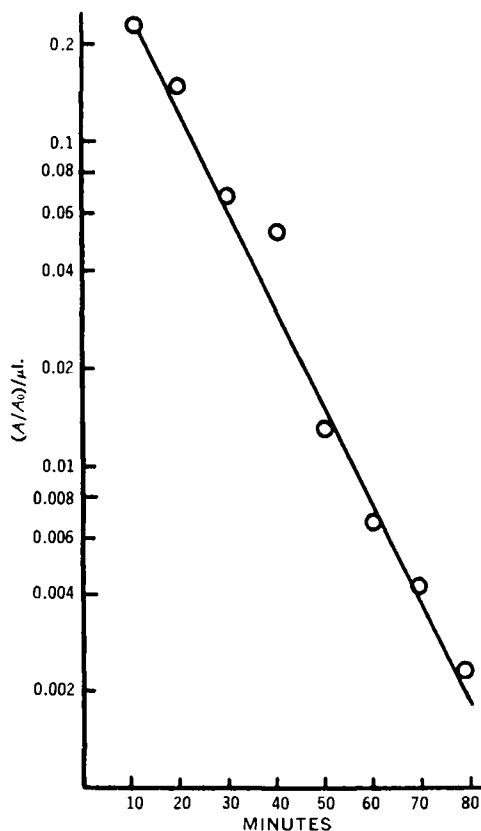
Figure 2— *Typical plot of the decline in isotopic activity of technetium versus time in unanesthetized rabbits. The specific run is for a 50-μl. instilled volume, in which the sampling technique was used to determine isotopic activity of technetium.*

studied to assess if the sampling technique was influencing the observed decline in activity of the isotope through irritation and lacrimation. Several different instilled volumes were used to ensure good mixing.

In the case of anesthetized animals, 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 anesthetized animals 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, for anesthetized animals it was concluded that the lacrimal turnover rate is either very small or nonexistent, which is not unusual considering that it is reported that humans do not lacrimate while sleeping (13-15). This observation has important bearing on animal studies, where time-concentration curves are generated in corneal absorption experiments. Anesthetized animals are generally used, and the results of these studies would be expected to be far different than results from unanesthetized animals considering the difference in turnover rate of lacrimal fluid.

Determination of Normal Lacrimal Fluid Turnover Rate and Instilled Volume Drainage Rate by Nonsampling Technique—*Unanesthetized Rabbits*—After instillation of technetium colloidal solution into the rabbit eye, monitoring of technetium radioactivity commenced no more than 2 or 3 sec. after instillation. A representative figure of the amount of tracer remaining as a function of time for various instilled volumes in unanesthetized animals is shown in Fig. 3. Also included in the figure are standard deviations, at random points, for the 5-μl. case to illustrate reproducibility of the procedure.

Initial inspection of Fig. 3 suggests that the data might fit a reaction sequence of the following type:

$$A \overset{B}{\underset{C}{\diagdown}}$$

where $A \to B$ represents the rapid drainage of the instilled solution phase and $A \to C$ represents turnover of lacrimal fluid. This can be visualized as follows: After instillation of a volume of tracer solution into the eye, the total volume (instilled plus lacrimal) drains out at a given rate, removing tracer solution, until the volume is reduced to the lacrimal volume. Further decline in tracer amount is then due to turnover of lacrimal fluid.

Although the curves in Fig. 3 are depicted as smooth, uniformly changing lines, the terminal portion (turnover of lacrimal fluid) is actually oscillatory. The amplitude of the wave is rather small, and the wave has a period of approximately 400 sec. A smooth line was drawn through the wave function to simplify analysis. The cause of this unexpected behavior could be one or a combination of factors such as an intermittent input of lacrimal fluid or pulsation of the eye musculature which forces lacrimal fluid out. It is not due to either blinking or head and eye movement. Mishima *et al.* (1) did not remark upon this behavior in humans, although the scatter in
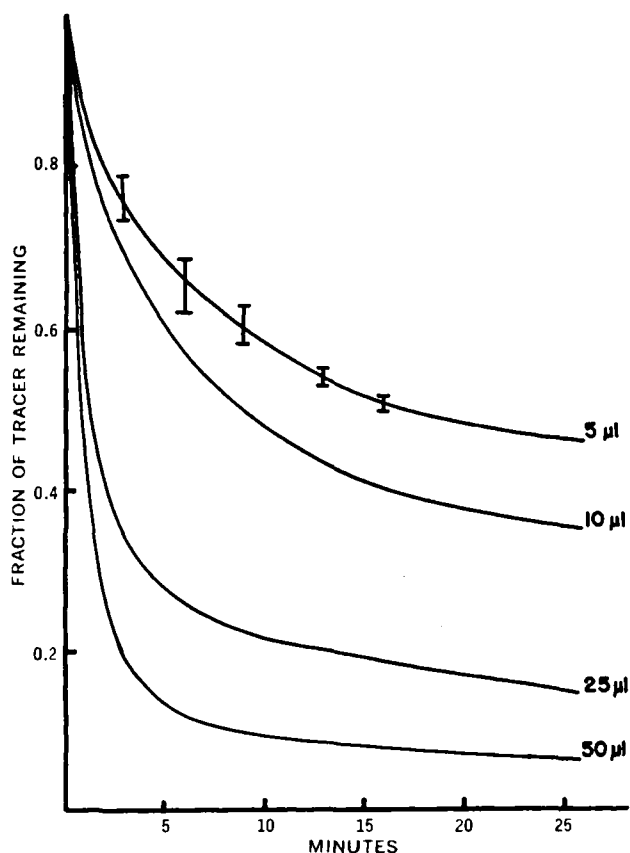
Figure 3—*Fractional amount of drug remaining as a function of time for various instilled volumes in unanesthetized rabbits. At least three separate experiments were conducted at each instilled volume, and the lines represent the mean values for these runs. Standard deviations, at selected time points, are shown only for the 5-μl. volume for the sake of clarity; the remaining runs show similar standard deviations.*

Table V—Volume of Solution Remaining at Various Times as a Function of Instilled Solution

Minutes	Volume Remaining[a], μl.			
	5[b]	10[b]	25[b]	50[b]
0[c]	12.5[d]	17.5	32.5	57.5
1	10.9	14.8	20.0	28.2
2	10.1	12.4	15.5	18.9
3	9.5	11.0	11.9	12.4
4	9.1	9.9	9.9	9.8
5	8.6	8.7	8.8	8.4
6	8.1	8.1	8.3	7.2

[a] Neglecting the inflow of lacrimal fluid which is small, approximately 0.50 μl./min., compared to instilled volume, particularly for the 25- and 50-μl. cases. [b] Instilled volume in microliters. [c] Time zero volume includes the instilled volume plus the normal lacrimal volume, which is assumed to be 7.5 μl. [d] All calculated volumes are median values with ±5% error.
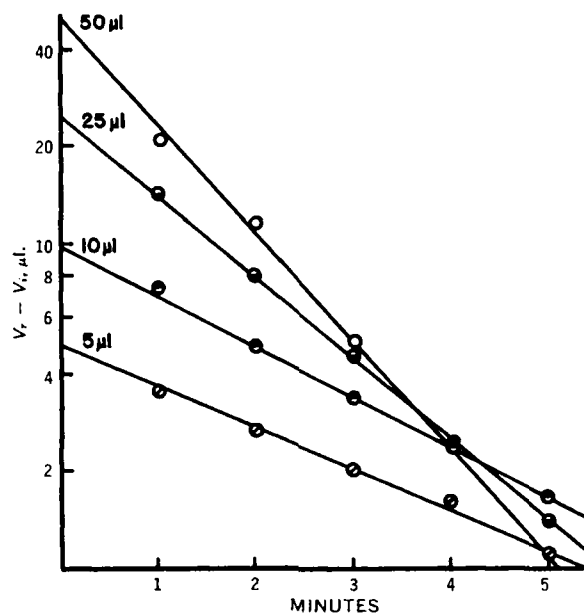
the initial instilled volume solution, the mathematical treatment is much simplified as shown in the *Appendix*. This assumption is not unreasonable, since the rate of lacrimal flow is small and the time period of concern is also small, *i.e.*, the first 5 min.

To illustrate the change in volume as a function of time during the initial drainage phase, Table V shows the volume remaining at various time intervals for the different instilled volumes as calculated from equations presented in the *Appendix*. The data in Table V are mean values from a minimum of three runs.

From Eq. A15 presented in the *Appendix*, it is possible to determine the rate constant for drainage. Good linearity is obtained when the logarithm of volume remaining is plotted *versus* time (Fig. 4). This indicates that drainage rate, for various instilled volumes, is directly proportional to the volume present, *i.e.*, apparent first order.

The different slopes of the lines in Fig. 4 suggest that drainage cannot be viewed as simple flow through a conduit or that it is merely proportional to the hydrostatic head developed by the presence of a given volume of solution. A change is occurring somewhere in the drainage mechanism as the volume of instilled solution changes.

To determine the relationship between the drainage rate constant and the instilled volume, the apparent first-order rate constants were determined from the slope of the lines in Fig. 4 (Table VI). The apparent first-order rate constants were then plotted against the instilled volume (Fig. 5). The excellent correlation shown in Fig. 5 suggests that the apparent first-order rate constant contains a volume-dependent term. In addition, the nonzero intercept sug-

their data suggests that such a phenomenon might exist. The present authors are currently exploring this phenomenon in more detail.

It is easy to provide an underlying mechanism to explain the apparent biphasic nature of the curves in Fig. 3. The terminal portion of each curve is relatively simple to describe in that the fractional decrease in tracer substance is due entirely to the normal turnover of lacrimal fluid; *i.e.*, the tear volume, composed of lacrimal fluid and instilled volume, is back to the normal lacrimal fluid volume. The turnover rate of lacrimal fluid is easily obtained from the slopes of the lines resulting from plots of the logarithm of fractional amounts of tracer remaining *versus* time (Table IV).

The agreement between these results and results obtained from the sampling technique (Table III) is excellent. This suggests that the sampling procedure can be used with confidence to determine lacrimal fluid turnover rate, as long as great care is used to minimize irritation to the eye.

The initial portion of the curves in Fig. 3 shows a rapid decline in the amount of tracer substance due to rapid drainage of the solution present. This decline is also influenced by inflow of additional solution (normal lacrimation). If no inflow is assumed, other than

Table IV—Normal Lacrimal Turnover Rate Obtained *via* Nonsampling Technique

Instilled Volume, μl.	Number of Determinations	F, μl./min.	SD
5	4	0.42	0.12
10	3	0.60	0.18
25	3	0.66	0.06
50	3	0.60	0.12
		Average F = 0.57	

Figure 4—*Change in volume of solution remaining from an instilled solution as a function of time in unanesthetized rabbits.*

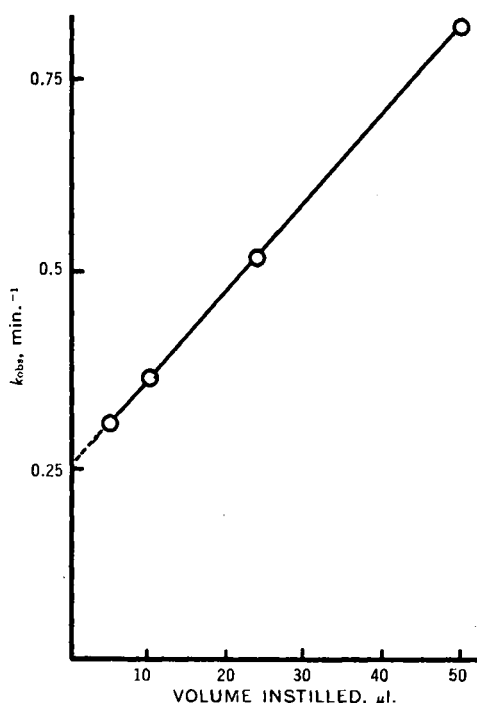**Table VI**—First-Order Rate Constants for Drainage of Instilled Solution

Volume Instilled, μl.	k_{obs}, sec.$^{-1}$
5	0.308
10	0.365
25	0.545
50	0.815

gests that it also has a volume-independent term. It was expected that if the lacrimal fluid secreted under normal conditions is drained by the same mechanism as that which removes an excess of tears or an instilled solution, the intercept in Fig. 5 would represent the rate constant for normal lacrimal fluid turnover (shown by the arrow in Fig. 5). In fact, the volume-independent term is some 200 times larger than the rate constant for lacrimal fluid turnover. This suggests that removal of normal lacrimal fluid is *via* a mechanism that undergoes some modification when larger volumes are present. Two distinct mechanisms for removal of fluid from the eye, one for small volumes and one for large volumes, are in accord with the description given by Maurice (15) for tear removal in humans.
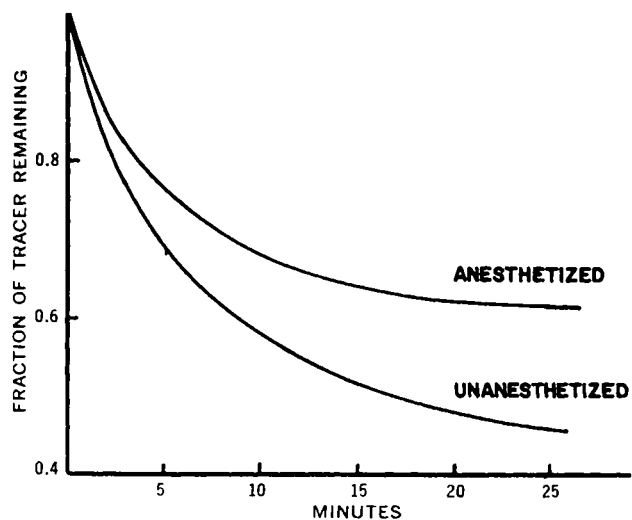
The drainage rate constant allows one to calculate the amount of tracer remaining from an instilled dose at various times, but this can be more easily seen from Figs. 2 and 4. It is observed that within 1 min. the 50-μl. instilled volume, which is slightly less than the average commercial drop, has diminished to approximately 50% of its initial volume ($V_i + V_l$). And at the end of 5 min., the eye still contains approximately 68% of the radioactivity from the 5-μl. instilled volume; whereas with 50 μl., only approximately 14% of the initial radioactivity remains. This finding corroborates the results with pilocarpine shown in Table I, namely, that instilled volume plays a substantial role in drug loss and, therefore, greatly influences drug activity.

Anesthetized Rabbits—Loss of tracer in anesthetized animals was significantly different from that in unanesthetized animals. Figure 6 illustrates the loss of tracer in both anesthetized and unanesthetized animals, using 5 μl. as the instilled volume.

Some qualitative observations on the total volume removal curve can be made. Whereas in unanesthetized animals the total volume was back to a constant volume in about 6 min., it takes considerably longer with anesthetized animals and the length of time varies

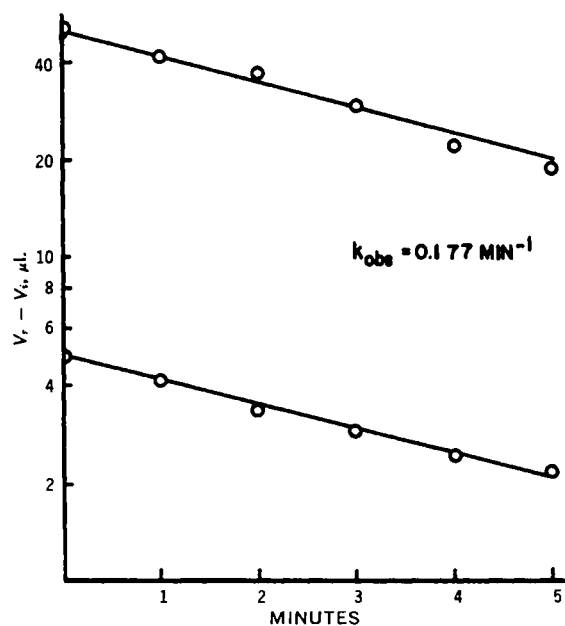**Figure 6**—*Fractional amount of drug remaining as a function of time for anesthetized and unanesthetized rabbits using 5-μl. instilled volume. The lines represent the average of four separate determinations.*

with the volume instilled. This is presumably due to a lack of eye movement and a relaxation of eye muscles.

Figure 6 shows that the percentage of isotope in anesthetized animals is less than in the unanesthetized case at all times. As the instilled volume gets larger, the percentage difference in tracer remaining between anesthetized and unanesthetized animals increases. Thus, for 50 μl. instilled volume, the unanesthetized animal shows roughly 14% of tracer remaining at 5 min. whereas the anesthetized animals shows approximately 33% remaining.

As expected, the terminal portions of the percent of tracer amount *versus* time plots for anesthetized animals reach a plateau. The sampling studies of turnover of lacrimal fluid showed that no lacrimation or drainage occurred while the animal was under anesthesia. Therefore, it was expected that the decline in drug amount or concentration would cease when drain-off was complete.

The change in volume as a function of time for various instilled solutions in anesthetized rabbits is shown in Fig. 7. Drainage of an

Figure 5—*Relationship between the observed rate constant for the decline in volume and volume instilled. The equation for the line is:*
$$k_{obs} = 0.25\,(min.^{-1}) + 0.0113\,(1\,\mu l./min.)(V_i).$$

Figure 7—*Change in volume of solution remaining from an instilled solution as a function of time in anesthetized rabbits. The top line is for an instilled volume of 50 μl. and the bottom line is for 5 μl. Both lines represent three different runs, and the data points are mean values $\pm 5\%$.*

$k_{obs} = 0.177\ MIN^{-1}$

instilled solution in anesthetized rabbits is strikingly different from unanesthetized animals, as can be seen by comparing Figs. 4 and 7 and can perhaps be related to the different mechanisms of drainage.

It has been suggested (16) that gravitational movement of solution is responsible for tear and instilled volume drainage in the rabbit. The data in Fig. 6 do not support this suggestion for instilled volume drainage in both anesthetized and unanesthetized rabbits. The fact that the rate of decline in radioactivity for identical instilled volumes is different in anesthetized and unanesthetized rabbits suggests that simple gravitational movement cannot be the mechanism in both cases. The conclusion with unanesthetized rabbits, based on the volume-dependent term in the apparent rate constant, was that gravitational movement was not the driving force for instilled volume removal. It can be seen from Fig. 7 that the slopes of the lines for the 50- and 5-μl. instilled solutions are identical and, therefore, the rate constants are identical. Since there is no instilled volume dependency in drainage of fluid from the eye of anesthetized animals, it was concluded that this supports the mechanism of drainage by gravitational movement in anesthetized rabbits.

DISCUSSION

It is quite clear from the pupillary diameter study in anesthetized animals that as instilled volume increases the resultant biological activity decreases for equivalent amounts of drug. This effect is expected to be much more dramatic in unanesthetized animals, since lacrimal fluid turnover is slow or absent in anesthetized animals and the drainage rate is considerably slower. Moreover, since human lacrimal fluid turnover rate (1) is twice as large as that found for rabbits and since humans blink considerably more than rabbits, a larger effect is expected in humans, assuming all other factors to be equal.

The various tear and instilled volume variables described in this report require some additional discussion.

Lacrimal Fluid Volume—The rabbit lacrimal fluid volume of 7.5 μl. is remarkably close to that of humans, i.e., 7.0 μl. (1). The rabbit eye globe is somewhat smaller than the human globe, but the rabbit eye possesses a third eyelid, the nictitating membrane, which occupies some of the eye area. Based on the somewhat smaller eye and the nictitating membrane, it was expected that rabbit lacrimal fluid volume would be less than humans, but this is apparently not the case.

It is, of course, possible that the 7.5-μl. lacrimal volume in unanesthetized rabbits is actually an underestimate of the true volume. The majority of lacrimal fluid is held under the upper and lower eyelids, and the methods of mixing technetium tracer with lacrimal fluid may not have reached all of this fluid. In the case of natural mixing, the majority of mixing occurred immediately postinstillation when the rabbit closed its eyelids in response to the instilled solution. The rabbit blinks infrequently and, therefore, the first blink and eye movement were responsible for mixing. In the case of manual mixing, the upper eyelid was not actively involved in the mixing process.

Turnover Rate of Lacrimal Fluid—The turnover rate of lacrimal fluid in rabbits is approximately one-half that of humans (16%/min. in humans and 7.1%/min. in rabbits). Considering the fact that rabbits blink infrequently and that, therefore, the evaporation rate of tears would be expected to be higher in rabbits than humans, it was expected that rabbits would have a larger turnover rate of lacrimal fluid. The fact that turnover rate determinations from both the sampling and nonsampling techniques are in such good agreement lends support to the validity of the turnover rate.

Absence of, or a severe reduction in, turnover rate in anesthetized animals is shown by both the sampling and nonsampling 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 an anesthetized rabbit was placed on its back with its head hanging down. It is possible that tears are produced under anesthesia and that tear removal by either absorption or evaporation maintains the constant volume. Neither of the techniques used would show this effect.

Drainage Studies—Movement of an instilled solution into the drainage apparatus in rabbits can be described by a first-order rate expression which contains both a volume-dependent and a volume-independent term. The volume-dependent term suggests that simple

gravitational movement of the solution is not the prime mechanism for fluid removal. It is not possible to elaborate on the mechanism for fluid drainage on the basis of the results from this study.

Practical Application of Results to Clinical Therapy—From the results presented in this report, considering lacrimal volume, lacrimal fluid turnover rate, and drainage of instilled solutions, it should be possible to determine an optimum volume for drug instillation in the rabbit eye. This optimum volume 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.

To illustrate the advantage of an optimum volume, consider the following hypothetical example. If 50 μl. of a 1% drug solution is needed to elicit a given biological response, perhaps 5 μl. of a 2 or 3% solution will generate the same response. In essence, the volume change has been from 50 to 5, a factor of 10, whereas the concentration change has been from 1 to 3%, a factor of 3. The difference between the two factors represents loss of solution through drainage.

For unanesthetized rabbits the appropriate volume would appear to be approximately 5 μl. Smaller volumes will cause the drug to be removed too quickly via turnover and are difficult to introduce accurately, whereas larger volumes are washed out too quickly. In anesthetized animals, since there is no turnover of lacrimal fluid, volumes of 1 μl. or less would be optimum. However, the difficulty of manually mixing this small volume with tears must be considered.

An appropriate or optimum volume in humans is difficult to establish without precise knowledge of drainage rates, but some qualitative statements can be made. According to Maurice[1], drainage of an instilled solution in humans is considerably faster than in rabbits. Presumably the rapid rate of blinking causes an acceleration in the rate of drainage. Thus, small volumes of 1–5 μl., provided they can be accurately delivered from an ophthalmic dropper arrangement, would allow for maximum results from an instilled drug.

An important benefit of using a smaller instilled volume, in addition to improved drug activity and lower cost, is a potential decrease in side effects from ophthalmic drugs. Consider, for example, epinephrine solution used to treat glaucoma. For stability reasons, these solutions are weakly buffered to approximately pH 3–4. Instillation of 50–75 μl. of this solution causes considerable pain due at least partially to the acidity, and it can create systemic side effects (17). Using 5–10 μl. as the instilled dose should decrease the pain on instillation and minimize side effects, since less drug will drain into the drainage apparatus and be absorbed. Instillation of 10 μl. of a 2% epinephrine solution to rabbits gave the same pupillary response as 50 μl. of a 1% solution and appeared to generate less pain and lacrimation, an observation that reinforces our suggestion.

Other considerations that result from this study are:

1. Blinking of the eyes, which many people do either as a reflex action or to mix the drug solution with lacrimal fluid, should be minimized if possible. Blinking quickly squeezes solution out of the eye, which accelerates drug loss.

2. Repeated application of drops, either of the same drug or different drugs, should be reconsidered and abandoned. It can easily be shown mathematically, using the drainage rate constants, that when one drop is followed by a second drop the rate of loss of the drug solution is extremely rapid and proportional to the volume present. When two drugs are involved and each is administered separately at a short interval, the first drug will be more rapidly eliminated from the eye as compared to the second, and this loss is a function of the instilled volumes and the time between dosings. This strongly argues for administering two drugs together when combination therapy is needed rather than administering them separately.

3. The suggestion of washing the eye with normal saline or buffer solutions prior to instillation of drug (18, 19) should be viewed in light of the results of this study. The time element between washing of the eye and instillation of drug solution will greatly influence the drainage rate of drug solution since the volume of rinse solution remaining in the eye can be large.

Further Studies—The technique and approaches used in this study can be applied to evaluation of ophthalmic vehicles. Contact time and drainage rate from various vehicles as a function of vis-

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cosity and other parameters are currently being studied and will be reported in a subsequent publication.

It is desirable to compare these animal studies to humans, and thus drainage studies in humans ought to be more fully explored. In addition, further studies on the drainage mechanism as well as movement of tears in the eye would be helpful.

APPENDIX

Lacrimal Volume Determination via Sampling Technique—When a known volume, V_i, and concentration, C_0 (counts/μl.), of technetium are instilled into the eye, the solution will be diluted by the lacrimal fluid, giving rise to a new concentration, C. From the concentration of the solution after dilution it is possible to calculate the volume of lacrimal fluid, V_l, that was present by use of Eq. A1a or A1b:

$$V_l = V_i \frac{C_0}{C} - V_i \qquad \text{(Eq. A1a)}$$

$$V_l = V_i \left(\frac{C_0}{C} - 1\right) \qquad \text{(Eq. A1b)}$$

The assumptions with this method are that complete mixing occurs and that there is no loss of unmixed solution. Both of these assumptions improve as the instilled volume gets smaller.

Normal Lacrimal Fluid Turnover Rate Determination—Disappearance of an applied dose of drug or tracer substance following instillation into the eye can be described by the following equation:

$$\frac{-dC}{dt} = k(C) = \frac{F}{V_l}(C) \qquad \text{(Eq. A2)}$$

where k = first-order rate constant for the decline in tracer concentration, F = turnover rate of lacrimal fluid, and V_l is the lacrimal volume. This equation assumes that loss of applied drug is only through normal lacrimal fluid turnover. Integration of Eq. A2 and rearrangement yield:

$$\log C_0 - \log C = -\frac{F}{2.303 V_l} t \qquad \text{(Eq. A3)}$$

A plot, therefore, of the log change in tracer concentration against time should be linear. The slope of the line yields the rate constant, k, for the decline in tracer concentration which, when multiplied by the lacrimal volume, V_l, yields the turnover rate. 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 nonsampling technique, activity (amount) can be substituted for concentration in Eq. A3.

Volume Remaining from Instilled Dose—After a drop of solution is instilled, the volume at various times up to 6 min. can be calculated. The assumption in the following derivation is that one must neglect the volume of inflow of lacrimal fluid, a reasonable assumption, for the first 6 min. postinstillation 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 a rearranged form of Eq. A1a or A1b:

$$C_0 V_i = C(V_i + V_l) \qquad \text{(Eq. A4a)}$$

or:

$$\frac{C_0}{C} = \frac{V_i + V_l}{V_i} \qquad \text{(Eq. A4b)}$$

The amount of tracer substance remaining at any time is given by:

$$A = C \times V_r \qquad \text{(Eq. A5)}$$

where A = amount of drug, and V_r = volume remaining. Substituting for C in Eq. A5 from Eq. A1 yields:

$$A = \frac{V_i C_0}{V_i + V_l} \times V_r \qquad \text{(Eq. A6)}$$

and since $V_i C_0$ is equal to A_0, one can substitute for $V_i C_0$ in Eq. A6 and, upon rearrangement, obtain:

$$V_r = \frac{A}{A_0}(V_i + V_l) \qquad \text{(Eq. A7)}$$

and at time zero:

$$V_r = V_i + V_l = V_0 \qquad \text{(Eq. A8)}$$

In this study, original and subsequent radioactivity was used to represent original amount of tracer, A_0, and amount of tracer remaining, A, respectively.

Drainage Rate Constant Determination via Nonsampling 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 through Eq. A9:

$$A_0 = V_i C_0 \qquad \text{(Eq. A9)}$$

where C 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:

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

Equation A10 is derived on the basis that the change in the amount of drug is due to both a continuous inflow of lacrimal fluid, which changes the concentration of tracer, as well as an outflow or drainage which changes the amount of tracer present.

A complete solution to Eq. A10 produces complex equations and will be presented at a later time. To simplify the treatment and allow solution, it was assumed that the inflow rate of lacrimal fluid is sufficiently small to be neglected for the first 5 min. postinstillation. If there is no inflow of lacrimal fluid, then dC/dt is zero and Eq. A10 reduces to:

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

Substituting for C produces:

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

or:

$$\frac{dA}{A} = \frac{dV_i}{V} \qquad \text{(Eq. A13)}$$

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:

$$dV_i/dt = -k_v V_i \qquad \text{(Eq. A14)}$$

to produce:

$$V_r - V_l = V_i e^{-k_v t} \qquad \text{(Eq. A15)}$$

which relates the change in volume to time.

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Effect of Bile Salts on Partitioning Behavior and GI Absorption of a Quaternary Ammonium Compound, Isopropamide Iodide

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Abstract ☐ The effect of various bile salts and, in particular, sodium glycocholate upon the partitioning behavior of the quaternary ammonium compound isopropamide iodide was studied *in vitro*. Absorption of this compound from the rat ileum *in situ*, in the presence of various concentrations of bile salt, was also studied. The results indicate that sodium glycocholate progressively increases the partitioning of isopropamide from a physiological aqueous buffer into *n*-octanol below the CMC of the bile salt, but increased partitioning is inhibited above this value. Isopropamide did not partition in the absence of the bile salt counterion. The formation of a lipid-soluble ion-pair between the bile salt anion and the quaternary cation is suggested as the mechanism by which enhanced partitioning occurs, the decrease in maximal transport being related to mixed micelle formation or adsorption of ammonium ions to the outer surface of the bile salt aggregate. Absorption from the rat ileum *in situ* in the presence of sodium glycocholate below and above its CMC appears not to follow a similar pattern. It is suggested that the GI absorption of the isopropamide cation

cannot be increased in the presence of bile salt molecules through ion-pair formation or mixed micelle formation. Above the CMC of bile salt, the absorptive process appears actually to be hindered through a decrease in the availability of the drug to the absorptive surface, either by a physicochemical interaction with the micellar phase or by decreased diffusivity of the drug in the presence of bile salt aggregates.

Keyphrases ☐ Isopropamide iodide—effects of bile salts on partitioning behavior (*n*-octanol–water) and GI absorption, possible ion-pair formation, rat ileum ☐ Bile salts—effects on partitioning behavior (*n*-octanol–water) and GI absorption of isopropamide iodide, possible ion-pair formation, rat ileum ☐ Ion-pair formation—isopropamide iodide—bile salts, isopropamide partitioning behavior (*n*-octanol–water) and GI absorption, rat ileum ☐ Partitioning—isopropamide iodide from buffer to *n*-octanol, effects of bile salts ☐ Absorption, GI—isopropamide iodide, effects of bile salts, possible ion-pair formation, rat ileum

The mucosal surface of the GI tract acts as a lipoidal barrier to nutrient and drug molecules. To characterize drug absorption through such a barrier, Schanker *et al.* (1) proposed the pH–partition hypothesis, relating the degree of absorption of weak acids and bases to their lipid solubility and degree of ionization. Quaternary ammonium compounds, being fully charged at physiological pH, cannot be characterized adequately using such criteria. However, many drugs of this class are known to be pharmacologically active in very small quantities when given *via* the enteral route.

Quaternary ammonium compounds may possibly be absorbed by combination with an endogenous substance in the lumen or wall of the gut (2). Combination with such a substance could conceivably enhance absorption by acting to facilitate diffusion or to initiate an active transport mechanism.

One might postulate the involvement of the bile salts in such an absorptive process. Bile salts, normally present within the lumen of the gut, form micellar solutions which enable fatty acids and monoglycerides to be absorbed (3). Drugs such as the quaternaries, many