Table II—Extraction Results of I as Percentage of II

Extract	Percentage I
Cream Extracts, Ultrasonication and Mechanical Shaking	
1	0.9
2	0.5
3	0.5
4	0.5
5[a]	1.2
Cream Extracts, Manual Shaking	
1	1.0
2	0.2
3	0.4
4	0.1
5	0.1
6	0.1
7	0.1
8[b]	1.2
Cream Extracts, Sodium Sulfate	
1	1.0
2	0.3
3	0.2
Cream Extracts, Water Dispersed	
1	4.1
2	4.8
3	6.4
4	6.2
II Paste	
1	0.2
2	<0.1
3	<0.1
4	<0.1
5[c]	0.2

[a] Overnight standing of extract in contact with cream. [b] Overweekend standing of extract in contact with cream. [c] Overweekend standing of extract in contact with paste.

than to rely on a reduced value for reference response. The small amount (0.5%) of acetic acid added to the mobile phase to suppress solute ionization was not a factor and did not appear to convert any extracted II significantly.

Linearity studies performed on both I and III showed each to be completely linear within the concentration range selected, 0.0008–0.008 and 0.008–0.03 mg/ml, respectively. For I, the y intercept was −0.07, the slope was 0.996, and the correlation coefficient was 1.000. For III, these values were 1.89, 1.49, and 0.999, respectively. An amount of I equivalent to 1.5% of II was added to 1 g of sample, which then was extracted. The recovery was 97.6%.

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Mechanistic Studies on Transcorneal Permeation of Fluorometholone

JAMES W. SIEG * and JOSEPH R. ROBINSON ˣ

Received December 1, 1980, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706.* Accepted for publication February 19, 1981. *Present address: College of Pharmacy, University of Kentucky, Lexington, KY 40506.

Abstract □ The mechanism of corneal fluorometholone penetration was studied using albino rabbits, and the apparent rate and extent of steroid accumulation in the various cell layers of the cornea and aqueous humor were determined for normal and abraded eyes. The results are compared and contrasted to the mechanism previously reported for pilocarpine. Fluorometholone readily penetrates the intact corneal epithelium and accumulates in the hydrophilic stromal layers of the cornea. The kinetic profile is similar to that of pilocarpine and is largely a result of the precorneal dynamic processes. Pharmacokinetic parameters for each tissue were determined to establish an overall mechanism for corneal permeation of the steroid.

Keyphrases □ Fluorometholone—mechanistic studies on transcorneal permeation, compared with pilocarpine, rabbits □ Corneal permeation—transport mechanism, fluorometholone, compared with pilocarpine, rabbits □ Pharmacokinetics—fluorometholone compared with pilocarpine, mechanistic studies on transcorneal permeation, rabbits

The ocular penetration of steroids has been widely studied (1–8), but with emphasis primarily on quantitating specific tissue levels of drug rather than on establishing the mechanism for drug movement through the cornea. Previous work with pilocarpine defined the role of various corneal tissue layers in the permeation of a drug with both water and oil solubility (9), and the techniques can be used to study a representative steroid with low water solubility

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such as fluorometholone. This report compares and contrasts the corneal transport mechanism of fluorometholone to that established for pilocarpine.

EXPERIMENTAL

Materials—Tritiated fluorometholone[1] was prepared using catalytic exchange. The specific activity of the steroid was 1.2 Ci/mmole, and the product was purified by vacuum distillation immediately prior to use.

Male albino rabbits, 2.0–2.5 kg, were fed a regular diet with unrestricted food and water.

Preparation of Fluorometholone Solution—A sufficient quantity of the tritiated steroid was added to sterile normal saline to make a 4×10^{-5} *M* solution. The saline containing the steroid was equilibrated on a shaker bath at room temperature for 24 hr to ensure complete solution. The small amount of added tritiated steroid did not significantly alter the osmolarity of the saline solution.

Removal of Corneal Epithelium—The epithelial layer of the cornea was removed under local anesthesia to simulate an abraded eye or was harvested following death of the animals. In the first case, 2 drops of 0.5% tetracaine hydrochloride was used as a topical anesthetic prior to epithelium removal.

Aqueous Humor Drug Concentration *versus* Time Profiles—The techniques used were presented previously (1, 9). Unanesthetized animals were used, and a standard 50-μl dose was instilled with a microliter syringe. The dose was placed carefully onto the upper margin of the corneal surface and allowed to collect in the lower cul-de-sac.

At specified times following dosing, animals were sacrificed using an overdose of pentobarbital sodium. Eyes were rinsed with saline, and aqueous humor was aspirated from the anterior chamber. Aqueous humor samples (100 μl) were delivered into plastic liquid scintillation vials[2] containing refrigerated liquid scintillation counting solution[3]. The samples were counted using a scintillation spectrometer[4], and the data were converted to micrograms of steroid per milliliter of aqueous humor.

Corneal Drug Concentration *versus* Time Profiles—Cornea samples were taken immediately after aqueous humor was aspirated from the anterior chamber. Each cornea sample was rinsed with saline and blotted on tissue to remove excess water. The corneas were placed into tared combustion cones[5], and the wet weight was determined using an analytical balance. The cornea-containing combustion cone then was placed in a plastic vial[6] containing scintillation solution[7]. A tissue oxidizer[8] was used to burn each cornea, and samples were analyzed for steroid content. The final count for each sample was converted to micrograms of steroid per gram of cornea.

The only modification to this basic procedure occurred when only specific tissue layers of the cornea were monitored for steroid content; the described techniques were used.

RESULTS

Aqueous Humor Steroid Levels after Dosing with Fluorometholone Solution—Aqueous humor steroid levels were monitored for 2 hr after topical instillation of fluorometholone solution into intact eyes (Fig. 1). A peak level was attained within 30 min. A semilog plot of the data gave a log-linear decline of concentration immediately following this peak, with an associated rate constant of 0.013 min^{-1}. Further pharmacokinetic analysis yielded an apparent absorption rate constant of 0.07 min^{-1}. In addition, estimates of the area under the curve for up to 2 hr postinstillation indicated that at least 20–30% of the instilled dose ultimately reached the aqueous humor.

Corneal Steroid Concentration following Dosing with Fluorometholone Solution—The corneal fluorometholone levels for the various tissue layers of intact eyes are presented in Figs. 1 and 2. The steroid reached a peak concentration in the whole intact cornea (Fig. 1) within 5 min; this result also was reflected by the data for the corneal epithelium (Fig. 2). A semilog plot of the data for the epithelium showed that drug concentration in this tissue was biphasic with two linear elim-

[1] New England Nuclear Corp., Boston, MA 02118.
[2] Mini-Vial, Research Products International Corp.
[3] Aquasol, New England Nuclear, Boston, MA 02118.
[4] Model 2002, Packard Instrument Co.
[5] Combusto-cone, Packard Instrument Co.
[6] The Vial, Research Products International Corp.
[7] Monophase-40, Packard Instrument Co.
[8] Model 306, Packard Instrument Co.

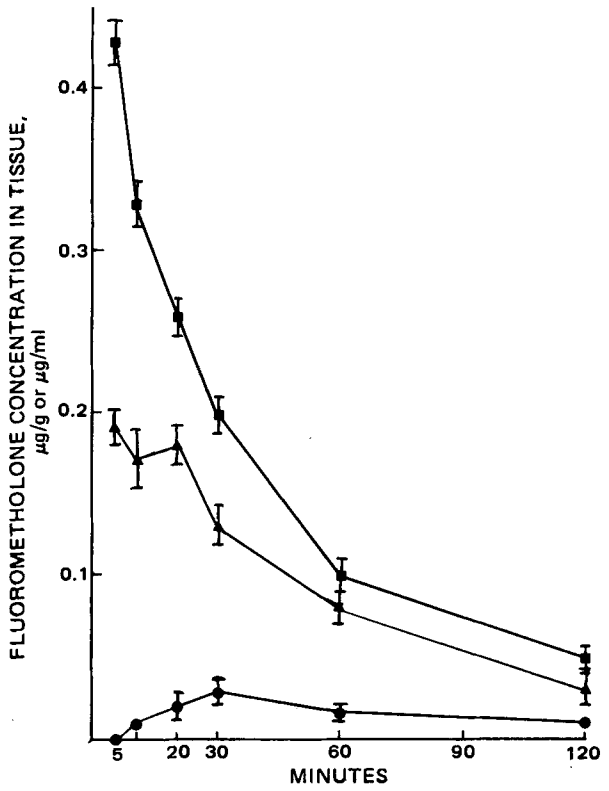

Figure 1—*Concentration of steroid in ocular tissues after dosing intact eyes with 4×10^{-5} M fluorometholone solution. Key: ●, aqueous humor; ■, intact cornea; and ▲, stroma–endothelium.*

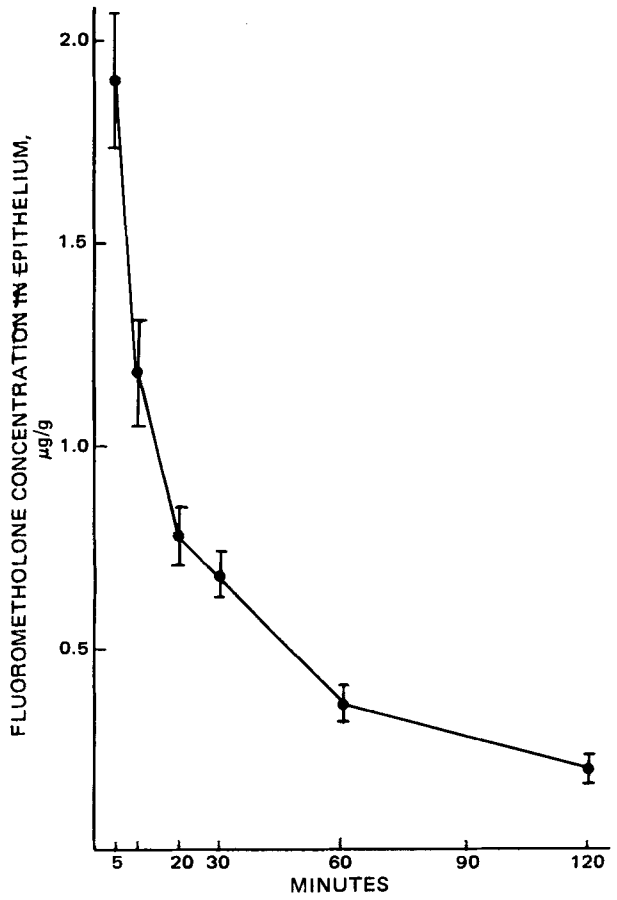

Figure 2—*Concentration of steroid in the epithelium after dosing intact eyes with 4×10^{-5} M fluorometholone solution.*

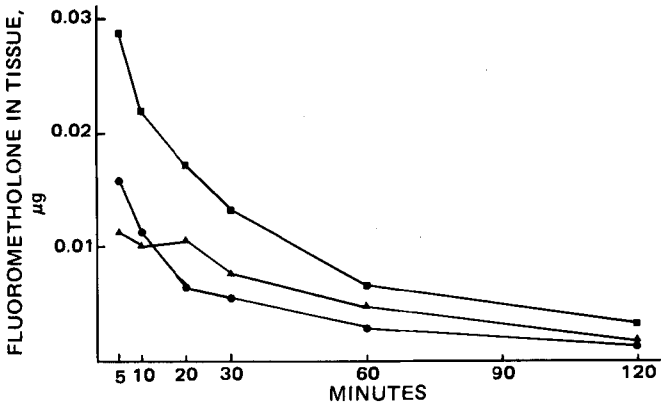

Figure 3—*Amount of steroid in ocular tissues after dosing intact eyes with 4 × 10^{-5} M fluorometholone solution. Key: ●, epithelium; ■, intact cornea; and ▲, stroma–endothelium.*

ination segments. This finding is almost identical to that previously seen with pilocarpine (9). The first-phase elimination rate constant was 0.06 min^{-1}, and the terminal phase rate constant was 0.014 min^{-1}.

The steroid also quickly reached the stroma–endothelium of the intact eye and built up to a relatively high concentration when compared with the aqueous humor levels. These levels decreased with an associated rate constant of 0.16 min^{-1} and remained higher than the respective aqueous humor concentrations at all times up to 2 hr after dosing.

The amount of steroid in each tissue was also calculated using the experimentally determined tissue weights. Data of this type often can provide a clearer understanding of drug movement through the various ocular tissues. Although drug concentration is the driving force for drug permeation through these tissues, the large differences in tissue weights and distribution volumes sometimes give a misleading representation of the actual amount of drug involved. A comparison of the various amount *versus* time profiles can be made by referring to Fig. 3. The amount of fluorometholone in the epithelium fell below that in the stroma–endothelium rather quickly, even though the epithelial concentration remained higher at all times up to 2 hr. These differences are due to the almost 10-fold difference in weights between these two tissue layers.

Ocular Penetration of Fluorometholone in Abraded Eyes—The concentration of fluorometholone in aqueous humor and the cornea following topical instillation of a 4 × 10^{-5} *M* solution into intact and abraded eyes is presented in Figs. 4 and 5. Removal of the epithelium increased the aqueous humor levels of fluorometholone by ~60%, even though the peak was shifted from 30 min in intact eyes to ~10 min in abraded eyes. In addition, the elimination rate from the aqueous humor was about twice that obtained for intact eyes, being ~0.027 min^{-1}. The net result of these effects was that the areas under the curve for intact and abraded eyes were nearly identical.

DISCUSSION

A true solution of the drug was selected as the dosing system to avoid slow absorption of undissolved drug in a suspension. Thus, the $t_{1/2}$ of fluorometholone in aqueous humor was ~54 min for intact eyes. This amount corresponds well with the turnover rate of aqueous humor, and

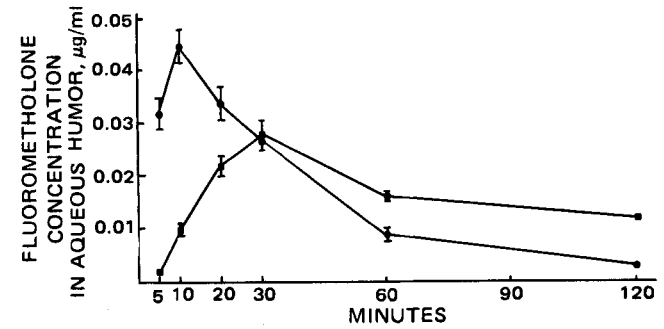

Figure 4—*Aqueous humor steroid concentration in intact (●) and abraded (○) eyes after dosing with 4 × 10^{-5} M fluorometholone solution.*

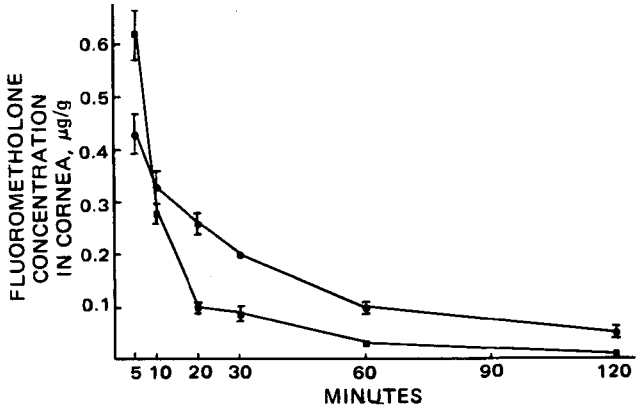

Figure 5—*Corneal steroid concentration in intact (●) and abraded (○) eyes after dosing with 4 × 10^{-5} M fluorometholone solution.*

presumably removal of fluorometholone from the anterior chamber is governed by this mechanism. However, previous corneal transport studies with fluorometholone suspensions reported a $t_{1/2}$ of ~100 min (10) because they did not extend beyond absorption of the suspension to obtain the true elimination rate constant.

The peak times for fluorometholone in the ocular tissues were similar to those previously obtained for pilocarpine (9). This observation would be somewhat puzzling when the vastly different water solubilities of these two drugs are considered. However, the effect of the parallel elimination process in the precorneal area on the observed apparent kinetic constants for ocular drug permeation already has been extensively discussed (9, 11). This parallel loss process is responsible for the apparent peak times and is essentially the same for both fluorometholone and pilocarpine.

The kinetic constants obtained for the various tissues are consistent with a mechanism consisting of a series of first-order absorption and elimination steps occurring in a succession of compartments with little or no lateral drug diffusion. The first-phase absorption rate constant calculated from the epithelium data was ~0.5 min^{-1} for fluorometholone. This value again coincides well with the apparent absorption rate constant derived from previous rinsing studies and further reinforces the premise that this apparent kinetic constant is a direct reflection of the precorneal drainage process (k_d = 0.545 min^{-1}). The first-phase corneal

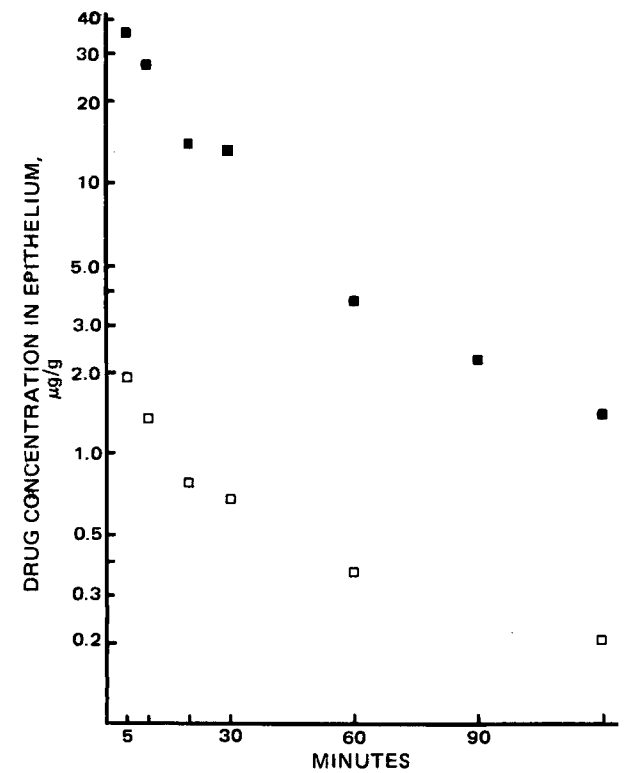

Figure 6—*Epithelial concentration* versus *time profiles for pilocarpine (▲) and fluorometholone (△).*

Table I—Percent of Total Corneal Drug Content Present in Epithelium at Various Times after Dosing with Pilocarpine and Fluorometholone Solutions

Minutes	Percent in Epithelium	
	Pilocarpine	Fluorometholone
5	92	56
10	85	52
20	68	40
30	72	42
60	67	45
120	73	44

elimination rate constant (0.06 min^{-1}) corresponds well with the apparent absorption rate constant into the aqueous humor (0.07 min^{-1}). In addition, the terminal phase elimination rate constant (0.014 min^{-1}) is almost equal to the measured aqueous humor elimination rate constant (0.013 min^{-1}).

The stroma–endothelium acts as a separate, distinct compartment from the aqueous chamber in the case of fluorometholone, unlike the situation for pilocarpine where free diffusion of drug between the aqueous humor and the stroma–endothelium occurred (9). The differences may be ascribed to possible binding of the steroid to some substance in the stroma (*e.g.*, collagen) or to an involvement of the endothelium. Owing to the oil solubility of fluorometholone, it is not unlikely that the drug may directly enter the cell membranes of the endothelium and pass *via* a transcellular route. On the other hand, being relatively fat insoluble, pilocarpine may pass directly into the anterior chamber *via* the intercellular spaces or pores. The difference in the elimination rate constants is particularly significant for the aqueous humor and cornea when comparing intact and abraded eyes. The increased elimination rate for abraded eyes suggests that the steroid leaves these tissues largely by back-diffusion into the tear film when the epithelium is not present. This effect was not apparent in the previous studies with pilocarpine. The extent of back-diffusion might argue against a protein binding mechanism for fluorometholone in the stroma in favor of a role for the endothelium.

The true absorption rate constant for pilocarpine is small, ~0.002 min^{-1} (9). The data for fluorometholone indicate a much larger true rate constant for the steroid. The initial precorneal conditions were similar for both drugs, yet the total bioavailability of fluorometholone from solution was much greater. Nearly 30% of the instilled fluorometholone dose appeared to penetrate the cornea as opposed to only ~3% of pilocarpine. This finding suggests that the true rate constant for absorption into the epithelium is about 10 times higher than that of pilocarpine, or on the order of 0.02 min^{-1}. However, this still would not change the observed apparent absorption rate constant (0.5 min^{-1}) since the overriding influence is the rate constant for drainage. Thus, even though the apparent rate constant is the sum of all rate constants in the precorneal area, the increased magnitude of k_{true} for fluorometholone is not sufficient to show up in the experimental measurement of $k_{apparent}$.

The results of the abraded eye studies (Fig. 4) show that the epithelium is not as great a barrier for fluorometholone as for pilocarpine. Thus, removal of the epithelium only increased the peak aqueous humor concentration by ~60%, whereas the observed increase with pilocarpine was a factor of 7 (9). This observation was noted previously (5) in an *in vitro* perfusion study where a three- to fourfold increase in corneal permeability was noted for water-soluble steroids and little or no increase was observed for lipid-soluble corticosteroids such as fluorometholone. This effect is also apparent when the percent of total corneal drug content present in the epithelium is calculated and compared for both drugs (Table I). Drug disposition in the cornea clearly favors the epithelium for pilocarpine, whereas fluorometholone distributes somewhat equally between the epithelium and stroma–endothelium. Previous work with pilocarpine showed the epithelium acting as both a barrier and a reservoir for pilocarpine, but this effect is less prominent with fluorometholone.

The epithelial drug concentration–time profile for fluorometholone is biphasic when plotted semilogarithmically. Similar circumstances exist for pilocarpine; in fact, the two drugs have remarkably similar profiles for this tissue (Fig. 6). Except for the absolute magnitude of the tissue concentrations, the shapes of the profiles are virtually identical. When the vastly different physical properties of fluorometholone and pilocarpine are considered, it seems likely that the explanation for this similarity is linked to some process involving the ocular tissues rather than to some specific property of both drugs.

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