Vehicle Effects on Ocular Drug Bioavailability I: Evaluation of Fluorometholone

JAMES W. SIEG and JOSEPH R. ROBINSON ˣ

Abstract □ The influences of drug concentration and vehicle composition on the corneal penetration of the steroid fluorometholone were studied in the albino rabbit. Aqueous dosing systems included a saturated solution and 0.1, 0.05, and 0.01% suspensions of micronized fluorometholone. Two different doses of a 0.1% oleaginous ointment were also studied. The results from the 0.1 and 0.05% suspensions show a peak aqueous humor steroid concentration at 30 min and a substantial sustaining effect with these two concentrations. The results also support the belief that moderate dilution of a suspension of a slowly soluble drug may not substantially lower the aqueous humor drug levels or, conversely, that use of a higher concentration suspension may not improve the aqueous humor drug concentration–time profile. The 0.01% suspension and the saturated solution did not produce a sustaining effect. The results demonstrate for the first time that the particles present in a dose of suspension are retained within the cul-de-sac of the eye and contribute significantly to the amount of steroid penetrating the cornea. This finding was confirmed by a study in which the eye was rinsed with saline solution 30 min after instillation of a dose of a 0.05% suspension. The rinsing procedure prematurely terminated the sustaining effect of the suspension. The results of the ointment studies show that partitioning of the lipophilic steroid from the oleaginous vehicle has a greater rate-limiting influence on corneal penetration than the dissolution rate parameter associated with the aqueous suspensions. Peak aqueous humor concentration was not achieved until 3 hr after dosing and was comparable to the 0.1 and 0.05% suspensions. Predosing of the eye with a saturated solution or the 0.1% suspension prior to dosing with ointment overcomes the inability of the ointment to provide adequate drug at short times following dosing. In this case, peak levels were achieved within 60 min and then maintained. The duration of aqueous humor levels and the amount penetrating from the ointment were greater than the suspensions, and these effects are discussed relative to the mechanism. Differences in aqueous humor levels produced by 25- and 50-mg doses of ointment were minimal. A discussion of the results from all studies is presented in the context of present theories regarding the role of the lipophilic epithelial layer of the cornea as a barrier to drug penetration.

Keyphrases □ Fluorometholone—effect of drug concentration and vehicle composition on corneal penetration, rabbits □ Ocular drug bioavailability, fluorometholone—effect of drug concentration and vehicle composition on corneal penetration, rabbits □ Bioavailability, ocular, fluorometholone—effect of drug concentration and vehicle composition on corneal penetration, rabbits

Steroids are widely used to treat various inflammatory disorders of the eye and to prevent progressive complications resulting from other ocular disease states. As a result, ophthalmic studies relating to the nature of topically administered steroids and to the extent to which they penetrate into ocular tissues have received increasing attention during the past 20 years. These investigations have provided information regarding the intraocular penetration of dexamethasone (1–6), prednisone (6–10), prednisolone (6–10), cortisone (11–13), hydrocortisone (11–13), and other related corticosteroids (12). Some studies have also included various derivatives of the parent compounds and have provided information pertaining to the influence of a drug's relative lipid and water solubilities on its ability to penetrate the epithelial layer of the cornea (5, 6, 9).

In addition, the dosing systems used in these studies have been formulated to include aqueous solutions (2–4, 10), suspensions (1, 7–9, 11–13), and various ointment vehicles (4, 5, 10). However, a mechanistic interpretation of the influence of these vehicles on the biological properties of the drug in ocular studies has not been explored in the literature. The present study examines the influence of vehicles on the intraocular penetration of the highly lipophilic steroid fluorometholone.

BACKGROUND

Whenever a true solution of a drug is administered to the eye, the ultimate aqueous humor drug concentration that may be attained is influenced by a number of controlling factors. These variables include the concentration of the drug in the vehicle (9, 10, 13), the volume of the instilled dose (14), the viscosity of the vehicle (15, 16), the influence of tear turnover and drainage on the instilled dose (14), and the absorption and elimination characteristics of the drug. For ophthalmic suspension systems, the additional parameters of intrinsic solubility and dissolution rate must be considered since the residence or contact time of the drug in the eye is usually brief.

The intrinsic solubility of the drug is important for suspension systems since it determines the amount of drug actually in solution and available for immediate absorption upon instillation of the dose. As the intrinsic solubility of the drug increases, the concentration of the drug in the saturated solution surrounding the suspended drug particles also increases. For this reason, any comparison of different drugs in suspension systems should include their relative intrinsic solubilities since the observed differences in their biological activities may be ascribed wholly or in part to their differences in this physical parameter.

The dissolution rate parameter must be considered for ophthalmic suspensions in light of the minimal residence time exhibited by most aqueous systems in the eye (14, 17). Upon instillation of the dose, drug from the saturated solution surrounding the suspended particles enters the lipophilic epithelium. It is then transported into the hydrophilic stromal layers of the cornea and ultimately penetrates the less lipophilic endothelium to the aqueous humor. As the initial saturated solution becomes depleted, the particles must dissolve to provide a further supply of drug. The requirement here is that the particles must undergo significant dissolution within the residence time of the dose in the eye if any benefit is to be gained from their presence in the dosing system.

For a drug whose dissolution rate is rapid, the dissolution requirement may present few problems; but for a slowly soluble substance, the dissolution rate becomes critical. If the dissolution rate is not sufficiently rapid to supply significant additional dissolved drug, there exists the possibility of a slowly soluble substance in suspension which provides no more drug to the aqueous humor than does a more dilute suspension or saturated solution of the substance in a similar vehicle (18, 19).

The particle size of the suspended drug affects the surface area available for dissolution. Apart from dissolution, particle size also plays an important role in the irritation potential of the dosing system. This consideration is important since irritation produces excessive tearing and rapid drainage of the instilled dose. It has been recommended that particles be less than 10 μm to minimize particle irritation to the eye.

Relative to an aqueous ophthalmic suspension or solution, the ultimate performance of an ointment system in the eye is controlled by a balance of positive and negative physicochemical considerations. On the positive side, there is the possibility of increased contact time for an ointment over that of an aqueous suspension or solution. In addition, for water-insoluble drugs, there exists a means of increasing the soluble drug concentration in the dosing system by choosing an oleaginous vehicle in which the drug is soluble. On the negative side, ophthalmic ointment systems present mixing problems between the ointment vehicle and the tears and also include an additional partitioning parameter for the drug between the ointment and the tear film.

The contact time of the ointment system with the precorneal tear film is largely determined by the viscosity of the vehicle and its other physical characteristics, in particular its miscibility with the ocular fluids. While the contact time can be extended by increasing the viscosity of the ointment vehicle or by decreasing its degree of miscibility with the tears, these same parameters also present mixing problems unfavorable for optimum partitioning and distribution of the drug from the ointment base to the tear film.

Mixing is an extremely important parameter since it determines the amount of surface area of instilled ointment that is available for partitioning within the time limitation imposed by drainage loss. The extent of this partitioning interface greatly influences the amount of drug released from the ointment and also affects the kinetics of drug distribution and the rate at which the drug partitions from the ointment to the precorneal tear film. At this point, it might seem reasonable to assume that an emulsion type of ointment base would be ideal in view of the large surface areas characteristic of this system. However, emulsion systems also have their own inherent problems involving stability, attainment of adequate viscosities, and the unpredictable effect of surfactants upon corneal drug transport and, most importantly, upon the integrity of the epithelial layer of the cornea (20, 21).

EXPERIMENTAL

Materials—Tritium-labeled fluorometholone preparations were formulated by the manufacturer[1] as a 0.1% aqueous micronized suspension and a 0.1% petrolatum-based ointment. The final products were of commercial quality, equivalent to other similar formulations currently available from the manufacturer. The specific activity of the two preparations was 40 μCi/mg of steroid.

Liquid scintillation counting vials and solutions were obtained from commercial sources[2,3]. All other chemicals used were either reagent or analytical grade, and water was double distilled from alkaline permanganate.

Male albino rabbits[4], 1.8–2.4 kg, were used. The rabbits were fed a regular diet with no restrictions on the amount of food or water consumed. Lighting and auditory stimulation (radio) were maintained in the caging facilities for 24 hr a day to provide a constant experimental environment.

Aqueous Humor Concentration *versus* Time Profile—*Fluorometholone Suspensions*—Male albino rabbits were placed into wooden restraining boxes to minimize movement. The normal upright posture of the animals was maintained at all times. Care was taken to permit normal eye movements, and head movement was only minimally restricted. The use of local and general anesthetics was avoided throughout the study. Recent work has demonstrated the effects of topical local anesthetics on tear dynamics and ocular drug bioavailability (22) and the influences of general anesthesia on lacrimal and instilled fluid dynamics (4) and on the corneal penetration of fluorometholone (19) in rabbits. Throughout each experimental run, the physical state of the test animals was as near normal as was experimentally feasible.

Fifty-microliter doses were administered, using a microliter syringe, by instillation directly onto the corneas of both eyes, collecting in the lower cul-de-sac. During instillation, the upper lid was slightly raised and the lower lid was pulled slightly away from the globe. The lids were immediately returned to their normal position after instillation was completed. Animals were then sacrificed at 20, 30, 60, 90, 120, 180, 240, and 300 min postinstillation by rapid injection of pentobarbital sodium into a marginal ear vein. The corneal surface was thoroughly washed with distilled water and dried with tissue. Aqueous humor samples were then removed from the anterior chamber using a 1-ml tuberculin syringe fitted with a 27-gauge needle.

One hundred-microliter samples of aqueous humor were transferred[5] to scintillation counting vials containing 5 ml of liquid scintillation solution. Vials were refrigerated at 0° for 24 hr prior to the addition of the samples and stored in the dark at room temperature for at least 72 hr before counting to minimize chemiluminescence. Ten-minute counts of each sample were made using a liquid scintillation spectrometer[6]. The final count rate obtained for each sample was then converted to a microgram of steroid per milliliter of aqueous humor basis by standardization techniques to facilitate the analysis and interpretation of results.

To investigate the effect of suspension concentration on the aqueous humor steroid levels, it was necessary to dilute the original 0.1% fluorometholone suspension to the desired concentrations. The potential influence of this procedure upon the final results will be discussed later. The 0.05 and 0.01% suspensions were prepared by diluting an aliquot of the original suspension with an appropriate volume of the suspension vehicle supplied by the manufacturer.

The diluted suspensions were then allowed to equilibrate on a shaker at room temperature for several days. Equilibration was verified by centrifuging samples taken from each suspension at various times and analyzing the supernatant liquids using liquid scintillation methods. Equilibration was assumed to be complete when the count rate for the supernatant samples reached a constant value.

Fluorometholone Saturated Solution—The saturated solution was prepared from the original 0.1% suspension of micronized fluorometholone. A laboratory clinical centrifuge[7] was used to separate the saturated solution and particulate components of the suspension. A tube containing 5 ml of the suspension was centrifuged at the highest speed setting until the supernate appeared clear by visual observation. The supernatant liquid was then removed and passed through a 0.22-μm biological filter[8]. The high initial specific activity of the original 0.1% fluorometholone suspension was required so that the saturated supernatant liquid obtained by centrifugation retained sufficient activity to give adequate counting rates in the aqueous humor studies.

Experimental runs using the saturated fluorometholone supernatant liquid were performed using the techniques previously described for the suspensions.

0.05% Fluorometholone Suspension Rinsed with Saline at 30 min Postinstillation of Dose—A modified suspension study was performed using a standard 50-μl dose of a 0.05% suspension. Thirty minutes after dosing, the eyes of the test animals were thoroughly flushed with isotonic saline injection containing no preservatives. A polyethylene wash bottle with a fine-drawn tip was used so that a stream of saline could be directed into all portions of the eye with sufficient force to remove any residual fluorometholone particles. The eyes were then left untouched and the animal was kept in a normal experimental posture for the completion of the run. No changes in the basic routine were made beyond the time of rinsing with saline.

0.1% Fluorometholone Ointment—Two different doses of fluorometholone ointment were used: 25 and 50 mg. The individual doses were weighed on an analytical balance prior to each experimental run. Dosing was carried out by means of a small laboratory microspatula. The weighed dose of ointment was carefully transferred to the spatula and then placed inside the center of the lower lid, with care being taken not to irritate the eye or touch the corneal epithelium with the instrument. The lower lid was gently moved upward to spread the dose uniformly over the corneal surface and then released. No further mechanical action was performed, and the remainder of the run was carried out using the same basic experimental techniques previously discussed.

[1] Allergan Pharmaceuticals, Irvine, Calif.
[2] Mini Vial, ICN Isotope and Nuclear Division, Cleveland, Ohio.
[3] Aquasol, New England Nuclear, Boston, Mass.
[4] Klubertanz, Edgerton, Wis.
[5] Biopette, Schwarz/Mann, Orangeburg, N.Y.
[6] Packard 2002, Packard Instrument Co., Downers Grove, Ill.
[7] Model CL, International Equipment Co., Needham Heights, Mass.
[8] Millipore, Millipore Corp., Bedford, Mass.

0.1% Fluorometholone Ointment (25 mg) Predosed with Fluorometholone Saturated Solution—This study involved a combination of the techniques described earlier for the fluorometholone saturated solution and the 0.1% fluorometholone ointment. Each run was initiated by dosing both eyes of the test animals with 50 μl of fluorometholone saturated solution. Ten minutes after instillation of the saturated solution, a 25-mg dose of 0.1% fluorometholone ointment was instilled and spread over the corneal surface as in the previous ointment study.

RESULTS

Aqueous Humor Steroid Levels following Dosing with Fluorometholone Saturated Solution—To determine the contribution of the micronized steroid particles to the aqueous humor drug levels, a study was conducted using only the saturated solution component of the suspension. These results would provide a baseline to evaluate the rate and extent to which the particles dissolve when instilled into the eye. Any observed differences in aqueous humor steroid levels between the saturated solution and the suspension could then be attributed to the particles, and the magnitude of this difference would provide some insight into their physical behavior in the eye.

The results of the study, using a 50-μl dose of saturated solution, are presented in Table I. The aqueous humor concentration *versus* time profile is depicted in Fig. 1. The results indicate that fluorometholone is a penetrating steroid which crosses the intact cornea to a significant extent within a short period following dosing, reaching a peak concentration in the aqueous humor at 30 min. The aqueous humor steroid levels then begin to decline rapidly. The apparent first-order rate constant for elimination was calculated to be 0.021 min^{-1}. A rigorous pharmacokinetic analysis of all data presented in this report will be presented in a subsequent publication.

Aqueous Humor Steroid Levels following Dosing with 0.1, 0.05, and 0.01% Fluorometholone Suspensions—Table I also presents the data from the studies using the fluorometholone suspensions. A comparison of the aqueous humor concentration *versus* time profiles obtained can be made by referring to Fig. 1. Error bars have been omitted for the sake of clarity. As in the case of the saturated solution, the peak time for each of the three suspension concentrations occurs at 30 min. However, in each case the peak aqueous humor steroid concentration was statistically higher than the baseline value obtained for the saturated solution. This finding may be attributed to the rapid dissolution and availability of small particles in the system or to protein binding of drug to the components of tears.

The 0.1% suspension maintains high aqueous humor levels for about 3 hr. Then the aqueous humor levels begin to decrease rapidly, indicating that the sustaining mechanism has lost its capacity to provide additional drug. A first-order kinetic analysis of the linear terminal portion of the curve results in an apparent elimination rate constant of 0.015 min^{-1}. This value is in good agreement with that obtained for the saturated solution.

The aqueous humor levels of fluorometholone presented in Table I for the 0.1% suspension are somewhat smaller than the values previously reported for a 0.1% suspension (19). The peak concentration at 30 min was approximately three times larger than the current value. These differences are due to the different methods of preparation of the two suspensions.

The previous work was performed using a suspension prepared in this laboratory, since a commercially prepared tritium-labeled suspension was not available. A 0.1% suspension was prepared by suspending 10 mg of micronized fluorometholone in 10 ml of the ophthalmic vehicle. An aliquot of tritium-labeled fluorometholone was then added, and the suspension was allowed to equilibrate on a shaker for several days. This method is similar to that used for the preparation of prednisolone suspensions (18). The suspension used in the current study was commercially prepared. The tritium label was first randomized by recrystallization. The steroid was then micronized to a particle size of not larger than 2.5 μm and reconstituted with ophthalmic vehicle according to the manufacturer's commercial specifications.

The results from the studies using the two suspensions demonstrate the importance of the physical properties of a suspension in regard to corneal drug penetration. While the qualitative aspects and the interpretation of the results of the previous study are not affected by these considerations, the values reported in the present study more accurately reflect the aqueous humor steroid levels achieved using a commercially available 0.1% fluorometholone suspension.

Table I—Concentration of Fluorometholone in Aqueous Humor following Topical Application of a Saturated Solution and 0.1, 0.05, and 0.01% Suspensions

t, min	Number of Eyes	Micrograms per Milliliter of Aqueous Humor
Fluorometholone Saturated Solution		
20	8	0.059 (0.003)[a]
30	6	0.077 (0.007)
60	8	0.043 (0.005)
90	9	0.017 (0.001)
120	6	0.014 (0.001)
0.1% Fluorometholone Suspension		
20	12	0.116 (0.010)
30	12	0.144 (0.016)
60	11	0.116 (0.016)
90	8	0.099 (0.010)
120	10	0.084 (0.012)
180	6	0.066 (0.010)
240	6	0.023 (0.005)
300	4	0.011 (0.001)
0.05% Fluorometholone Suspension		
20	10	0.097 (0.014)
30	8	0.120 (0.011)
60	6	0.103 (0.011)
90	8	0.085 (0.009)
120	8	0.073 (0.006)
0.01% Fluorometholone Suspension		
30	4	0.111 (0.007)
60	6	0.053 (0.006)
90	4	0.043 (0.007)
120	6	0.020 (0.003)

[a] Numbers in parentheses represent standard error of the mean.

The results of the study using the 0.05% fluorometholone suspension demonstrate that moderate dilution of the original suspension does not significantly lower the aqueous humor concentration of steroid. A statistical analysis of the data from the two studies failed to show a significant difference between the 0.1 and 0.05% suspensions. These results support the belief that higher concentrations of suspensions containing slowly soluble substances do not necessarily provide increased amounts of drug to the aqueous humor. Further work with more concentrated fluorometholone suspensions will be necessary to confirm this observed behavior over a wide range of concentrations.

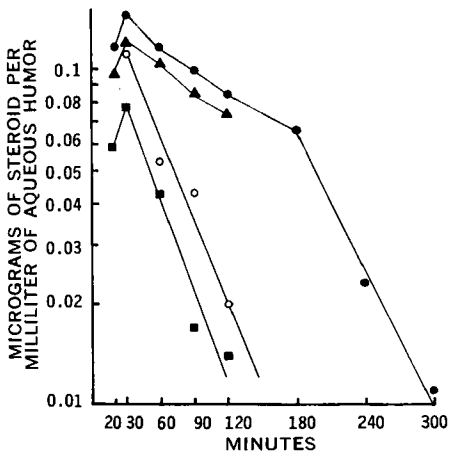

Figure 1—*Aqueous humor steroid concentrations following topical dosing with aqueous fluorometholone suspensions and saturated solution. Key:* ■, *0.05 ml of saturated solution;* ●, *0.05 ml of 0.1% suspension;* ▲, *0.05 ml of 0.05% suspension; and* ○, *0.05 ml of 0.01% suspension.*

Table II—Concentration of Fluorometholone in Aqueous Humor following Dosing with 0.05% Suspension and Rinsing with Saline at +30 min

t, min	Number of Eyes	Micrograms per Milliliter of Aqueous Humor
60	4	0.100 (0.017)[a]
90	8	0.065 (0.008)
120	6	0.043 (0.002)
180	7	0.014 (0.004)

[a] Numbers in parentheses represent standard error of the mean.

Although the experimental design for the 0.01% suspension was the same as that used for the 0.1 and 0.05% suspensions, the resulting aqueous humor concentration *versus* time profile is substantially different. The peak time and peak aqueous humor concentration are in good agreement with the two previous suspension concentrations, but Fig. 1 clearly indicates that the 0.01% suspension does not show the sustaining effect. Once the peak aqueous humor concentration is achieved, the 0.01% suspension undergoes a rapid decline in aqueous humor steroid levels. This decline is similar to that of the saturated solution and the linear terminal portion of the profile for the 0.1% suspension. First-order kinetic analysis of the curve for the 0.01% suspension gives an apparent elimination rate constant of 0.019 min^{-1}.

Aqueous Humor Steroid Levels following Dosing with 0.05% Fluorometholone Suspension and Rinsing with Saline—To confirm that the sustaining mechanism of the suspension systems is due to steroid particles retained within the cul-de-sac of the eye, a study was performed in which the eyes of the test animals were thoroughly rinsed with saline solution 30 min after instillation of 50 μl of the 0.05% fluorometholone suspension. The data obtained are presented in Table II.

The first sample point chosen for this study was 30 min after rinsing of the eyes with saline. This time corresponds to a sample time of 60 min postinstillation of the initial dose of suspension. It can be seen in Fig. 2 that the aqueous humor steroid concentration at this time point coincides with that obtained from the previous study using the 0.05% suspension. However, beyond this point the aqueous humor steroid levels begin a characteristic decline as observed in previous cases. The apparent first-order rate constant for elimination was calculated to be 0.016 min^{-1}, in good agreement with those values calculated for the 0.1 and 0.01% suspensions and the saturated solution.

Aqueous Humor Steroid Levels following Dosing with 0.1% Fluorometholone Ointment—Since studies with the aqueous fluorometholone suspensions demonstrated the importance of particle dissolution in achieving increased aqueous humor steroid levels over those produced by a simple saturated solution, it was considered worthwhile to investigate another physical parameter. Fluorometholone is a steroid with very low water solubility and strong lipophilic character. It was felt that incorporating this steroid in an oleaginous ointment vehicle would yield valuable information regarding its behavior in the eye. Partitioning of the drug from the oleaginous vehicle to the precorneal tear film would then be a potentially rate-limiting step in the corneal penetration process and would be reflected in the results of studies performed in the same manner as those using the aqueous suspensions.

Table III—Concentration of Fluorometholone in Aqueous Humor following Topical Application of 0.1% Ointment

t, min	Number of Eyes	Micrograms per Milliliter of Aqueous Humor
	50-mg Dose	
30	5	0.017 (0.007)[a]
60	4	0.065 (0.014)
120	4	0.105 (0.023)
180	4	0.136 (0.023)
240	4	0.114 (0.022)
300	4	0.106 (0.027)
360	4	0.062 (0.021)
420	4	0.026 (0.006)
480	4	0.009 (0.001)
	25-mg Dose	
120	4	0.102 (0.016)
180	4	0.146 (0.006)
240	4	0.102 (0.010)
300	4	0.053 (0.010)
360	4	0.029 (0.003)

[a] Numbers in parentheses represent standard error of the mean.

The results of the studies using two different doses of the 0.1% ointment are presented in Table III and illustrated by the curves in Fig. 3. Runs using the 50-mg dose gave a slow rise in aqueous humor steroid levels, with the peak concentration occurring at 180 min. The aqueous humor steroid concentration is then maintained at high levels for up to 360 min, giving the curve a broad bell shape in the interval from 120 to 360 min. The declining steroid levels beyond the 360-min time point were analyzed using first-order kinetics, and the linear terminal portion of the curve yielded an elimination rate constant of 0.018 min^{-1}. This value is again in accordance with the value obtained for the aqueous fluorometholone systems.

To assess the effect of smaller doses of ointment, a study was performed using a 25-mg dose of the same 0.1% fluorometholone ointment. A comparison of the aqueous humor concentration *versus* time profiles for the two doses can be made by referring to Fig. 3. The 25-mg dose produces statistically equivalent levels of aqueous steroid in the time interval from 120 to 240 min.

Aqueous Humor Steroid Levels of 0.1% Fluorometholone Ointment Predosed with Fluorometholone Saturated Solution—Since the results from the ointment studies indicated that partitioning of the lipophilic steroid from the oleaginous ointment base was limiting the rate at which peak aqueous humor steroid levels were being achieved, the effect of providing immediately available steroid to the precorneal tear film in conjunction with a dose of ointment was investigated. To effect this dosing requirement, a 50-μl dose of fluorometholone saturated solution was in-

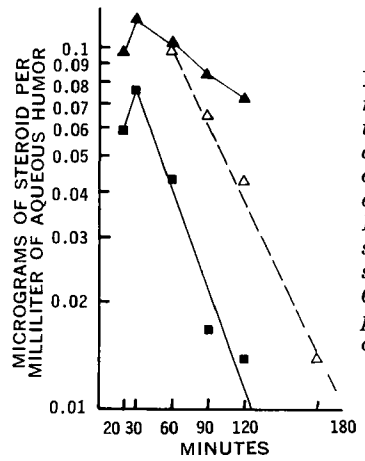

Figure 2—*Aqueous humor steroid concentrations following topical dosing with aqueous fluorometholone suspensions and saturated solution. Key: △, 0.05 ml of 0.05% suspension rinsed with saline at +30 min; ▲, 0.05 ml of 0.05% suspension; and ■, 0.05 ml of saturated solution.*

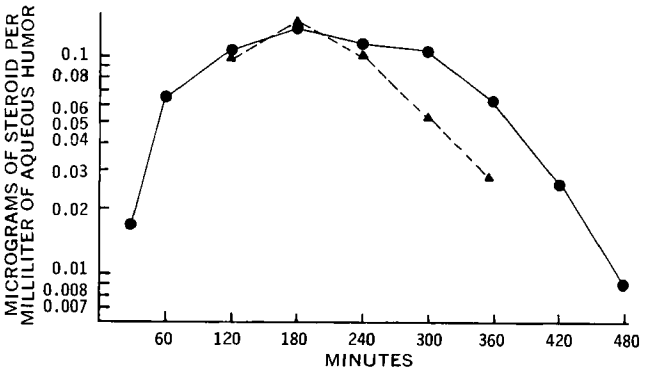

Figure 3—*Aqueous humor steroid concentrations following topical dosing with 0.1% fluorometholone ointment. Key: ●, 50-mg dose; and ▲, 25-mg dose.*

Table IV—Concentration of Fluorometholone in Aqueous Humor Using 0.1% Ointment Predosed with Fluorometholone Saturated Solution

t, min	Number of Eyes	Micrograms per Milliliter of Aqueous Humor
30	4	0.065 (0.013)[a]
60	4	0.130 (0.014)
120	4	0.100 (0.011)
180	4	0.098 (0.010)

[a] Numbers in parentheses represent the standard error of the mean.

stilled into the eyes of the test animals 10 min prior to the instillation of a 25-mg dose of the 0.1% ointment.

The results of this study are presented in Table IV. A comparison of the aqueous humor steroid concentration *versus* time profiles for this study and the two previous ointment studies can be made by referring to Fig. 4. It is clear that the immediate availability of the steroid from the saturated solution allows for a rapid rise in aqueous humor steroid levels relative to the other two ointment studies. The 30-min steroid level is significantly higher than that obtained for the nonpredosed ointments, and the peak aqueous humor steroid concentration is achieved 60 min after instillation of the dose—2 hr prior to the peak in the previous studies. The aqueous humor steroid concentration is then maintained at high levels as in the case of the nonpredosed ointment studies.

DISCUSSION

Contribution to Aqueous Humor Steroid Levels by Fluorometholone Particles in Aqueous Dosing Systems—The formulation of a drug as an aqueous suspension presumably has the advantage that the micronized particles persist within the cul-de-sac of the eye for prolonged periods. Particle dissolution then provides a sustaining source of drug to the precorneal tear film. Since fluorometholone possesses such a high degree of lipophilic character, one would not intuitively expect an aqueous dissolution rate rapid enough to provide significant amounts of additional steroid within the residence time of the particles in the eye. With this assumption, one would then expect that a suspension of this steroid would not provide significant increases in aqueous humor steroid levels over that of a simple saturated solution. In the absence of significant particle dissolution, the drug that penetrates the cornea would come only from the steroid actually in solution upon instillation of either dosing system. Since the solution surrounding any suspension of particles is in a state of saturation, the two dosing systems would be essentially equivalent in their ability to provide drug to the precorneal tear film.

The results of the suspension and saturated solution studies presented in this paper demonstrate that this is only partially correct. A comparison of the curves in Fig. 1 clearly shows that a significant amount of fluorometholone is provided by the particles in the suspensions. The aqueous humor steroid levels are statistically higher for the suspensions relative to the saturated solution. These results require an explanation as to the mechanism by which the particles are able to provide additional drug to the eye rapidly. The manner in which this dissolution process occurs can perhaps be explained on the basis of the extremely large surface areas associated with micronized suspensions. Considering the small volume of fluid actually in the eye (14) and the extensive surface area available for dissolution, it does not seem unreasonable that the suspension is able to provide additional steroid quickly.

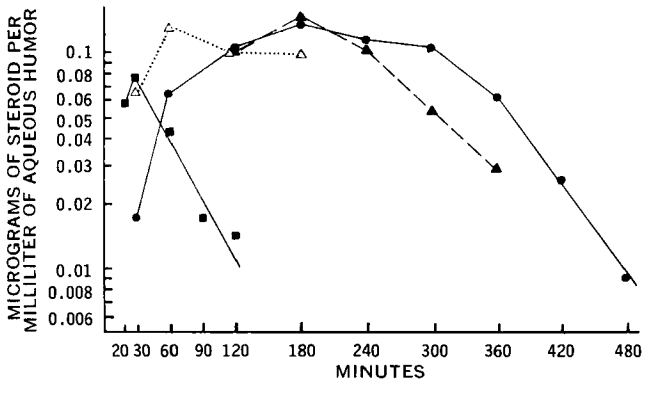

Figure 4—*Aqueous humor steroid concentrations of fluorometholone following topical dosing with 0.1% ointment and aqueous saturated solution. Key: △, 25 mg ointment predosed with 0.05 ml saturated solution; ●, 50 mg ointment; ▲, 25 mg ointment; and ■, 0.05 ml of saturated solution.*

The natural surface-active properties of the tears and the conjunctival mucin (23, 24) would also be expected to exert a favorable influence on the dissolution rate of the instilled particles. This additional steroid would then be available to enter the lipophilic epithelial layer of the cornea. Hence, one sees significant increases in aqueous humor steroid levels with the suspensions over those produced by the saturated solution, even at very short times following instillation of the doses. However, the interplay of contact time and dissolution rate is illustrated by the fact that the 0.1% suspension does not significantly improve the aqueous humor steroid levels over those produced by the 0.05% suspension.

The results obtained from the study using the saline-rinsed 0.05% suspension (Fig. 2) confirm for the first time that particles in an instilled dose of suspension are retained within the cul-de-sac of the eye. These particles are also responsible for the sustaining effect produced by the 0.1 and 0.05% suspensions. Evidence comes from the observation that the sustaining effect seen with the suspension is prematurely terminated by the rinsing process. The data for the rinsed suspension also show that steroid is provided to the aqueous humor for approximately 30 min beyond the point that the particles are removed from the cul-de-sac. This evidence strongly supports the existence of a reservoir somewhere in the cornea.

To explain the nonsustaining results from the 0.01% suspension study, it is worthwhile to note that this is an extremely dilute system and contains quite a small fraction of particles. Preliminary data from this laboratory indicate that this system contains only about 10 times the amount of steroid present in the saturated solution. A certain degree of particle contribution is evidenced by the fact that the 30-min point for the 0.01% suspension is statistically higher than that for the saturated solution. However, this contribution is not sufficient to sustain the aqueous humor levels. A reasonable explanation would be rapid removal of the small number of particles through the processes of drainage and tear turnover.

It is appropriate at this point to mention the possible influence on the results due to the method of preparation of the 0.05 and 0.01% suspensions. Since the 0.1% suspension was the only formulation available from the manufacturer at the time this study was performed, it was necessary to dilute the original suspension to produce the two lower concentrations. Although the vehicle itself was not altered in any way, subsequent equilibration after dilution was probably achieved at the expense of the smaller particles in the systems. Thus, the range of particle sizes was perhaps not consistent throughout the various suspension systems, with the lower concentrations possibly having a smaller percentage of the finer particles and a narrowed size distribution. This could have also influenced the behavior of the 0.01% suspension, since this system would have been affected the most by the dilution and equilibration process, especially if the sustaining mechanism is primarily maintained by the finer particles in the suspension.

If the method of preparation does have an effect on the results, the implication is that particle size has an influence on the aqueous humor drug levels produced by a suspension system. Current studies in this laboratory are being performed to investigate the influence of particle size on the corneal penetration of steroids and the results will be reported.

Effects of Vehicle on Aqueous Humor Levels of Fluorometholone—The epithelial layer of the cornea is highly lipophilic in character, whereas the stromal region tends to be more hydrophilic. It does not seem unreasonable to expect that a highly lipophilic steroid such as fluorometholone will tend to concentrate in the epithelial layer, perhaps even reaching a saturation concentration prior to passage through the hydrophilic layers of the cornea. Such a mechanism is not unlikely and was recently speculated upon for other steroidal compounds in a study using prednisolone acetate (9). In such a process, the rate and extent to which the epi-

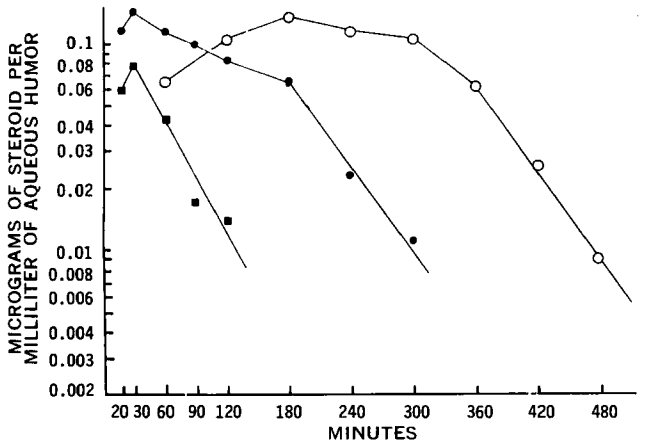

Figure 5—*Comparison of the aqueous humor steroid concentrations of fluorometholone following dosing with two aqueous dosing systems and 0.1% ointment. Key:* ■, *0.05 ml of saturated solution;* ●, *0.05 ml of 0.1% suspension; and* ○, *50-mg dose of ointment.*

thelial layer is saturated with steroid as well as the duration of this condition will influence the peak time, the peak concentration, and the maintenance of high steroid levels in the aqueous humor.

Figure 5 provides a perspective for a comparison of the results from the aqueous dosing systems and the oleaginous ointment. These results show several important aspects regarding the influence of the vehicles used in the present study:

1. The suspension does not obey strict sustained-release principles as evidenced by the absence of a true plateau in its aqueous humor concentration *versus* time profile.

2. The oleaginous vehicle causes an increase in the time required to reach peak steroid levels in the aqueous humor.

3. The area under the curve for the 0.1% ointment is considerably greater than that produced by a similar dose of the 0.1% suspension.

Upon instillation of a dose of the aqueous systems, the drug is immediately available from the steroid present in solution, as reflected by the relatively short peak time seen with these systems. For the suspensions, additional steroid becomes available through the process of particle dissolution. This process serves to maintain high levels in the aqueous humor but is not sufficient to provide a true sustaining mechanism. The ointment does sustain relatively constant levels of steroid but suffers from a delay in the time required to achieve these levels. Failure of the suspension to produce a true plateau indicates that a constant drug concentration in the tear film could not be maintained, due presumably to the slow dissolution rate of drug and/or drainage loss of drug.

The shift of the peak time seen with the ointment indicates a change in the rate-limiting mechanism. Unlike the aqueous systems, the ointment does not provide immediately available steroid. Partitioning of the steroid from the oleaginous vehicle decreases the availability of drug for a period of time following dosing. This partitioning process becomes the rate-controlling step in this case and causes a delay in the time to reach peak aqueous humor steroid levels. This delay can be easily overcome, as shown by the predosed ointment study. The dose of saturated solution compensates for the inability of the ointment to provide steroid immediately following dosing. This finding suggests that an oil-in-water or water-in-oil emulsion system might generate a better drug concentration–time profile. Furthermore, the delayed nature of the partitioning process for the ointment is reinforced by the fact that the 30-min steroid levels for the original saturated solution study and the predosed ointment study are essentially the same (Fig. 4).

Future studies will be performed in this laboratory using higher concentrations of fluorometholone ointment to investigate the influence of this parameter. Higher concentrations of steroid should provide greater amounts of steroid to the precorneal tear film (assuming it is less than saturated), and the early portion of the profile should show a steeper rise of higher levels of aqueous humor steroid at the earlier time points.

Although the peak time is extended in the case of the ointment, the duration of the aqueous humor levels is longer relative to the suspension. Increased contact time of the ointment is a possible explanation for this finding, but this cannot be stated with certainty. It is clear, however, that the area under the curve for the ointment is greater than that for the suspension. It must be concluded that the availability of fluorometholone from the ointment is greater. Therefore, it appears that for fluorometholone the greatest ocular bioavailability would be obtained by predosing the eye with a saturated solution or the 0.1% suspension followed by a dose of ointment.

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[x] To whom inquiries should be directed.