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Effect of pH and buffer on the precorneal disposition and ocular penetration of pilocarpine in rabbits

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Summary

An in vivo technique was utilized to study the effect of pH and buffer capacity on the precorneal disposition and ocular penetration of pilocarpine in the rabbit eye. Tear film pH, tear drug concentration and aqueous humor levels were measured at various times following the instillation of 25 μl of a 1×10^{-2} M isotonic pilocarpine solution. Test solutions were prepared in 0.0667 M phosphate buffer at various pHs (4.5, 6.0, 7.2) and at various phosphate buffer concentrations (0 M, 0.00667 M, 0.0667 M, 0.1 M) at pH 4.5. It appears that following the instillation of pilocarpine nitrate solutions buffered below the physiological pH of the lacrimal fluid, the extent of depression of the tear film pH and the tear pH re-equilibration time depends not only on the pH of the solution, but also on the precorneal fluid dynamics, and the buffer capacities of the instilled solution and that of the tears. As the buffer capacity of the instilled solution is increased, the ability of incoming tears to raise the pH in the precorneal area to its physiological value is reduced. The increase in the drainage rate due to reflex tear production is an effective mechanism by which the tear film pH re-equilibrates and is also responsible for the large reduction in the drug concentration in the precorneal area. This study examines this mechanism in detail.

The depression in the tear film pH can also reduce the ocular penetration of pilocarpine by reducing its corneal permeability. Buffering of the pilocarpine nitrate solution with 0.0667 M phosphate buffer at pH 4.5 causes a two-fold reduction in the aqueous humor level as compared to an unbuffered solution at the same pH. Such a decrease is also predicted based on tear pH–time and tear concentration–time

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measurements of pilocarpine. This study shows the practical utility of such information in estimating the ocular penetration of drugs. Based on such information it is expected that at least a two-fold reduction in pilocarpine absorption may be caused by reflex tear production. In order to ensure optimum ocular penetration of pilocarpine, the system should not depress the tear film pH appreciably, and should allow rapid tear pH re-equilibration.

Introduction

The pH and the buffer system in which a topically applied drug solution is formulated are important factors to consider in ophthalmic drug delivery. Many drugs are formulated below physiological pH because of stability or solubility constraints. Marked and prolonged depression of tear film pH may cause irritation and tissue damage in the eye. A more concentrated acid buffer causes a stronger sensation of stinging or burning than does a less concentrated buffer of the same acid pH (Longwell et al., 1976). In addition, the cornea has been shown to be less permeable to the ionized than to the unionized drug molecule (Francoeur et al., 1983; Mitra and Mikkelson, 1983). Since basic drug molecules exist predominantly in their ionized form below physiological pH, prolonged tear film pH depression can reduce corneal permeability (Francoeur et al., 1983).

Bioavailability of drugs applied topically to the eye is poor due to the rapid loss of drug from the precorneal area via drainage (Chrai et al., 1973; Sieg and Robinson, 1977). Drug loss from the precorneal area can be exacerbated following instillation of drug solutions buffered below physiological pH due to increased drainage and induced lacrimation (Conrad et al., 1978). A complete understanding of the effect of pH and buffer capacity on the ocular disposition and the ultimate bioavailability of ionizable drugs is not available.

In this study, the effect of the pH and buffer concentration of a pilocarpine solution on the tear film pH, precorneal disposition and the ocular penetration of the drug are examined in vivo. Tear film pH measurements are combined with tear and aqueous humor concentration–time data in an attempt to explain how the solution pH and the concentration of the buffer influences the disposition of pilocarpine in the eye. This information can be utilized in the evaluation and development of a rational approach to ophthalmic drug formulation.

Materials and Methods

Materials

Pilocarpine nitrate (Sigma Chemicals, St. Louis, MO) was obtained commercially and used as received. Tritiated pilocarpine alkaloid (New England Nuclear, Boston, MA, spec. act. 6.75 mCi/mmol) was received as an ethanolic solution and [^{14}C]inulin was received as a dry powder (New England Nuclear, spec. act. 2.47 mCi/mg).

Approximately 0.05 mCi was accurately weighed and dissolved in 1 ml of water to serve as a stock solution. All radiolabelled materials were stored at −20°C until needed.

Lacrimal fluid pH was determined using sensitive indicator sticks (ColorpHast, E. Merck, Darmstadt, F.R.G.). Solution pH was measured with a digital electrode (Orion Research, Model 701A). One microliter microcapillary tubes (Microcap, Drummond Scientific, Broomall, PA) were used for collecting tear samples. Samples were counted on a Beckman LS-7000 (Beckman Instruments, Irvine, CA) scintillation counting system.

Sodium phosphate (dibasic heptahydrate and monobasic monohydrate) used for making buffers were obtained from Fisher Scientific. All other reagents used were either chemical or analytical grade and were used as received.

The rabbits used in these studies were white New Zealand rabbits (Small Stock Industries, Pea Ridge, AK). At the time of use their age ranged from 55–64 days. No restrictions were placed on food or water intake by the rabbits prior to experimentation.

Solution preparation

Pilocarpine nitrate solutions were prepared in isotonic sodium phosphate buffers of different molar concentrations at pH 6.2. These buffers were made isotonic with sodium chloride. In all these studies the concentration of pilocarpine nitrate used was 1×10^{-2} M. The pH was checked and adjusted as necessary. 'Cold' solutions of pilocarpine nitrate were prepared preceding each series of experiments and discarded following use. Prior to each experiment, approximately 50 μl of tritiated pilocarpine alkaloid was transferred to a microevaporation flask using a Hamilton microsyringe. Two 1-ml portions of ethanol U.S.P. were added and the solution was evaporated to dryness under a constant stream of nitrogen. The residue was reconstituted with 1 ml of the 1×10^{-2} M 'cold' pilocarpine nitrate solution. The final solution had an activity of about 150,000 counts per minute per microliter. The small amount of isotopic material added did not affect the molarity or the pH of the final solution.

The [^{14}C]inulin solutions were prepared similarly. This solution did not contain any unlabelled inulin. Inulin served as a non-absorbable marker in these experiments. Although inulin has been found to be absorbed into the eye (Longwell et al., 1976), its rate of absorption is slow and its extent of absorption is small. Over the time course in which inulin was used in these studies, any absorption would be negligible.

Blockage of the drainage duct

In one series of experiments, in order to prevent instilled solution drainage, the drainage ducts of the rabbits were blocked using techniques previously described (Patton and Robinson, 1976). Polyethylene tubing (PE 50), 0.61 mm i.d. and 0.96 mm o.d. cut into sections of approximately 5 mm in length were made into plugs and inserted into the punctum. The plugs were inserted 1 min prior to the instillation of the drug solution.

Lacrimal fluid pH–time profiles

The pH indicator sticks were cut into small strips approximately 1 mm in width and 5 mm in length. Unanesthetized rabbits were placed in restraining boxes in a normal upright posture. A test solution of 1.00×10^{-2} M pilocarpine nitrate was prepared in pH 6.24 isotonic phosphate buffer. In one set of experiments the buffer concentration was held constant at 0.0667 M and the final pH was varied (pH 4.5, 6.0 and 7.2). In the other case the final solution pH was held constant at pH 4.5 and the total concentration of the phosphate buffer was varied (0 M, 0.00667 M, 0.0667 M and 0.1 M). The 0 M phosphate buffer solution consisted of 1.00×10^{-2} M pilocarpine nitrate in distilled water, pH adjusted with HCl and made isotonic with sodium chloride. The solution was administered as a 25-μl dose with a Hamilton microsyringe. Tear pH was determined in vivo by placing these strips in the lower cul-de-sac for 3 s and reading the color against a color chart provided by the manufacturer. The accuracy of pH paper measurements was checked by comparing the pH paper readings with that of a standard pH electrode using standard solutions ranging from pH 4–8. Over this pH range, the pH electrodes and the pH paper measurements were within ±0.2 pH units.

Loss of drug from the precorneal area

Tear concentration of pilocarpine and inulin were measured to investigate the influence of instilled solution pH and buffer capacity on the factors responsible for the loss of drug in the precorneal area. A 25-μl dose of 1×10^{-2} M tritium-labelled pilocarpine nitrate solution, unbuffered at pH 4.5 and buffered in 0.0667 M phosphate at pH 4.5, 6.0 and 7.2, was topically instilled in the eye. One μl tear samples were withdrawn at 1 min intervals for 5 min. Each eye was subjected to a single treatment. Experiments were also performed where the tear drainage duct of the rabbit was blocked. The test solutions contained either tritium-labelled pilocarpine or [^{14}C]inulin. Tear samples were collected into 1 μl microcapillary tubes by procedures described previously (Miller, 1980). After sampling, the entire microcapillary tube was placed in a liquid scintillation vial prefilled with 5 ml of liquid scintillation cocktail (Aquasol II). Standards were prepared by taking a series of 1 μl samples of the dosing solution in microcapillaries. Samples and standards were stored in a cool, dark place for 24 h to eliminate photoluminescence, and counted on a Beckman LS7000 scintillation counter. Withdrawing small volumes of tears from the precorneal pocket at early times post-instillation did not significantly alter the measured drug concentration.

Aqueous humor concentration of pilocarpine

The effect of pH and buffer on the ocular penetration of pilocarpine in rabbits was studied by measuring the aqueous humor concentration of the drug. A 25-μl dose of 1.00×10^{-2} M tritium-labelled pilocarpine nitrate solution was topically instilled in the eye. Studies were done both in the absence and the presence of buffer at pH 4.5, and at pHs 4.5, 6.0 and 7.2 while holding the total phosphate concentration constant at 0.0667 M.

Animals were killed 10, 20, 30 and 40 min following drug administration with an

overdose of sodium pentobarbital injected into the marginal ear vein. A given volume of aqueous humor was collected by techniques described previously (Miller, 1980). Standards were prepared using the aqueous humor collected from two untreated eyes and spiking them with the dosing solution. The aqueous humor samples were transferred to a polyethylene vial containing 5 ml of pre-refrigerated liquid scintillation cocktail. Samples were stored for 24 h in a dark, cool place to minimize photoluminescence and subsequently counted for activity.

Results and Discussion

The resident tear pH of rabbits was found to be 7.3 ± 0.1. It was observed that the lower the pH of the instilled solution, the greater the initial depression in the tear film pH from its normal resident value (Table 1). Following the instillation of 25 μl of 0.01 M pilocarpine nitrate buffered in 0.0667 M isotonic Sorensen's phosphate, pH 4.5, the tear pH 1 min post-instillation was 5.7, over one pH unit higher than that of the solution instilled. It thus appears that the instilled solution pH is not the only formulation variable that influences the tear film pH. In this regard, the buffer capacity of the instilled solution must also be considered. Phosphate has a poor buffer capacity at pH 4.5, whereas the tears have better buffer capacity below pH 6.5 (Keller et al., 1980). Therefore, the tears can effectively resist the depression of pH in this region.

Following instillation, the tear pH returns with time to its normal resident pH value. The rate of rise in tear pH depends on many factors including the factors affecting fluid dynamics in the precorneal area, along with formulation variables and the physical-chemical characteristics of the tears. Induced lacrimation caused by acidic instilled solutions results in the influx of fresh tears and an increase in the drainage of the tears away from the precorneal area (Conrad et al., 1978). This could account for the rapid change in the lacrimal fluid pH following the instillation of a solution buffered below the physiological pH of the tears. This is particularly true

TABLE 1

LACRIMAL FLUID pH VS TIME IN RABBITS FOLLOWING TOPICAL INSTILLATION OF 25 μl OF 0.01 M ISOTONIC PILOCARPINE NITRATE SOLUTION OF VARYING pH. TOTAL BUFFER CONCENTRATION HELD CONSTANT AT 0.0667 M

Time (min)	Instilled solution pH		
	4.5	6.0	7.2
1	5.7 (0.1, 7) [a]	6.1 (0.2, 7)	7.2 (0.1, 7)
3	6.1 (0.2, 7)	6.5 (0.1, 7)	7.3 (0.1, 7)
6	6.7 (0.3, 7)	6.8 (0.2, 7)	7.3 (0.1, 7)
10	7.2 (0.1, 7)	7.3 (0.2, 7)	7.3 (0.1, 7)
15	7.3 (0.2, 7)	7.4 (0.1, 7)	7.4 (0.1, 7)

[a] The numbers in parentheses refer to the S.E.M. and the number of determinations, respectively.

when the instilled solution pH is 4.5 because of the poor buffer capacity of phosphate at this pH. Thus, the influx of fresh tears into the precorneal area can rapidly change the pH. When the instilled solution pH is 6.0 and 7.2, a slower change in the tear film pH is observed because the extent of induced lacrimation is reduced.

The effect of buffer concentration of the instilled solution on the tear film pH was studied similarly. Lacrimal fluid pH was measured in vivo at 1, 3, 6, 10 and 15 min following the instillation of 25 μl of 1×10^{-2} M pilocarpine nitrate solution. The solutions tested were either unbuffered or buffered in Sorensen's phosphate with total buffer concentrations (C_T): 0.00667 M, 0.0667 M and 0.1 M and pH held constant at 4.5. The extent to which the tear film pH is depressed appears to vary with C_T — the higher the total phosphate concentration, the greater the extent to which the tear film pH is depressed (Fig. 1). This is probably due to an increase in the buffer capacity of the instilled solution as C_T is increased. A slower rate of increase in the tear film pH is observed following the instillation of pilocarpine nitrate solutions of increasing phosphate concentration. The ability of incoming tears to promote a rapid return of the tear film pH to its resident value is diminished when the solution is strongly buffered. This is verified by the data presented in Fig. 2. In this case a series of experiments were performed using rabbits in which normal tear drainage was prevented by plugging the drainage duct. Solutions of 1×10^{-2} M pilocarpine nitrate, pH 4.5, were prepared in 0.0667 M and 0.1 M isotonic phosphate buffer, as well as in the absence of buffer. The tear film pH was measured following instillation of a 25 μl drop. In these studies, the increase in the tear film pH

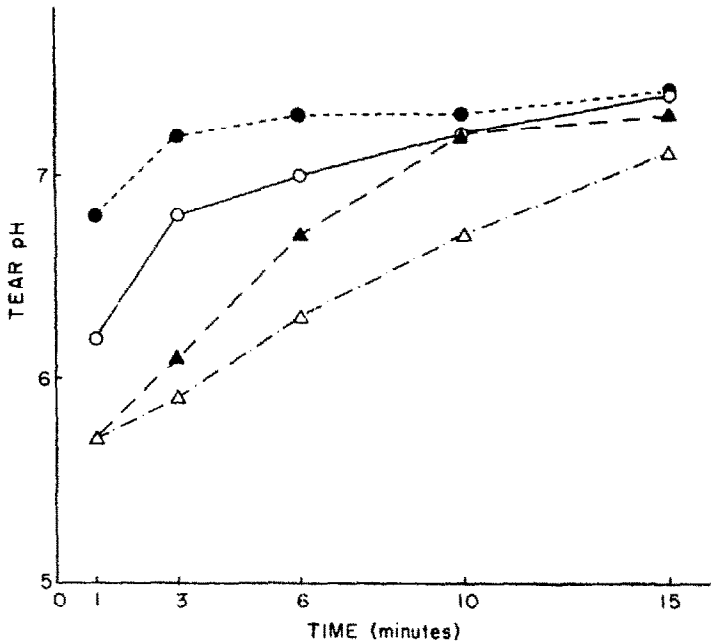

Fig. 1. Lacrimal fluid pH vs time profile after topical instillation of 25 μl of 0.01 M pilocarpine nitrate solutions of different buffer concentration (isotonic Sorensen's phosphate), the pH held constant at 4.5. Total buffer concentration: 0 M, ●; 0.00667 M, ○; 0.0667 M, ▲; 0.1 M, △.

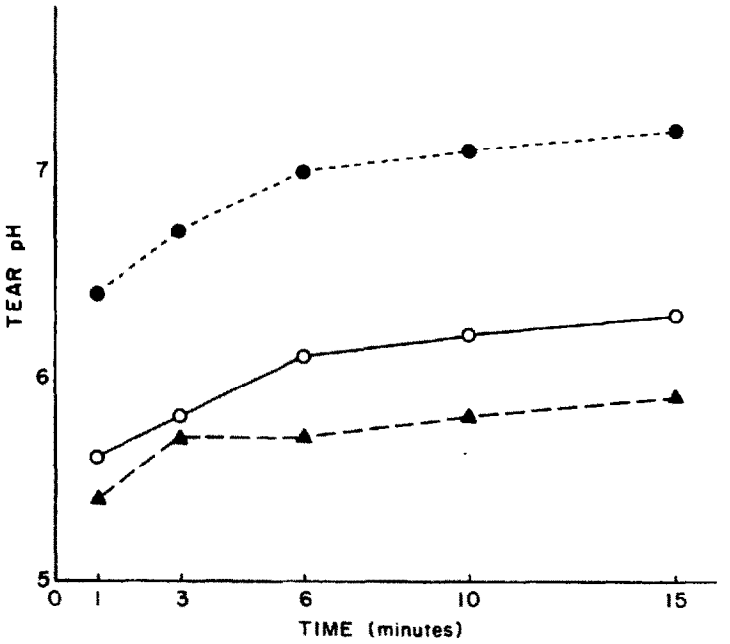

Fig. 2. Lacrimal fluid pH vs time in rabbits with blocked drainage duct following the topical instillation of 25 μl of 0.01 M pilocarpine nitrate solutions of different buffer concentrations (isotonic Sorensen's phosphate), the pH held constant at 4.5. Total buffer concentration: 0 M, ●; 0.0667 M, ○; 0.1 M, ▲.

post-instillation is primarily attributed to an influx of tears into the precorneal area. The results clearly show that the rate of change in tear pH is slower with increasing buffer concentration. This fact also establishes the importance of drainage on the tear pH re-equilibration rate.

These studies on the lacrimal fluid pH as a function of the formulation pH and buffer capacity have several important implications regarding the disposition of pilocarpine in the eye. It is evident that following the instillation of a solution buffered below the physiological pH of the tears, the extent of depression of the tear pH and the tear pH re-equilibration time depends not only on the pH of the solution, but also on precorneal fluid dynamics and the buffer capacities of the instilled solution and that of the tears. Prolonged depression of the tear film pH could reduce the corneal permeability of an ionizable, basic drug such as pilocarpine. Furthermore, induced lacrimation stimulated by a non-physiological condition in the eye could dilute and cause rapid removal of the drug from the precorneal area. Therefore, a substantial reduction in ocular bioavailability can occur when the instilled solution is strongly buffered.

The effect of pH and buffer capacity on the precorneal loss of pilocarpine is shown in Table 2. The concentration of pilocarpine in the precorneal area post-instillation was determined and was found to decline monoexponentially. This behavior is in agreement with previous studies (Patton, 1976). From plots of log-concentration versus time, pseudo-first-order rate constants for the decline in concentration of pilocarpine were obtained.

There is a slight but statistically insignificant decrease in the apparent rate

TABLE 2

APPARENT FIRST-ORDER RATE CONSTANTS FOR THE DECLINE OF PILOCARPINE CONCENTRATION IN THE TEARS IN RABBITS FOLLOWING THE TOPICAL INSTILLATION OF 25 μl OF 0.01 M PILOCARPINE NITRATE SOLUTIONS BUFFERED AT VARIOUS pHs WITH 0.0667 M ISOTONIC PHOSPHATE BUFFER AND AT pH 4.5 IN THE ABSENCE OF BUFFER

pH of the instilled solution	First-order rate constants for the decline in pilocarpine concentration in the tears (min^{-1})	
	Buffered	Unbuffered
4.5	0.672 (0.097, 13) [a]	0.643 (0.070, 21)
6.0	0.620 (0.042, 8)	
7.2	0.429 (0.075, 7)	

[a] Numbers in parentheses refer to the S.E.M. and number of determinations, respectively.

constant when the instilled solution was buffered at pH 6.0 as opposed to pH 4.5, and a much slower rate of decline is observed when the instilled solution pH is 7.2. Pilocarpine has a higher permeability coefficient in its unionized form compared to the ionized drug molecule (Francoeur et al., 1983). Increasing the concentration of unionized pilocarpine in the precorneal fluid should facilitate the absorption of pilocarpine. Accordingly, a faster rate of decline in the tear concentration of pilocarpine would be expected as the pH of the instilled solution is increased. Therefore, the trend seen in Table 2 cannot be attributed to absorption processes.

To further examine the effect of instilled solution pH on the precorneal disposition of pilocarpine, the normal tear removal mechanism in the rabbit was eliminated by blocking the tear drainage duct. The concentration of inulin and pilocarpine in the precorneal area was measured following instillation of 25 μl of 1×10^{-2} M pilocarpine nitrate solutions, buffered at pH 4.5 and 7.2 in 0.0667 M Sorensen's phosphate buffer. The drug solution was spiked with either [^{14}C]inulin or [^{3}H]pilocarpine. The data was treated as described previously and apparent rate constant values were obtained (Table 3). Under these conditions, the decline in the inulin concentration in the tears is primarily due to tear production, while for pilocarpine, absorption in addition to tear production is responsible. The results show that the rate of decline in the tear concentration of inulin are similar at pHs 4.5 and 7.2. The decline in pilocarpine concentration in the tears is two-fold faster at pH 7.2 than at pH 4.5. Since unlike inulin, pilocarpine is absorbable and exists primarily in its ionized form at pH 7.2, these observations can be explained by a faster absorption of pilocarpine at the higher pH.

However, when there is normal tear drainage, the apparent rate of decline in tear concentration of pilocarpine is greater at pH 4.5 than at 7.2. Tear drainage in association with reflex tear production appears to have a large effect on drug concentration in the precorneal area. These factors at pH 4.5 have a greater effect on the tear concentration of pilocarpine than does an increase in the absorption rate at pH 7.2. Consequently, in this case the apparent rate constant for decline in

TABLE 3

APPARENT FIRST-ORDER RATE CONSTANTS FOR THE DECLINE IN PILOCARPINE CONCENTRATION AND INULIN ACTIVITY IN THE PRECORNEAL AREA OF 60-DAY RABBITS WITH PLUGGED TEAR DRAINAGE DUCT FOLLOWING TOPICAL INSTILLATION OF 25 μl OF 0.01 M PILOCARPINE NITRATE SOLUTION BUFFERED AT EITHER pH 4.5 OR pH 7.2 WITH ISOTONIC 0.0667 M SORENSEN'S PHOSPHATE BUFFER.

	pH of instilled solution	
	4.5	7.2
Inulin	0.097 (0.004, 14) [a]	0.106 (0.044, 8)
Pilocarpine	0.121 (0.023, 6)	0.242 (0.037, 6)

[a] The numbers in parentheses refer to the S.E.M. and to the sample size, respectively.

pilocarpine concentration in the tears is larger at pH 4.5 than at pH 7.2.

The value for the apparent rate constant for the decline in pilocarpine concentration in the tears following the instillation of an unbuffered solution at pH 4.5 is similar to that of a buffered solution at the same pH (Table 2). Initially, this observation is surprising since more reflex tear production and a faster decline in the pilocarpine concentration in the precorneal area is expected when the solution is buffered.

In order to examine the formulation buffer effect on the precorneal disposition of pilocarpine, rabbits were dosed with 25 μl of 1×10^{-2} M pilocarpine nitrate solutions spiked with [^{14}C]inulin. The solutions administered were either buffered at pH 4.5 in 0.0667 M sodium phosphate or unbuffered at pH 4.5. The inulin concentration in the tear chamber was measured following instillation (Table 4). The faster decline in inulin concentration for the buffered solution indicates that buffering increases tear production and tear drainage. The similar magnitude for the rate constants for the decline in pilocarpine concentration in the absence and in the presence of buffer is probably due to an increase in the rate of absorption of

TABLE 4

APPARENT FIRST-ORDER RATE CONSTANTS FOR THE DECLINE OF PILOCARPINE CONCENTRATION AND INULIN ACTIVITY IN RABBITS FOLLOWING THE TOPICAL INSTILLATION OF 25 μl OF 0.01 M PILOCARPINE NITRATE SOLUTION BUFFERED AT pH 4.5 WITH 0.0667 M ISOTONIC PHOSPHATE BUFFER AND AT pH 4.5 IN THE ABSENCE OF BUFFER

	First-order rate constants for the decline in pilocarpine concentration and inulin activity in the tears (min^{-1})	
	Inulin	Pilocarpine
Buffered	0.586 (0.078, 15) [a]	0.672 (0.097, 13)
Unbuffered	0.456 (0.049, 19)	0.643 (0.070, 21)

[a] Numbers in parentheses refer to the S.E.M. and number of determinations, respectively.

pilocarpine when the formulation is unbuffered. This partially off-sets precorneal fluid factors when the solution is buffered.

Following the instillation of 25 μl of a pH 4.5 drug solution, there is a 6–8-fold reduction in the rate of decline in pilocarpine and inulin concentration in the precorneal area when tear drainage is blocked (Tables 3 and 4). A similar but more modest two-fold reduction is observed when the instilled solution pH is 7.2 (Tables 2 and 3). These findings are surprising since drainage is not expected to alter drug concentration in the tears directly (Patton and Robinson, 1976). Teorell (1937) showed that the first-order rate constant associated with the movement from one compartment to the next is inversely related to the fluid volume in the donor compartment. The resident tear volume in awake rabbits is approximately 7.5 μl (Chrai et al., 1973). Following the instillation of a 25 μl dose, the lacrimal fluid volume will rapidly decline from 32.5 μl to 7.5 μl due to instilled solution drainage. However, when tear drainage is occluded, the lacrimal fluid volume remains essentially constant at 32.5 μl post-instillation. Therefore, the larger lacrimal fluid volume in the precorneal area can cause a 4-fold reduction in the rate constant value when the drainage duct is blocked. While this explains the two-fold reduction in the rate in the absence of drainage when the instilled solution is pH 7.2, it does not completely account for the 6–8-fold reduction in the rate constant value when the pH of the dose is 4.5. Induced lacrimation is expected to be greater following the instillation of a pH 4.5 solution than a pH 7.2 solution and is reflected by a larger drainage rate at the lower pH (Conrad et al., 1978). When tear drainage is prevented, induced or reflex tear production does not appear to significantly alter the tear concentration of inulin (Table 3). Therefore, when tear drainage is not blocked, the maximum expected value for the rate constant for the decline in inulin concentration in the tear chamber following instillation of a 25 μl dose, due to a volume effect, would be about 0.44 min^{-1}. However, the measured value of 0.586 min^{-1} (Table 4) when the instilled solution pH is 4.5 is considerably larger. In fact, this value is in good agreement with the reported drainage rate constant value of about 0.62 min^{-1} at pH 4.5 (Conrad et al., 1978). Therefore, it appears that induced lacrimation not only increases the rate of tear drainage, but the interplay of induced tear production and drainage also causes a substantial decline in the drug concentration in the tears. However, neither induced lacrimation nor drainage alone has much effect on the drug concentration in the tear chamber. The interaction between reflex tear production and drainage is also an effective mechanism by which the tear film pH re-equilibrates. Tear re-equilibration time is substantially prolonged in the absence of tear drainage (Fig. 2) and in anesthetized rabbits (Longwell et al., 1976).

Decline in pilocarpine concentration in the tear chamber is due to both precorneal fluid dynamics and absorption processes. On the contrary, decline in inulin levels in the precorneal area is due to fluid dynamics only, since inulin is not appreciably absorbed across the intact cornea (Maurice, 1973). The difference in the rate constant for the decline in inulin and pilocarpine concentration in the tears is a measure of the apparent absorption rate. Several such parameters have been calculated from the data in Tables 3 and 4 and are compiled in Table 5. The meaning of these 'apparent' absorption rate constants must be interpreted cautiously

TABLE 5

'APPARENT' ABSORPTION RATE CONSTANTS FOR PILOCARPINE AS A FUNCTION OF THE INSTILLED SOLUTION pH AND BUFFER CAPACITY

Condition	'Apparent' absorption rate constant (min^{-1}) [a]
Open tear drainage duct; solution buffered at pH 4.5	0.086
Open tear drainage duct; solution unbuffered at pH 4.5	0.187
Blocked tear drainage duct; solution buffered at pH 7.2	0.136
Blocked tear drainage duct; solution buffered at pH 4.5	0.024

[a] Obtained by subtracting the values for inulin from those of pilocarpine in Tables 3 and 4.

because these values reflect not only corneal absorption, but also the 'non-productive' absorption of pilocarpine (Patton and Robinson, 1976). Furthermore, since these parameters were calculated based on tear concentration data, they reflect and are influenced by precorneal fluid dynamics. For instance, the inflated values for the 'apparent' absorption rate constants in the case where the tear drainage duct is open are probably due to the 'parallel loss' of drug caused by instilled solution drainage (Makoid and Robinson, 1979). However, these parameters do provide qualitatively useful information and may be utilized to compare the relative extent of absorption under various conditions. Based on data in Table 5, buffering the drug solution at pH 4.5 may cause a two-fold reduction in the ocular penetration of pilocarpine as compared to when it was not buffered.

The effect of pH and buffer capacity on the ocular penetration of pilocarpine was also studied. Aqueous humor levels of pilocarpine were measured following the instillation of 25 μl of 1×10^{-2} M pilocarpine solution. The peak time remained at about 20 min regardless of the formulation (Table 6). Induced lacrimation can significantly reduce pilocarpine absorption when the instilled solution pH is below the physiological pH of the lacrimal fluid. For this reason much higher aqueous humor levels are seen when the pH of the dose is 7.2, because of minimal induced lacrimation at this pH. Therefore, increasing the concentration of unionized pilo-

TABLE 6

PEAK AQUEOUS HUMOR CONCENTRATION OF PILOCARPINE IN RABBITS FOLLOWING THE TOPICAL INSTILLATION OF 25 μl OF 0.01 M PILOCARPINE NITRATE SOLUTIONS BUFFERED (WITH 0.0667 M ISOTONIC SORENSEN'S PHOSPHATE) AT pH 4.5, 6.0, 7.2, AND IN THE ABSENCE OF BUFFER, pH 4.5.

pH of the instilled solution	μg of pilocarpine per ml of aqueous humor	
	Buffered	Unbuffered
4.5	0.72 (0.05, 7) [a]	1.77 (0.18, 6)
6.0	1.11 (0.11, 8)	
7.2	2.02 (0.22, 8)	

[a] Numbers in parentheses refer to the S.E.M. and number of determinations, respectively.

carpine in the precorneal area causes a substantial increase in ocular penetration only when reflex tear production is negligible.

The aqueous humor level of pilocarpine was two-fold higher when the instilled drug solution was not buffered at pH 4.5 as compared to when it was buffered at the same pH (Table 6). This increase in ocular penetration is in agreement with the previous results shown in Table 5. Based on the results shown in Tables 5 and 6 and from the information presented throughout this study, it is clear that buffering a pilocarpine solution below the physiological pH of the tears reduces ocular penetration both by suppressing the absorption of the drug and by increasing reflex tear production and tear removal.

Conclusion

In summary, the buffer used in formulating an ophthalmic solution below physiological pH plays a crucial role in determining the ocular penetration of pilocarpine. In this regard, both precorneal fluid dynamic factors and drug absorption based on the pH-partition hypothesis are important. In systems that do not depress lacrimal fluid pH appreciably and allow a rapid return of the tear film pH to its normal resident value post-instillation, drug absorption depends primarily on the pH-partition effect.

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