Concentration-Dependent Enhancement of 1-Dodecylazacycloheptan-2-one on the Percutaneous Penetration Kinetics of Triamcinolone Acetonide

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Received October 7, 1983, from the *Department of Pharmaceutics, College of Pharmacy, University of Houston, Houston, TX 77004.* Accepted for publication December 5, 1983.

Abstract □ The enhancing effect of 1-dodecylazacycloheptan-2-one (I) on the penetration kinetics of triamcinolone acetonide (II) and the possible mechanisms of enhancement were studied using nonreinforced and reinforced silicone elastomer membranes and full-thickness hairless mouse skin as penetration barriers. Lactam I, at test concentrations of 0.1–10%, significantly promoted the extent and the rate of penetration of the acetonide II. Regardless of the barrier used the effect was concentration dependent, but the penetration profiles were different. The enhancement with synthetic membranes was attributed solely to the effect of lactam I doubling the diffusion constant (D), which resulted in the increase of the permeability coefficient (K_p) and the shortening of the lag time (τ); the partition coefficient (K_m), however, was not affected. With mouse skin, I exerted effects on both D and K_m. Compound I potentiated the retention of II in skin (a reservoir effect) about sevenfold; however, the enhancing reservoir effect was independent of the concentration of I. Pretreatment with I was found to be more efficient than the coapplication of I and II in the vehicle. The coexistence of I and II was not required for the enhancement, and the reduced enhancing efficiency suggested an interaction between I and II in the vehicle. Penetration impedance was observed after exposure to I with skin, but not with synthetic membranes. Interaction of I with the skin component, or the coexistence of I and II in the skin, could be responsible for such an impedance.

Keyphrases □ Penetration kinetics—triamcinolone acetonide, influence of 1-dodecylazacycloheptan-2-one, concentration dependence □ Mechanisms of enhancement—reservoir effect, diffusion constant, partition coefficient, promotion of skin hydration □ 1-Dodecylazacycloheptan-2-one—influence on penetration kinetics of triamcinolone acetonide, concentration dependence

1-Dodecylazacycloheptan-2-one[1] (I) enhances the biological activity of various topically active agents (antibiotics and antifungals, glucocorticoids, and antipsoriatic agents) using corresponding bioassays, the zonal inhibition of microorganism growth, vasoconstriction, and lesion clearance, respectively (1, 2). The enhancing effect of I was observed with concentrations as low as 1% (1, 2), which is much lower than that required with other enhancers, such as urea, dimethyl sulfoxide, and *N*,*N*-dimethylformamide. In addition, lactam I causes practically no irritation to human skin even at concentrations >50% (2). In the light of these advantages, this substance is a promising new enhancer for topical pharmaceutical formulations.

Therapeutic activity of a topical agent is known to be the result of drug release from the dosage form, penetration of released molecules through skin barrier, and intrinsic activity of the penetrating agent at the target sites (3). The reported enhancement may be due to the I effect on any one or combination of the three components. A few studies have attempted to evaluate the I effect on the individual processes. Using triamcinolone acetonide (1), methanol (4), 8-bromoadenosine 3′,5′-hydrogenphosphate (8-bromo-cAMP; III)[2], 9-β-D-arabinofuranosyladenine (vidarabine, ara-A; IV) (5), arabinofuranosyladenine 5′-valerate (ara-A-5′-valerate; V) (6), 5-fluorouracil, indomethacin, and hydroquinone (2), the enhancing effects of I have been demonstrated to be on the penetration process, with (6) or without the mechanism of drug release. Stoughton (1) reported that percutaneous penetration of triamcinolone acetonide (II) in the presence of 10% I (within 7 and 24 h) was 10- and 5-fold higher than that without I. Greater enhancement was observed with 5-fluorouracil and indomethacin at I concentrations of 1.8–45%, yet only a two- to threefold increase was observed with hydroquinone at 2 and 5% I (2). Stoughton also found that in the case of III, the same level of penetration enhancement induced by 1% I was barely achievable with 50% dimethylformamide[2].

Behl *et al.* (4) demonstrated that the permeability of methanol was promoted 200–400-fold by I (asymptotically with I concentrations) with maximal enhancement at 10–25% I. Vaidyanathan *et al.* (5) showed that I enhanced the delivery of IV with systematic increase in the presence of 0.1–3% I. The permeability of IV with 3% I was 1.4×10^{-7} cm/s, 10-fold greater than the control. The absolute amount that penetrated at 24 h was a 170-fold increase over the control value with 3% I and a 42-fold increase with 0.1%. Vaidyanathan *et al.* (6) also reported that 20% I promoted the total fluxes of IV and V from a suspension with emulsified I or from gel formulation, 20-fold greater than those found using dimethyl sulfoxide as the vehicle. Nevertheless, no complete profile of the penetration due to I has been established. In addition, the mechanisms of such enhanced penetrations have not been elucidated in terms of the modifications of I on the basic parameters of percutaneous penetration kinetics [other than the permeability coefficient (5)]: lag time, diffusion constant, and partition coefficient.

Penetration enhancement appeared to be dependent on the concentration of I. The extent of II penetration increased with increasing I up to 10%; further increase of I (40%) diminished the extent of enhancement (1). The highest level of enhancement on 5-fluorouracil penetration was achieved with 1.8% I among test cencentrations, 1.8, 9, and 45%. With indomethacin as a penetrant, 2% I exerted an effect similar to that of 8%; both were higher than that of 24%. Similarly, 2% I had a greater effect on hydroquinone penetration than 5% I (2). Optimal enhancement was also observed with methanol (4) at 10–25% I. The enhancing effect of I on biological activity, determined either by the vasoconstriction assay or other bioassays, seemed to be concentration dependent as well (1, 2), and the pattern of such a dependence also seemed to vary with test compounds. For instance, erythromycin and clindamycin had increasing antibacterial effect with increasing I, while glucocorticoids and fusidic acid gave the greatest biological effect with the lowest examined concentrations of I (1–3%). Therefore, we attempted to characterize such a dependence and to explore its possible mechanism.

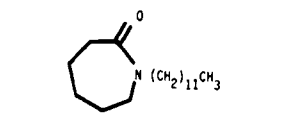
One of the most frequently prescribed topical glucocorti-

[1] Azone; Nelson Research and Development Co., Irvine, Calif.
[2] R. B. Stoughton, unpublished data, personal communication, 1981.

0022-3549/84/1200-1794$01.00/0

coids, II (7), was selected for the study. Unlike antifungal and other antimicrobial agents, glucocorticoids exert pharmacological effects only after the agents have penetrated through the skin barrier and reached the target cells in the epidermis and dermis. In regard to therapeutic efficacy, bioavailability of the active ingredient at the site of target cells is of more concern than the concentration of the drug in the dosage form. The importance of the bioavailability of glucocorticoids in various topical drug-delivery systems has received attention from both the pharmaceutical and medical communities during the past decade. However, vehicles in use are still inefficient or only marginally effective in providing satisfactory bioavailability (8, 9). Maibach studied the percutaneous penetration of radioactively labeled hydrocortisone in 18 subjects at a dose of 4 $\mu g/cm^2$ and found that only ~1% of the dose was actually absorbed from the forearm of a normal subject (10). This problem of low bioavailability of glucocorticoids called for an urgent need to develop more efficient systems for delivering this therapeutically useful agent.

This study is intended to evaluate the penetration enhancement of II by I (in terms of the modifications of I on the individual penetration parameters) and to elucidate the possible mechanisms of concentration-dependent effects of I using an *in vitro* penetration study with excised full-thickness hairless mouse skin. In addition, two synthetic, nonreinforced and reinforced, silicone elastomer membranes were evaluated for use as artificial skin models.



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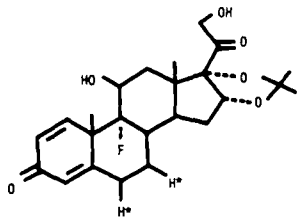

II

EXPERIMENTAL SECTION

Materials—Compound I[3] was used as supplied. Tritiated II[4] in benzene-ethanol (9:1), with a specific activity of 37.0 Ci/mmol, was used as the penetrant. Test solutions of II [3.01×10^{-10} mol/mL in propylene glycol[5] (11)], with or without 0.1, 0.5, 1, 2, 3, 4, 5, 8, and 10% (v/v) I were prepared with methanol[6] as an intermediate solvent (12).

Skin Preparation and Synthetic Membranes—Skin preparations from homozygous Hr/Hr hairless mice (HRS/J strain)[7] (age, 2–4 months) were used. The skin of all animals appeared normal and was free of bites, scratches, or bruises. The mice were sacrificed by ether inhalation, and the skin was dissected. Full-thickness skin was used for the penetration study. Tritiated water[8] served as a standard penetrant to check the intactness of the skin preparation. The thickness of the skin preparation was measured with a micrometer[9]. Membranes of silicone elastomer[10], nonreinforced and reinforced with plain weave fabric of polyethylene terephthalates, were 127.0 and 177.8 μm thick, respectively.

***In Vitro* Penetration Study**—The skin sample or synthetic membrane was mounted between the donor and receptor chambers of the Franz diffusion cell[11] (5 mL) with a 9 mm i.d. O-ring flange. The stratum corneum was exposed to ambient conditions, and the dermal side was oriented toward the saline-phosphate buffer (pH 7.4) (13), simulating the physiological pH of the dermis [pH 7.3–7.4 (14)], in the receptor chamber.

Prior to the application of the test sample, the solution in the receptor chamber was equilibrated by circulating water through a jacket surrounding the chamber at 32°C, which was chosen to reflect the temperature of the stratum corneum (15). The solution in the receptor chamber was stirred with a polytef-coated magnetic bar driven by a magnetic stirrer. A sample (0.2 mL) of II test solution, with or without I, was spread across the exposed stratum corneum surface of the mounted skin. Aliquots of saline-phosphate buffer containing the penetrated II (0.2 mL) were withdrawn from the side-arm of the receptor chamber; this volume was replaced with fresh buffer at 32°C. Samples were taken every 30 min for the initial 2 h and hourly thereafter to 12 h. The amount of II penetrated was measured by liquid scintillation counting of the withdrawn diffusate (0.2 mL in 4 mL of xylene-based cocktail[12]).

The reservoir effect of the skin to retain II was examined after the 12-h penetration study. The exposed skin was blotted dry, dissolved in 1 mL of quaternary ammonium hydroxide tissue solubilizer[13] at 55°C for 2 h, and decolorized with 0.1 mL of 30% hydrogen peroxide[14] for 30 min. The radioactivity in the solubilized skin was monitored by liquid scintillation counting in 10 mL of toluene-based cocktail[15]. The background counts and quenching effects of blank diffusate aliquot and solubilized skin were negligible. No quench correction was employed.

Data Analysis (16, 17)—The penetration parameters of II [lag time (τ), permeability coefficient through the membrane (K_p), diffusion constant within the membrane (D), and partition coefficient between the membrane and the vehicle (K_m)] were calculated from the penetration data. The penetration profiles were constructed by plotting the total amount of II penetrated *versus* time. The x-intercept of the extrapolated linear region of the curve gives τ. D was calculated from τ with known thickness of the penetration barrier (δ), using Eq. 1. The slope of the linear portion of the profile, determined by linear regression analysis, was J_s. K_p was calculated by dividing J_s by the employed concentration of II, C_s (Eq. 2), and K_m was calculated with Eq. 3:

$$\tau = \delta^2/6D \qquad \text{(Eq. 1)}$$

$$J_s = (K_m \cdot D \cdot C_s)/\delta = K_p C_s \qquad \text{(Eq. 2)}$$

$$K_p = (K_m \cdot D)/\delta \qquad \text{(Eq. 3)}$$

The significance of the difference in parameter values among groups with and without I was tested by a nonpaired Student's t test.

RESULTS AND DISCUSSION

Effects of I on Penetration Profiles and Kinetic Parameters of II—The penetration profiles of II, with and without I, through the nonreinforced and reinforced silicone elastomer membrane and full-thickness hairless mouse skin for 12 h, are shown in Figs. 1, 2, and 3, respectively. Regardless of the type of membrane used, both the extent and the rate of II penetration through the membrane were significantly enhanced with I. The penetration profiles through synthetic membranes always consisted of a lag phase followed by the linear rise. This profile was obtained with hairless mouse skin only when the concentration of I was ≤2% (Fig. 3). With I > 2%, the steady-state penetration was somewhat impeded after 8 h of exposure to I, as reflected by the plateau in the profile. The impedance was noticed only with the skin; this cautioned the use of synthetic membranes as artificial skin models for studies of I.

The percentage of II penetration at 12 h through synthetic membranes increased from two- to fourfold the control value with increasing concentrations of I (0.1, 2, and 10%, Table I). A different concentration dependence was observed with hairless mouse skin (Table II). Compound I effectively

[3] Azone, lot 0486K0610; kindly supplied by Nelson Research and Development Co.
[4] Lot 1227-060 and 14611-204; New England Nuclear, Boston, Mass.
[5] Lot A-6M04; MCB, Norwood, Ohio.
[6] OmniSolv, glass distilled; MCB, Gibbstown, N.J.
[7] Jackson Laboratory, Bar Harbor, Me.
[8] Lot 272-980, 2.16×10^6 dpm/mL; New England Nuclear, Boston, Mass.
[9] Hi-precision, Model 221; the Starrett Co., Athol, Mass.
[10] Silastic sheets, Lot H049156 (nonreinforced) and H021446 (reinforced); Dow Corning Co., Midland, Mich.
[11] FDC 108 series with FDC 128 manifold and FDC-127 magnetic bar; Crown Glass Co., Somerville, N.J.
[12] Aquasol-2, 079AT1; New England Nuclear.
[13] Protosol, 092PR1; New England Nuclear.
[14] Analytical Reagent, Lot KJGP; Mallinckrodt Inc., Paris, Ky.
[15] Econofluor, 231EC1; New England Nuclear.

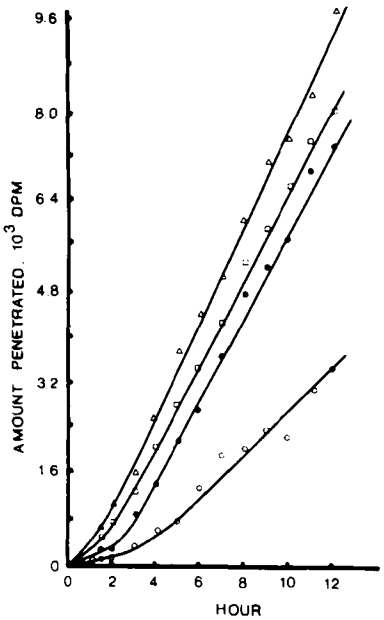

Figure 1—*Penetration profiles of II through nonreinforced silicone elastomer membrane with various concentrations of I. Key: (○) 0%; (●) 0.1%; (□) 2%; (△) 10%.*

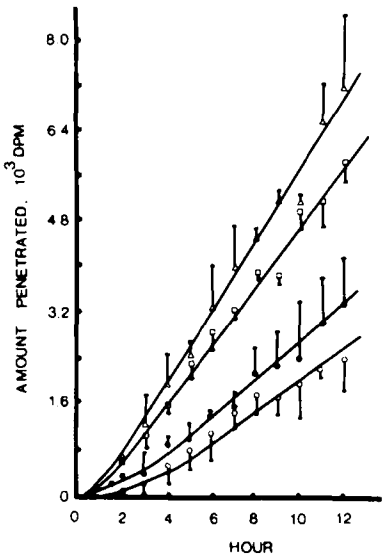

Figure 2—*Penetration profiles of II through reinforced silicone elastomer membrane with various concentrations of I. Key: (○) 0%; (●) 0.1%; (□) 2%; (△) 10%, (n = 3 each).*

elevated the percent of II penetrated through the skin with concentrations >0.5% ($p < 0.02$). Penetration increased 8-, 28-, and 124-fold over that observed without I, at 0.5, 1, and 2-3% I, respectively. However, increased concentrations of I (4-10%) reduced the promotion ($p < 0.005$, except $p < 0.1$ with 5% I) to 76-98-fold the control value (*i.e.*, only 61-79% of the maximum enhancement achieved at 2-3% I). This was a result of late-stage penetration impedance.

The steady-state penetration rate of II (J_s) through nonreinforced silicone elastomer membrane was doubled with as low as 0.1% I, but increases to 2 and 10% did not further promote the penetration rate. Similar results were observed with reinforced membrane, except that the effective concentration was >0.1% (Table I). The J_s value of II through the hairless mouse skin was also concentration dependent (Table II). The rate was 3.38×10^{-18} mol/cm^2/s without I, doubled with 0.1% I, and drastically increased 14-, 46-, and 138-fold with 0.5, 1, and 2-3% I, respectively. Increasing I to 4% reduced the effect to ~66% of the observed maximal enhancement (4.67×10^{-16} mol/cm^2/s) with 2-3% I) or ~90-fold greater than the control penetration rate. Relatively constant penetration rates ($3.05\text{-}3.38 \times 10^{-16}$ mol/cm^2/s) were obtained with I concentrations of 4-10% (Table II).

The effect of I on the gross penetration process can be explained by individual parameters of penetration kinetics as shown in Tables I and II. With synthetic membranes τ values were not appreciably altered with 0.1% I, but were shortened to about one-half with I concentrations of 2 and 10%. K_p values similarly increased with J_s values as a function of I concentrations. Since K_m values were not significantly affected by test concentrations of I, the increase in K_p and reduction in τ were due solely to the I effect of increasing D in synthetic membranes.

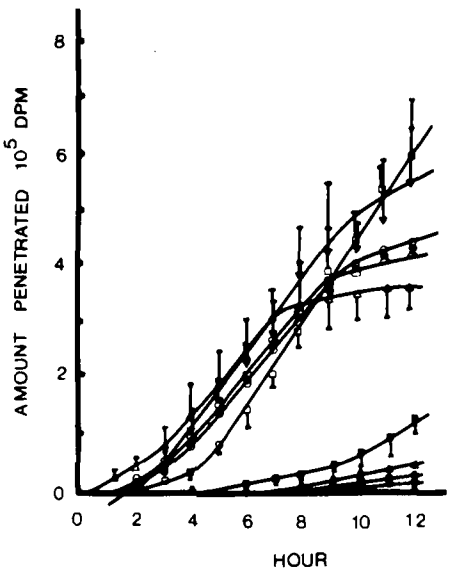

Figure 3—*Penetration profiles of II through full-thickness hairless mouse skin with various concentrations of I. Key: (○) 0%, n = 6; (●) 0.1%, n = 3; (▲) 0.5%, n = 1; (■) 1%, n = 4; (□) 2%, n = 6; (▼) 3%, n = 2; (●) 4%, n = 4; (○) 5%, n = 4; (◯) 8%, n = 8; (△) 10%, n = 7.*

Table I—Percutaneous Penetration Parameters of Triamcinolone Acetonide Through Silicone Elastomer Membranes with Various Concentrations of 1-Dodecylazacycloheptan-2-one (I)

I, %	Percent Penetrated at 12 h	Penetration Rate (J_s), 10^{-18} mol/cm^2/s[a]	Lag Time (τ), h	Permeability Coefficient, (K_p), 10^{-8} cm/s[b]	Diffusion Constant (D), 10^{-9} cm^2/s[c]	Partition Coefficient (K_m), 10^{-2} [d]
			Nonreinforced Membrane			
0	0.04	1.97	2.27	0.73	3.29	2.82
0.1	0.07	4.32	2.04	1.60	3.66	5.55
2.0	0.12	4.42	1.23	1.63	6.07	3.43
10.0	0.14	5.02	1.06	1.86	7.04	3.35
			Reinforced Membrane			
0	0.06	1.41	1.96	0.53	0.75	1.24
0.1	0.15	1.87	1.82	0.69	0.80	1.53
2.0	0.16	3.08	0.98	1.14	1.49	1.36
10.0	0.20	3.72	1.09	1.38	1.34	1.83

[a] J_s = slope/(A × 3600 × specific activity × 10^6 × 2.2 × 10^6), where slope was in the unit of dpm/h, A = 0.636 cm^2, and specific activity of tritiated II was 37 Ci/mmol. [b] $K_p = J_s/C_s$, where $C_s = 3.01 \times 10^{-10}$ mol/mL. [c] $D = \delta^2/6\tau$, where δ was 127 μm (0.005″) as reported by the manufacturer for nonreinforced membrane and δ was 177.8 μm (0.007″) for reinforced membrane. [d] $K_m = K_p\delta/D$.

Table II—Percutaneous Penetration Parameters of Triamcinolone Acetonide Through Full-Thickness Hairless Mouse Skin with Various Concentrations of 1-Dodecylazacycloheptan-2-one (I)

I, %	n	Percent Penetrated at 12 h	Penetration Rate (J_s), 10^{-16} mol/cm^2/s[a]	Lag Time (τ), h	Permeability Coefficient (K_p), 10^{-6} cm/s[b]	Diffusion Constant (D), 10^{-8} cm^2/s[c]	Partition Coefficient (K_m)[d]	Adjusted Diffusion Constant (D'), 10^{-10} cm^2/s[c]	Adjusted Partition Coefficient (K_m')[d]
0	6	0.11 ± 0.05	0.04	>12	0.01	<1.25	0.06-0.46	<0.62	0.81-6.57
0.1	3	0.18 ± 0.04	0.07	>12	0.03	<1.25	0.12-0.46	<0.62	1.65-6.57
0.5	1	0.88	0.46	7.20	0.17	2.09	0.46	1.03	6.57
1.0	4	3.14 ± 1.12	1.56	7.46	0.58	2.02	1.64	0.99	23.43
2.0	6	13.57 ± 1.22	4.65	4.39	1.72	3.43	2.86	1.69	40.71
3.0	2	12.59 ± 3.56	4.67	3.11	1.73	4.84	2.04	2.38	29.08
4.0	4	10.75 ± 0.73	3.07	2.08	1.14	7.23	0.90	3.56	12.81
5.0	4	10.27 ± 4.07	3.14	2.13	1.16	7.06	0.94	3.48	13.33
8.0	8	9.54 ± 1.85	3.38	2.43	1.25	6.18	1.15	3.04	16.45
10.0	7	8.32 ± 0.09	3.05	1.48	1.13	10.20	0.63	5.01	9.03

[a] J_s = slope/(A × 3600 × specific activity × 10^6 × 2.2 × 10^6), where slope was in the unit of dpm/h, A = 0.636 cm^2, and specific activity of tritiated II was 37 Ci/mmol. [b] $K_p = J_s/C_s$, where $C_s = 3.01 \times 10^{-10}$ mol/mL. [c] $D = \delta^2/6\tau$, where δ was measured as 570 μm for full-thickness skin, and D' was similarly calculated with δ estimated as 40 μm for hydrated stratum corneum. [d] $K_m = K_p\delta/D$, and K_m' was similarly calculated with D'.

With hairless mouse skin, τ values were significantly shortened from >12 h to 7.20, 4.39, and 1.48 h by increasing I from 0 to 0.5, 2, and 10%, respectively (Table II). K_p values gradually increased from 1.25×10^{-8} cm/s to a maximum of 1.73×10^{-6} cm/s (a 138-fold increase over the control value) by increasing I to 2-3%. Values then declined with I ≥4%, leveling off at 66% of maximum ($1.13-1.25 \times 10^{-6}$ cm/s, Table II). D and K_m values were calculated first with the measured full-thickness of the skin (570 ± 104 μm, n = 15). However, since the major penetration barrier of the skin is in the stratum corneum (18, 19), the derivations of D and K_m, considering the skin a uniform and invariant cellular matrix, might have overestimated D values, yet underestimated K_m values. A second attempt was therefore made to estimate D' and K_m', using the literature thickness value (20) for fully hydrated stratum corneum (40 μm). The D' value thus obtained with no I ($< 6.2 \times 10^{-11}$ cm^2/s) was similar to the literature values for steroids [ranging from 2×10^{-11} for progesterone to 3×10^{-13} cm^2/s for hydrocortisone (21)]. The K_m' value obtained without I, 0.81-6.57, was comparable to the literature value of 5 for hydrocortisone (19).

Unlike the cases with synthetic membranes, I exerted effects on both D' and K_m' (Table II). D' was significantly promoted with I at as low as 0.5%; the extent of enhancement increased with increased I concentrations. On the other hand, K_m' increased with increasing I up to a maximum of sevenfold greater than the control value with 2% I while further increase in I from 3 to 10% reduced the enhancement from 67 to 21% of the maximum. The reduction in τ was a result of promoting D', whereas the increase in K_p was the net activity of I effects on D' and K_m'. With ≤2% I, both parameters were increased with increasing I; however, with I concentrations of ≥3%, K_m' dwindled from the maximum value, offsetting the I promoting effect on D'. The less favored partitions of II to stratum corneum with 3-10% I in vehicle, as compared with that at 2% I, dominated other effects of I and accounted for the decline in K_p at these high concentrations of I.

***In Vitro* Pretreatment of Hairless Mouse Skin with I**—To determine whether the coexistence of I and II in the vehicle is essential for penetration enhancement and impedance, the excised hairless mouse skin was pretreated *in vitro* with I prior to the penetration of II (instead of coapplication of I and II). Compound I (0.2 mL) in propylene glycol (2% or 10%), was applied on the skin mounted on the diffusion cell for 8 h. After the removal of I, 0.2 mL of II in propylene glycol without I was applied and the penetration profile was monitored. Controls were samples with pretreatments of 0.2 mL of propylene glycol for 8 h, followed by 0.2 mL of II with 0, 2, 4, 5, and 10% I.

The penetration profiles of II with I pretreatment in propylene glycol or with propylene glycol alone were established (Fig. 4), and the penetration parameters were evaluated (Table III). The 8 h contact of skin with propylene glycol, a skin-hydrating solvent, did not appreciably alter, or only marginally increased, the percent penetrated and K_p of II ($p > 0.2$, except for 0% I which had a significant increase, $p < 0.025$). Pretreatment of the skin with 2 or 10% I in propylene glycol, on the other hand, significantly augmented the I enhancements on the extent (percent penetrated), rate (J_s), and K_p of the penetration of II ($0.05 < p < 0.1$). [Compare the paired values C and B (obtained with the skin pretreatment of propylene glycol) or C and A (obtained without any skin pretreatment) in Table III.] The τ values were drastically shortened to 0.7 h after pretreatment with 2 or 10% I, as a result of the substantial promotion of D' within the skin by an 8-h pretreatment. Two percent I was as effective as 10% for pretreatment in increasing D', and significantly more effective than 10% ($p < 0.001$) in enhancing other parameters; both 2 and 10% pretreatments were more effective ($0.05 < p < 0.1$) than the coexistence of II and I of corresponding concentrations in the vehicle (Table III). This observation suggested that the penetration enhancement did not require the coexistence of I and II in the vehicle. Actually, penetration was more efficiently promoted with pretreatment of I than with coapplication of I and II, suggesting a possible interaction between I and II in the vehicle (unfavorable for the penetration process at the I concentrations tested). K_m values were low and of the magnitude obtained with coexisting 0.5% I; therefore, the great extent of penetration enhancement with pretreatment was mainly due to the D' promotion by I.

In addition, the previously reported penetration impedance of II with ≥3% I, still occurred with pretreatment of 10% I, and a similar onset of the impedance (~8 h) was observed (Fig. 4). With the pretreatment of 2% I, no plateau of the penetration profile (similar to that with coexisting 2% I) was evident after 12 h of penetration (Fig. 4). These observations suggested that 8-h skin exposure to I alone was unable to induce the penetration impedance; otherwise the impedance should have been observed from the initiation of the penetration study after pretreatment. Pretreatment of I, without the coexistence of I and II in the vehicle, could still bring about the impedance, again at 8 h after penetration began. The observations that coexistence in the vehicle (and consequently the simultaneous penetration of I and II) was not essential, yet I alone could not cause the impedance, suggested that the coexistence of II and a sufficient amount of I in the reservoir site (probably the stratum corneum of the skin) might be responsible for the observed penetration impedance by an as yet undefined mechanism.

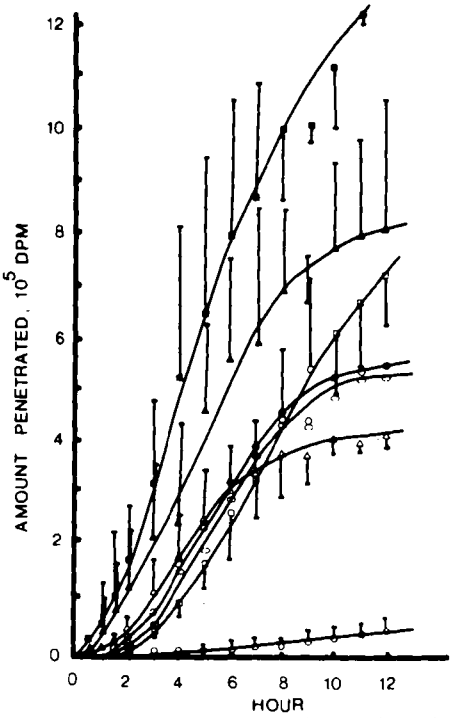

Figure 4—*Penetration profiles of II through full-thickness hairless mouse skin pretreated with I or propylene glycol for 8 h. Key: open symbols, pretreated with propylene glycol; (○) 0% I, n = 2; (□) 2% I, n = 2; (◯) 4% I, n = 1; (○) 5% I, n = 1; (△) 10% I, n = 2. Solid symbols, pretreated with I: (■) 2%, n = 3; (▲) 10%, n = 10.*

Table III—Effect of Pretreatments on Percutaneous Penetration Parameters of Triamcinolone Acetonide Through Full-Thickness Hairless Mouse Skin

1-Dodecyl-azacyclo-heptan-2-one, %	Pre-treatment[a]	n	Percent Penetrated at 12 h	Penetration Rate (J_s), 10^{-16} mol/cm^2/s[b]	Lag Time (τ), h	Permability Coefficient (K_p), 10^{-6} cm/s[c]	Diffusion Constant (D), 10^{-8} cm^2/s[d]	Partition Coefficient (K_m)[e]	Adjusted Diffusion Constant (D'), 10^{-10} cm^2/s[d]	Adjusted Partition Coefficient (K_m')[e]
0	A	6	0.11 ± 0.05	0.04	>12	0.01	<1.25	0.06-0.46	<0.62	0.81-6.57
	B	2	1.06 ± 0.73	0.33	>7.20	0.12	<2.09	~0.33	<1.03	~4.74
2	A	6	13.57 ± 1.22	4.65	4.39	1.72	3.43	2.86	1.69	40.71
	B	2	16.50 ± 2.16	5.21	2.97	1.93	5.10	2.17	2.49	30.95
	C	3	30.28 ± 4.97	8.61	0.69	3.19	21.80	0.83	10.74	11.89
4	A	4	10.75 ± 0.73	3.07	2.08	1.14	7.23	0.90	3.56	12.81
	B	1	12.13	5.24	2.28	1.94	6.60	1.68	3.25	23.89
5	A	4	10.27 ± 4.07	3.14	2.13	1.16	7.06	0.94	3.48	13.33
	B	1	12.57	4.53	1.80	1.68	8.40	1.15	4.12	16.33
10	A	7	8.32 ± 0.90	3.05	1.48	1.13	10.20	0.63	5.01	9.03
	B	2	9.53 ± 0.90	3.94	1.27	1.46	11.84	0.70	5.83	10.01
	C	10	18.51 ± 4.37	5.78	0.77	2.14	19.54	0.62	9.62	8.90

[a] Key: (A) II with I as penetrant, without any pretreatment. Data were abstracted from Table II; (B) skin pretreated with propylene glycol for 8 h and II with I as penetrant; (C) skin pretreated with I for 8 h and II alone as the penetrant. [b] J_s = slope/(A × 3600 × specific activity × 10^6 × 2.2 × 10^6), where slope was in the unit of dpm/h, A = 0.636 cm^2, and specific activity of tritiated II was 37 Ci/mmol. [c] $K_p = J_s/C_s$, where C_s = 3.01 × 10^{-10} mol/mL. [d] $D = \delta^2/6\tau$, where δ was measured as 570 μm for full-thickness skin, and D' was similarly calculated with δ estimated as 40 μm for hydrated stratum, corneum. [e] $K_m = K_p\delta/D$, and K_m' was similarly calculated with D'.

Table IV—Influence of 1-Dodecylazacycloheptan-2-one on Reservoir Effect of Hairless Mouse Skin in Retaining Triamcinolone Acetonide

I, %	n	Recovery at 12 h, % Skin	Diffusate	Total
0	4	1.4 ± 0.5	0.1 ± 0.1	1.5 ± 0.5
1	3	9.8 ± 4.2	3.1 ± 1.1	12.9 ± 4.3
2	4	8.9 ± 3.1	13.6 ± 1.2	22.5 ± 3.3
5	3	10.6 ± 3.3	10.3 ± 4.1	20.9 ± 5.3
8	2	10.6 ± 5.6	9.5 ± 1.9	20.1 ± 5.9
10	2	8.4 ± 4.2	8.3 ± 0.9	16.7 ± 4.3
Mean		9.7		
SD		3.5		
0[a]	1	3.3	1.1 ± 0.7	4.4 ± 0.7
2[a]	2	33.1 ± 2.2	30.3 ± 5.0	63.4 ± 5.5
10[a]	2	19.5 ± 3.6	18.5 ± 4.4	38.0 ± 5.7

[a] Pretreatment for 8 h.

Reservoir Effect—The so-called "reservoir effect" has been reported qualitatively or semiquantitatively for various topically applied glucocorticoids (19). The stratum corneum retains the penetrated drug molecules and makes the agents available for additional absorption even when the drug administration is ceased. To examine the I effect on the reservoir effect of II in hairless mouse skin, the II retentions in skin after 12-h penetration (with various concentrations of I) were evaluated. The presence of I in the vehicle significantly promoted the retention of II in full-thickness skin ($0.05 < p < 0.1$); the mean was 9.7 ± 3.5%, as compared with that without I, 1.4 ± 0.5% (Table IV). However, the enhancing effect was independent of the concentration of I. With the concentrations of I studied (1-10%), the II retained in the skin ranged from 8.4 ± 4.2% to 10.6 ± 5.6% (Table IV); variations were insignificant ($p > 0.7$).

The pretreatment of the skin with 2 or 10% I for 8 h further increased the I enhancing effect on II reservoir in the skin, from 8.9 ± 3.1% to 33.1 ± 2.2% for 2% I ($p < 0.001$) and from 8.4 ± 4.2% to 19.5 ± 3.6% ($0.1 < p < 0.2$) for 10% I (Table IV).

The promotion of skin hydration has been reported as a major mechanism of penetration enhancement induced by 8 M urea and dimethyl sulfoxide, as evidenced by the respective 280 and 150% weight gain of the epidermis (22). The possible I-induced hydration of stratum corneum was accordingly explored. The weights of skin samples, with an area of 0.636 cm^2, in contact with various concentrations of I for 12 h were measured (mean, 108.3 ± 17.7 μg; range, 84.9–146.5 μg; n = 12) and were not significantly different ($p > 0.6$) from the samples without I (mean, 103.5 ± 16.8 μg; range, 84.3 − 124.8 μg; n = 4).

Another possible mechanism of leaching out normally impermeable components of stratum corneum (lipoprotein or phospholipid) and leaving a permeable channel for easier penetration, reported by Allenby *et al.* (22) for urea and dimethyl sulfoxide, was not explored for I.

CONCLUSIONS

The penetration profiles of II with I through synthetic membranes were different from those through hairless mouse skin. This observation cautioned the use of synthetic membranes as skin models in the study of I.

Compound I significantly enhanced the extent of II penetration through hairless mouse skin, from 7-fold with 0.5% I, to 124-fold with 2-3% I, and then leveled off at 76-98-fold with 4-10% I. A similar pattern was obtained for the I enhancement on the penetration rate of II; a 2-fold increase with 0.1%, increasing to 138-fold with 2-3% and leveling off at 90-fold with 4-10%. The enhancing effect on the penetration of II was dependent on the concentration of I. With I concentrations >2%, penetration impedance was observed after an 8-h skin exposure to I. Optimal concentration of I on the penetration of II was 2% (v/v). Compound I exerted increasing promoting effects on the diffusion constant of II within the stratum corneum with increasing concentrations of I, manifested by the continuous reduction in lag time. On the other hand, I increased the partition coefficient of II between stratum corneum and propylene glycol with an optimal concentration of 2%. Further increment in I >2% diminished the enhancing effect.

Compound I potentiated the retention of II in skin (reservoir effect) about sevenfold; however, such an increase was independent of the concentration of I. The difference between the weights of the skin samples with and without I contact was insignificant, suggesting that the promotion of skin hydration was not a major mechanism in the penetration enhancement induced by I.

The pretreatment of I was found to be more efficient than coapplication of I and II in the vehicle. The pretreatment of skin with 2 or 10% for 8 h further augmented the enhancing effect of I on the extent and the rate of II penetration, and on the reservoir effect, due to the substantial promotion of the diffusion constant; also, pretreatment with 2% I was more effective than with 10% I. The observations indicated that coexistence of I and II was not required for penetration enhancement and that there was a possible interaction between I and II in the vehicle to lessen the enhancing efficiency.

The penetration impedance after 8-h skin exposure to I was observed only with skin, not with synthetic membranes. Interaction of I with the skin component could be responsible for such an impedance. In the pretreatment study, the penetration impedance after 8 h was still observed with 10% I, suggesting that I and II were required to coexist (not in the vehicle but probably in the stratum corneum) to elicit this impedance.

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ACKNOWLEDGMENTS

This research was supported by a Research Initiation Grant, University of Houston and Biomedical Research Support Grant, National Institutes of Health. Presented in part at the 130th Annual Meeting of APhA, New Orleans, La., April 1983. The authors acknowledge the supply of Azone given by Nelson Research and Development Co. and the assistance of the University of Houston Word Processing Center in preparing the manuscript.

Pectin–Gelatin Complex Coacervates II: Effect of Microencapsulated Sulfamerazine on Size, Morphology, Recovery, and Extraction of Water-Dispersible Microglobules

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Abstract □ Spherical medicated microglobules were prepared by complex coacervation of Type A gelatin with pectin, having nominal diameters of 5, 10, and 25 μm and containing 37.3, 44.9, and 45.2% (w/w) sulfamerazine, respectively. They were recovered as water-insoluble powders and were spontaneously revertible to highly disperse systems when reconstituted in water or physiological electrolyte solution. The conditions affecting microglobule formation were studied. For complete formation, the crystals must be dispersed at ≥pH 5. The effect of the sulfamerazine mass added on microglobule morphology, yield, and contents were investigated. As much as 37.3, 44.5, and 69.1% (w/w) sulfamerazine in 5-, 10- and 25-μm microglobules could be formed without loss of spherical shape. The microglobule yield *versus* drug-to-colloid ratio curves were nonlinear below the critical drug-to-colloid ratio for loss of sphericity. Addition of sulfamerazine suppressed coacervation by 10–15% but it had no significant effect on microglobule size. The extraction of medicated microglobules in various media demonstrated the existence of a porous matrix that required hydration to facilitate extraction of the microglobular drug. Fifteen percent of the encapsulated sulfamerazine was extracted from 25-μm microglobules as opposed to 9% from 10-μm microglobules after equilibration for 24 h in replacement electrolyte solution.

Keyphrases □ Microencapsulation—pectin–gelatin coacervates, sulfamerazine □ Coacervates—pectin–gelatin, sulfamerazine, microglobules, recovery, size

Little consideration of the morphological character of complex coacervate-coated particles appears in the pharmaceutical literature until Newton *et al.* (1) produced spherical microglobules of uniform size with centrally located drug. The process of medicated microglobule formation is to be differentiated from microencapsulation; the latter usually yields nonspherical particles. Complex coacervates of gelatin–acacia or gelatin–pectin readily wet and surround suspended particles and emulsified droplets. On cooling and denaturation with formaldehyde, the liquid coacervate droplets congeal, forming microglobules that are recoverable as water-dispersible powders after flocculation in 2-propanol (1, 2).

Certain physicochemical conditions for preparing and recovering nonmedicated microglobules from type A gelatin–pectin complex coacervates were detailed previously (2). The purpose of this investigation was to characterize conditions for sulfamerazine microglobule formation in complex coacervates of pectin and gelatin, yielding microglobules of uniform size with centrally located drug that were recoverable as a dry powder. Medicated microglobules with diameters of <10 μm, spontaneously revertible to a polydisperse system in aqueous fluids, were sought for potential use in parenteral dosage forms.

EXPERIMENTAL SECTION

Materials—Type A gelatin[1] and pectin NF[2] were used in sols containing 1% benzyl alcohol for preservation. Sulfamerazine USP[3], micronized powder (≤5 μm); formaldehyde solution, USP; glycerin, 99.6%; 2-propanol, 99%; replacement electrolyte solution[4]; collagenase B[5]; pancreatin[5]; and pepsin[6] were used as received. Other reagents were of analytical reagent grade.

Preparation and Recovery of Microglobules – The method of preparation and recovery of microglobules employed by McMullen *et al.* (2) was used. Coacervates were prepared at 45°C from 40.0-g batches stirred by a magnetic stirrer at a speed sufficient to produce a vortex without air bubbles. Appropriate weights of 2% (w/w) stock solutions of pectin and gelatin were individually adjusted to the mixing pH (pH 8–10) with 1.0 M NaOH at 45°C to

[1] 275 Bloom, isoelectric point 8.6; Fisher Scientific Co., Fair Lawn, N.J.
[2] Sunkist Growers Inc., Ontario, Calif.
[3] Matheson, Coleman & Bell, Norwood, Ohio.
[4] Normosol-R pH 7.4; Abbott Laboratories, North Chicago, Ill.
[5] Calbiochem, San Diego, Calif.
[6] Wilson Labs, Chicago, Ill.

0022-3549/84/1200-1799$01.00/0