In Vitro Release and *In Vivo* Penetration Studies of a Topical Steroid from Nonaqueous Vehicles

ROBERT E. DEMPSKI, JOEL B. PORTNOFF, and ARTHUR W. WASE

Abstract ☐ A method is described for studying the *in vitro* release of dexamethasone and some of its esters from gelled isopropyl myristate, petrolatum USP, and two other nonaqueous vehicles. The effect of steroid solubility in the major component of each vehicle is discussed. *In vivo* penetration of dexamethasone-^{14}C through stripped human skin showed good correlation with *in vitro* studies. No difference in the vehicles was observed with intact skin.

Keyphrases ☐ Topical vehicles—drug release, penetration ☐ Dexamethasone-^{14}C release, penetration—ointments ☐ Penetration, *in vivo*, correlation—*in vitro* release rates ☐ Vehicle solubilizing effect—dexamethasone release

In recent years many reports have been published on the *in vitro* release of drugs from topical vehicles (1–4), and on the *in vivo* penetration or absorption of drugs through human skin (5–9). A few investigators attempted to correlate selected physical properties, such as solubility or partition coefficients, of the penetrant with the rate or degree of cutaneous penetration (10–13). Generally these latter studies were conducted with drugs dissolved in specific solvents rather than incorporated in finished topical formulations. None of the papers found in the literature attempted to correlate *in vitro* release data with *in vivo* penetration studies using a practical topical vehicle.

This paper describes a method for studying the *in vitro* release of a steroid and its esters from nonaqueous vehicles, and compares the release rates with steroid solubility in the major component of each vehicle evaluated. Further studies using ^{14}C-labeled steroid show the *in vivo* penetration studies compare favorably with *in vitro* release rates.

EXPERIMENTAL

Solubility Determinations—The solubility of dexamethasone and its esters was determined in isopropyl myristate or mineral oil at 37°. These solubility parameters were used as an indicator of drug solubility in the complete formulation. This procedure was adopted because isopropyl myristate or mineral oil was the major component of each vehicle studied and also because the vehicles were semisolid at 37°.

Solubilities were determined by adding an excess of the steroid to about 100 ml. of distilled water, isopropyl myristate (cosmetic grade), or mineral oil in a 120-ml. glass bottle. The bottles were tightly capped and placed on a rotating-bottle apparatus in a 37° water bath for periods of not less than 24 hr. or more than 72 hr. Equilibrium was determined by repetitive sampling.

Before the bottles were sampled for assay, the rotating apparatus was turned off to allow the excess steroid to settle in the solvent. The liquid was filtered through a filter[1] (0.45-μ pore size) to ensure the absence of any solid particles. The filtration and sampling equipment were maintained at 37° to eliminate any variation due to temperature differences. One-milliliter samples were withdrawn from the filtrate, and diluted sufficiently with methanol when necessary, for assay. The UV absorbance of each solution was determined with a recording spectrophotometer,[2] and the steroid concentration was calculated from the absorptivity (a) for dexamethasone or its esters previously determined from standard solutions.

Preparation of Ointments—The gelled isopropyl myristate consisted of 50% isopropyl myristate, 27% Wax B white square, 22% lanolin alcohols, and 1% inorganic buffer salts. This vehicle was made by melting together all components in a suitable vessel, cooling with agitation till the entire mass congealed, and then milling to produce a smooth homogeneous product.

All other vehicles were obtained commercially.

The steroid was incorporated into each vehicle by hand levigation on an ointment tile. The dexamethasone concentration was calculated in terms of its free alcohol so that all steroid esters were present in each base in the same concentration with respect to the alcohol (0.1%).

In Vitro Release Procedure—The steroid-containing ointments were filled into 15-cm. diameter Petri dishes until the ointment was flush with the surface of the dish. To prevent the dish from floating during the studies, a thin brass weight was placed in the dish prior to adding the formulation. The entire mass was then transferred to a Pyrex crystallizing dish 9 cm. high and 17 cm. in diameter. Four hundred milliliters of distilled water was added cautiously and the supernatant liquid agitated at 60 r.p.m. after placing the entire apparatus in a 37° water bath. The agitation was only enough to mix the drug throughout the aqueous phase after its release from the vehicle; it was not enough to permit much of the base to dissolve. Samples were withdrawn for analysis at 1, 2, 4, 6, and 8 hr. and assayed spectrophotometrically for steroid content. Blanks were run to correct for interference by components of the vehicles.

In Vivo Penetration Studies—The cutaneous penetration of dexamethasone-^{14}C incorporated in gelled isopropyl myristate and petrolatum USP was observed in human beings by an external counting technique. A group of nine human volunteers with ages ranging from 23 to 46 years was included in this crossover study. Both preparations, each containing 0.1% dexamethasone equivalent to about 1 μc. radioactivity per gram, were applied to stripped skin of all subjects.

Approximately 15 mg. of each formulation (equivalent to 1.5×10^{-3} μc.) was applied to adjacent areas of stripped skin on the palmar surface of the forearm. The skin was stripped by the classical adhesive cellophane tape method until the glistening area was reached. This required between 10 and 31 strippings on each subject.

The 15 mg. of each preparation was rubbed into the stripped skin of a circular area approximately 3.18 cm. (1.25 in.) in diameter for exactly 2 min. All excess materials were then removed with tissue paper and the initial radioactivity was immediately counted (with a G-M detector and a Baird-Atomic Abacus G-M Scaler model 123). Metal collars were designed to be attached to the window end of the G-M detector tube. These collars were 2.54 cm. (1 in.) in height and maintained a constant counting distance between the tube and skin surface. A pair of collars was assigned to each subject; the collars were decontaminated between readings.

Preliminary experiments indicated best results were obtained by counting backgrounds for 10-min. periods and skin areas for 5-min. periods. The monitoring intervals were then standardized at 1, 3, 5, 24, 48, 72, and 96 post-application hours. Each treated area was protected with a vaccination shield, and each subject was instructed not to wash his forearm during the 4-day period.

[1] Millipore Filter Corp., Bedford, Mass.

[2] Cary model 14.

Table I—Solubility of Steroid Forms in Three Solvents

	Solubility (mg./100 ml.) at 37° Isopropyl Myristate	Mineral Oil (Light)	Distilled Water
Dexamethasone alcohol	23.3	0.01	11.6
Dexamethasone acetate	124.0	nil	2.7
Dexamethasone TBA	60.5	nil	1.0
Dexamethasone sodium phosphate	0.2	nil	>8500.0

RESULTS AND DISCUSSION

Solubility Data—There is always the question of which steroid form should be used in a topical vehicle. Solubility studies show the phosphate is quite soluble in water, but almost insoluble in isopropyl myristate and mineral oil. Other forms that may be useful topically include the alcohol, acetate, or tertiary butyl acetate (TBA); their solubility in three solvents at 37° are summarized in Table I. Only dexamethasone alcohol shows similar solubilities in both distilled water and isopropyl myristate even though its solubility in the latter solvent is about twofold greater.

In Vitro Release Data—The *in vitro* release rates for dexamethasone alcohol, acetate, TBA, and phosphate from gelled isopropyl myristate are illustrated in Fig. 1. The results show the release rates of both the dexamethasone alcohol and acetate are fairly rapid and essentially the same, whereas the release rates of the phosphate and TBA are very poor. On the basis of these data, either the alcohol or acetate should be a suitable candidate for *in vivo* evaluation. Since the alcohol form was released slightly faster than the acetate, the former was selected for further evaluation both *in vitro* and *in vivo*.

The release rates of dexamethasone alcohol from three other anhydrous vehicles containing petrolatum as the major component were also determined; these vehicles included petrolatum USP, hydrophilic petrolatum USP, and an anhydrous absorbant base.[3] The *in vitro* results are illustrated in Figs. 2–4, respectively. In each of these examples, the release of dexamethasone alcohol was almost nil when compared to its release from gelled isopropyl myristate. During the 8-hr. period, about nine to ninety times more steroid was released from the isopropyl myristate vehicle than from the three other vehicles tested in this study.

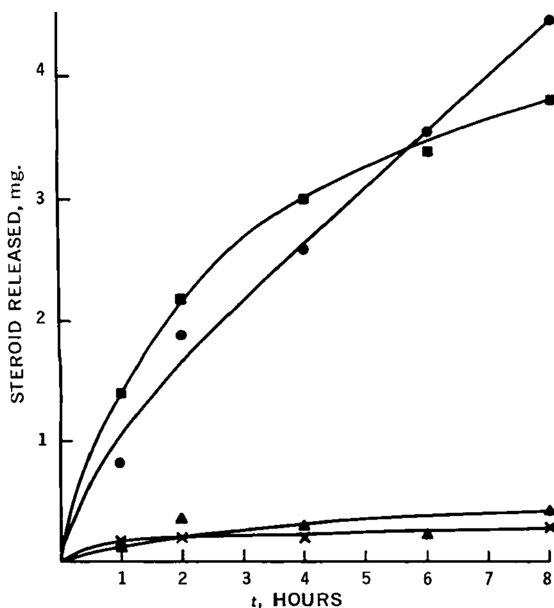

Figure 1—*Release profiles from gelled isopropyl myristate. Key:* ●, *dexamethasone alcohol;* ■, *dexamethasone acetate;* ▲, *dexamethasone phosphate;* ×, *dexamethasone TBA.*

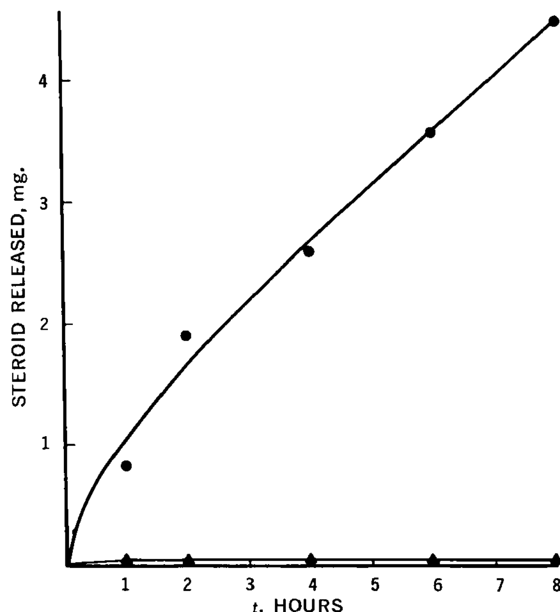

Figure 2—*Release profile of dexamethasone alcohol from gelled isopropyl myristate* (●) *and petrolatum USP* (▲).

The four vehicles employed in this study have only one characteristic in common: they are all anhydrous oily-type bases. The gelled isopropyl myristate consists of isopropyl myristate, wool alcohols, and white wax. Petrolatum, of course, contains no surfactants and will pick up very little water by itself. Both hydrophilic ointment USP and the anhydrous absorbant base are petrolatum-type bases, but they contain surfactants to make them water-absorbable; only in this respect are they similar to the isopropyl myristate gel. The presence of petrolatum as a major ingredient in these formulations may account for their comparatively poor performance as topical vehicles.

These studies show that a drug must be partially soluble in its vehicle to provide good release into an aqueous environment. This would seem to explain the dramatically superior release rate of

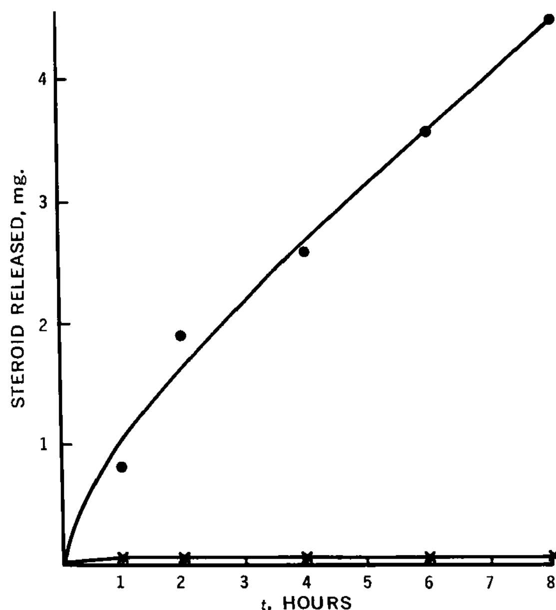

Figure 3—*Release profile of dexamethasone alcohol from gelled isopropyl myristate* (●) *and hydrophilic petrolatum USP* (×).

[3] Polysorb made by E. Fougera and Co.

dexamethasone alcohol from isopropyl myristate gel; the dexamethasone is partly soluble in this base, while it is essentially insoluble in the petrolatum-type vehicles of petrolatum USP, hydrophilic petrolatum, and the anhydrous absorbant base from which it does not release well.

If the drug is insoluble in its vehicle, it appears that only the drug particles available at the surface of the vehicle will dissolve into an aqueous medium. If the drug is partly soluble in the vehicle, it seems to dissolve and diffuse throughout the medium as it dissolves from the surface, and then returns to the surface for release into the surrounding medium.

The solubility experiments indicate dexamethasone alcohol is soluble at 37° to about 12 mg./100 ml. in distilled water and to about 23 mg. per 100 ml. in isopropyl myristate. The solubility in mineral oil, a vehicle very similar to petrolatum, is about 0.01 mg. per 100 ml. Consequently, if the steroid were incorporated into a vehicle such as mineral oil or petrolatum, in which it is insoluble, the steroid could not diffuse through the medium, and therefore could create poor release performance from the finished product. On the other hand, since dexamethasone alcohol is appreciably soluble in gelled isopropyl myristate, the steroid will saturate the base, permitting the particles to dissolve and diffuse to the surface, while maintaining a driving gradient for promoting entry into an aqueous environment.

In Vivo Penetration Data—The penetration rates of dexamethasone-^{14}C alcohol from petrolatum and gelled isopropyl myristate were determined by the decrease in radioactivity from the sites of application. The average results for the nine subjects were plotted on semilog graph paper as percent residual dexamethasone-^{14}C remaining *versus* time in hours.

Figure 5 shows the penetration rates for the two preparations tested. It is evident from the linearity of the plots that the penetration follows an apparent first-order rate. The graph further shows the presence of two slopes for the plots of log percent steroid remaining *versus* time for both preparations studied. In both cases, the initial penetration rate was greater than that obtained from the later portion of the curve.

The initial portion of the curve for both preparations shows a rapid penetration rate for the first 5 hr. However, the total penetration of the steroid using gelled isopropyl myristate as the vehicle is much greater than when petrolatum USP is used. If Fig. 5 is used to estimate the total penetration for the initial portions of the curves, the amount is about five times greater when gelled isopropyl myristate is the vehicle. Qualitatively, these results agree with the data previously illustrated in Fig. 2 which compares the *in vitro* release rates of both products. Therefore, gelled isopropyl myristate appears to exert a greater effect than petrolatum USP on the penetration of dexamethasone through stripped skin of human beings.

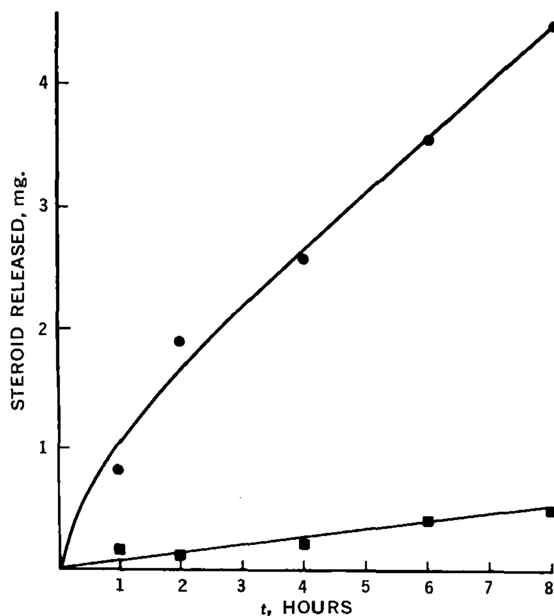

Figure 4—*Release profile of dexamethasone alcohol from gelled isopropyl myristate* (●) *and an anhydrous absorbant base* (■).

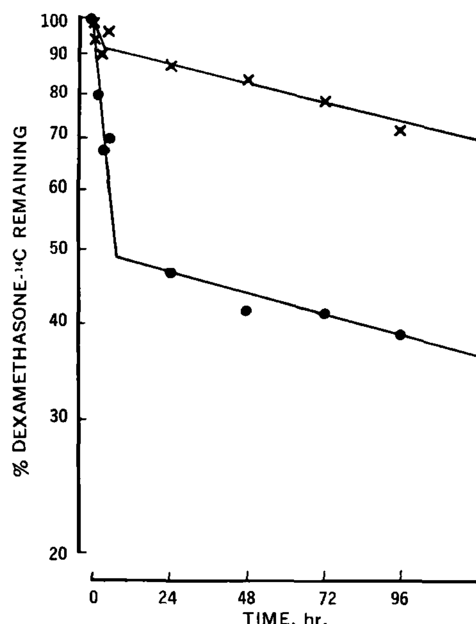

Figure 5—In vivo *penetration plots for dexamethasone-^{14}C from gelled isopropyl myristate* (●) *and petrolatum* (×) *on stripped skin.*

At some time after the 5-hr. but before the 24-hr. monitoring interval, a decrease in the penetration rate for both preparations is evident in Fig. 5. In all likelihood, this decrease in the rate of penetration was caused by regeneration of the skin barrier. On the basis of penetration studies with other steroids, Malkinson reported barrier regeneration had begun within 24 hr. after stripping and complete reconstitution required 72 hr. or more (6). Matoltsy *et al.* described similar regeneration times on stripped skin using rate of water loss as an indicator (14). Regardless of the technique employed to measure barrier regeneration time, variations can occur because of biological differences among normal subjects, the lack of a clear-cut end point in the stripping procedure, and gentle *versus* traumatic stripping (6).

It is interesting to note that after the epidermal barrier regenerated, the slopes for each curve were essentially parallel. Apparently, in this instance, the gelled isopropyl myristate had little influence on the penetration of dexamethasone through "intact" skin. Similar results on stripped skin were published by Feldmann and Maibach (5). In their studies the percent penetration and absorption was determined by measuring the urinary excretion rate of ^{14}C-hydrocortisone following topical application on intact and stripped skin. On stripped skin, initially there was a much higher excretion rate, but within 24 hr. the excretion rate tapered off and then remained essentially parallel to the rate for normal skin for the remaining 9 days of their study. Their data also indicate a rapid regeneration of the skin barrier, possibly within 24 hr.

CONCLUSIONS

These studies demonstrated that the *in vitro* release of medicinal agents from topical bases is a function of the degree of solubility of that agent in both the base and its surrounding media. The medicinal must be sufficiently soluble in a nonaqueous base to allow for its release into an aqueous medium but not so soluble to preferentially remain in that base.

In vivo data indicate correlation to the extent that the amount of dexamethasone penetrating stripped human skin over a period of time was approximately sevenfold greater from gelled isopropyl myristate than from petrolatum. The absorption patterns were apparent first-order and became essentially parallel for the petrolatum and isopropyl myristate bases after regeneration of the skin barrier.

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Investigation of Normal and Acne Skin Surface Lipids

RICHARD A. RUNKEL, DALE E. WURSTER, and GARRETT A. COOPER

Abstract □ Human skin surface lipid samples from both acne patients and normal individuals were examined with respect to both total acidity and the detailed composition of the free acid fraction. On the basis of this study, there is no apparent relationship between the total acidity of the surface lipid and the severity of the acne condition, nor is there a gross difference in the composition of the free acid fraction between the normal and the acne condition. *Corynebacterium acnes* has been cultured in a medium containing certain glycerides normally found in sebum and its esterase activity established.

Keyphrases □ Skin surface lipids—analysis □ Acne, normal skin—surface lipid acidity □ Microbiological analysis—artificial sebum □ GLC—analysis □ IR spectrophotometry—analysis

The composition of the mixed free fatty acid fraction of lipids from both the surface of human skin and the hair has been studied using a gas-liquid chromatography technique (1, 2). In 1959 Bougton *et al.* considered the possibility that the composition of the skin lipid might be different in the normal and acne conditions and, therefore, studied the total fatty acid mixture (3). They concluded that there was no important difference between the mixture of acids (free and esterified) found on normal skin and those of acne skin. The concept persisted, however, that the free acids might play a role in the inflammation associated with the acne lesion when the follicular contents are discharged into the dermis. Indeed, Strauss *et al.* indicated this to be the case (4, 5). It then became important to know whether the composition of just the free acid fraction is different in the acne state, and if so, the manner in which it differs. In this investigation the detailed composition of the free acid fraction has been studied and the results obtained with both normal and acne lipid samples compared. The relationship of the acid to ester fractions was followed with the aid of IR spectroscopy. An attempt was also made to determine if there was a relationship between the total acidity (acid number) of the lipid and the severity of the acne condition.

The presence of acne bacillus in and on the skin was established in 1911 (6). Subsequently this anaerobic bacteria was designated as *Corynebacterium acnes* (7). In 1946 Douglas and Gunter (8) suggested that it should more appropriately be called *Propionbacterium acnes.* General esterase activity on the glycerides of sebum present in and on the skin has been shown (9, 10); however, the current work shows specifically that *C. acnes* is capable of causing hydrolysis when cultured in the presence of glyceride esters.

EXPERIMENTAL

Materials and Apparatus—All of the solvents and chemicals used were analytical reagent grade. The ethyl ether was purged before using by bubbling nitrogen gas through it. The standard methyl esters were prepared from standard free acids (Eastman Organic Chemicals). The diazomethane was prepared with *N*-methyl-*N*-nitroso-para-toluenesulfonamide[1] (11). The IR spectra were run on a spectrophotometer (Beckman model I.R. 5A). The GLC work was performed on an Aerograph (model 204-B) equipped with dual columns, a dual-flame ionization detector, and a linear temperature programmer. A recorder (Leeds and Northrup Speedomax H) was also used.

The Lipid Samples—The lipid samples were obtained from human volunteers[2] having skin conditions classified as normal, mild, moderate, and severe acne. The patients were considered untreated if they had not received topical applications of any kind, or the systemic administration of antibiotics, sulfas, or steroids. The "deep" lipid was obtained 30 min. after the first collection and is assumed to be essentially the material in the follicles (12, 13). A series of collections was made and pooled for each of the four categories. Another series was also collected but here the sample from each individual was labeled and maintained separate. The

[1] Diazald, Aldrich Chemical Co., Milwaukee, Wis.
[2] The authors are indebted to Dr. Eli Packman of the Philadelphia College of Pharmacy and Sciences, Philadelphia, Pa., for providing a large portion of the lipid samples.