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The effect of solvents on solute penetration through fuzzy rat skin *in vitro*

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Synopsis

Solvents common to cosmetic and pharmaceutical formulations, including water, dimethylisosorbide, three aliphatic alcohols, and three polyols, were studied using theophylline and methylparaben as model permeants. The integrity of fuzzy rat skin was maintained for about two days when fully hydrated and only one day when exposed to methanol. The maximal permeation rate of each solute was markedly dependent on the solvent, indicating that solvent/skin interactions contributed significantly to permeation. Flux ratios for the solvents relative to water were computed for each permeant. A perfect rank order correlation between permeants was observed. Among the alcohols, methanol was most effective in enhancing flux of both compounds, followed by ethanol and then 1-propanol. Propylene glycol had a small negative effect, while the more polar polyols markedly reduced the flux ratio. Flux-concentration profiles for methylparaben and flux differences between the alcohols suggest that the interaction mechanism differs from that with polydimethylsiloxane membranes.

INTRODUCTION

In addition to industrial toxicologic considerations, the permeability characteristics of skin are of paramount importance in the development of pharmaceuticals and cosmetics. Topically applied drug entities may be used to achieve either local or systemic pharmacologic actions (1). Often, to avoid allergic or toxic reactions, it is desirable to prevent or limit the penetration of materials that have been applied to the skin. Cosmetic preparations should be formulated to reduce penetration of components that are irritating or can induce allergic responses.

Contact with solvents can markedly alter the physical-chemical properties, including permeability, of membranes. Solvent interaction may alter membrane resistance by affecting solute mobility and/or capacity of the membrane to contain solute. In order to quantitate interactive effects it is necessary to account for any changes in solute release from the vehicle. Suspensions should yield equivalent solute flux in the absence of solvent-induced membrane damage. The literature on solvent effects on skin perme-

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ability is confusing and sometimes contradictory because changes in the thermodynamic activity of solute between vehicles were not considered (2,3).

In previous studies (4–6), the enhancement in solute flux by aliphatic alcohols through polydimethylsiloxane membranes was investigated. Alcohols were sorbed by the membrane, resulting in increases in solute partitioning. Diffusion coefficients were not significantly changed owing to initially high solute mobility within the "dry" polymer matrix. Saturated solutions maintained constant activity of solute but reduced the activity of the solvent. Alcohol sorption, and hence membrane interaction, exponentially declined as the alcohol content was reduced. As a result, flux did not increase proportionally with solute concentration but instead reached a peak and then declined. Diffusion experiments at infinite dilution provided data that generated a "solvent index" allowing solute independent quantitation of permeation enhancement. The delineation of alcohol effects on the synthetic membrane was made possible due to its stability and homogeneity.

In this study, similar experiments employing excised fuzzy rat skin have been performed. The objectives of this preliminary work include the identification and quantitation of interactive solvents, evaluation of concentration effects, and comparison to polydimethylsiloxane interactive systems. Fuzzy rat skin as a model for human skin offers several advantages, including its ready availability, known history, and reduced inter-subject variability. Also, limited studies indicate that the permeability of fuzzy rat skin to n-alkanols is qualitatively similar to that of human skin (7).

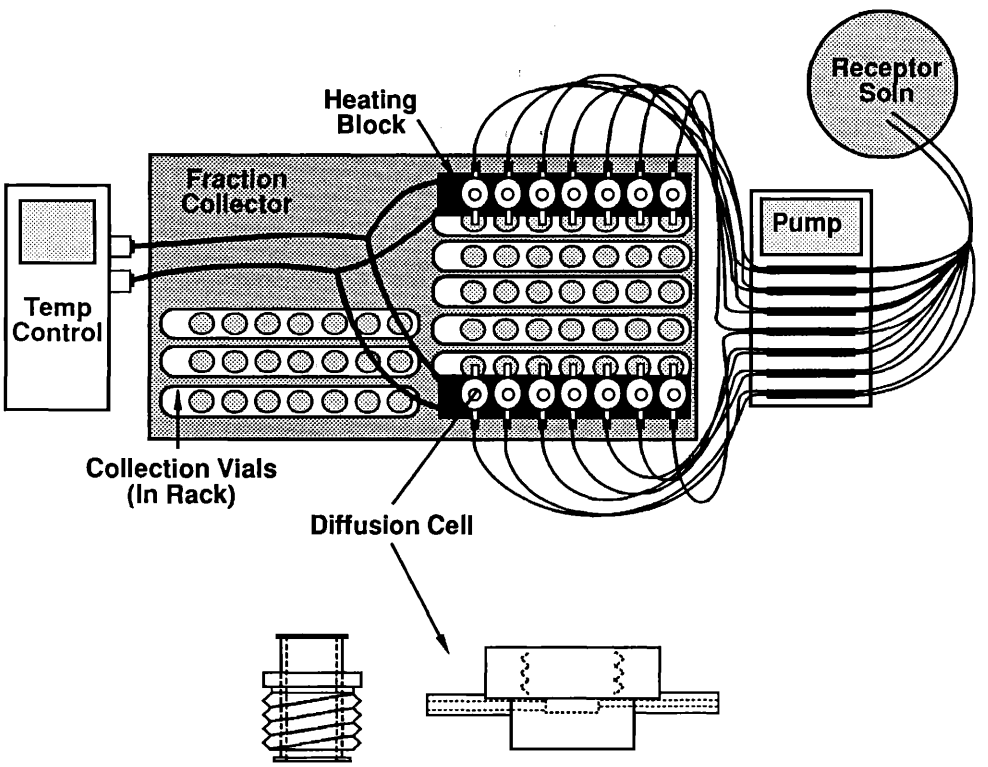

Figure 1. Skin permeation apparatus used in this study.

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EXPERIMENTAL

MATERIALS

The solutes utilized were methylparaben, propylparaben (Fisher Scientific Company, Fairlawn, NJ) and theophylline (Eastman Kodak Company, Rochester, NY). Solute melting points and HPLC chromatograms were used to establish purity. The solvents were distilled water, spectrograde alcohols, propylene glycol, polyethylene glycol 400 (PEG 400), glycerin (Fisher Scientific), and dimethylisosorbide (ICI Americas, Wilmington, DE).

EQUIPMENT

A teflon flow-through cell mounted into a temperature controlled aluminum block (Crown Glass, Somerville, NJ) was used in the permeation studies (Figure 1). The body portion of the cell contained the inlet and exit ports and a glass window allowing inspection for air bubbles. Prepared skin was mounted stratum corneum side up in the body of the cell. A cylinder was locked into the cell body (using a threaded ring) and formed the donor compartment. The cylinder was modified by increasing its length in order to reduce skin torque during mounting and to increase donor compartment volume. The area available for diffusion was 0.64 cm^2. Receptor solution was delivered to the cells via a peristaltic pump, and effluent was collected into tared vials. A fraction collector allowed the unattended collection of samples at specified time intervals.

METHODOLOGY

Male fuzzy rats (Skh:fz; Temple University, Philadelphia, PA), 10-weeks-old, weighing 275–300 g, were used in this study. The animals were sacrificed by CO_2 asphyxiation. Excised skin samples were stored in a freezer for less than eight weeks. The samples were sufficiently thawed before use, and the dorsal region was removed and dermatomed to a thickness of 320 μm or 450 μm (Pagett Dermatome, Kansas City, MO). After gross examination to eliminate damaged skin specimens, the skin was then mounted into the diffusion cell as previously described. The cells were heated to maintain a 37°C temperature at the dermal side of the skin. The receptor solution was the same throughout the study and consisted of normal saline with 0.25% w/v chlorobutanol. The receptor reservoir was maintained at 40°C to reduce formation of air bubbles under the dermal surface. Entrapped air was removed by careful tilting of the cell. Receptor fluid was pumped through the cell at a controlled rate to allow detection of

Table I
Inter- Versus Intra-Subject Variation in Flux for Aqueous Suspensions of Theophylline (450-μm Skin Thickness)

	Flux ± SD (μmol/cm^2/h)	CV (%)
Within an animal (n = 6)	0.12 ± 0.02	12
Between animals (n = 6)	0.14 ± 0.01	10

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Table II
Influence of Skin Thickness for Saturated Aqueous Systems

Thickness (μm)	Theophylline		Methylparaben	
	Flux ± SD ($mg/cm^2/h$)	Lag time ± SD (h)	Flux ± SD ($mg/cm^2/h$)	Lag time ± SD (h)
320	0.024 ± 0.003	1.8 ± 0.7	0.11 ± 0.02	2.9 ± 0.7
450	0.024 ± 0.003	1.2 ± 0.5	0.096 ± 0.02	3.0 ± 0.3

permeant (approximately 3 ml/h). The mounted skin was allowed to equilibrate for one hour before application of the donor solution. The donor compartment was sealed with parafilm to eliminate evaporative loss.

Effluent samples were collected periodically and weighed to determine the actual amount of solution delivered. Samples were filtered through a 0.45 μm filter, and 20 μl were injected onto a reversed phase HPLC column (Zorbax ODS-3 column, Dupont, Wilmington, DE) with UV detection-theophylline at 272 nm, parabens at 254 nm.

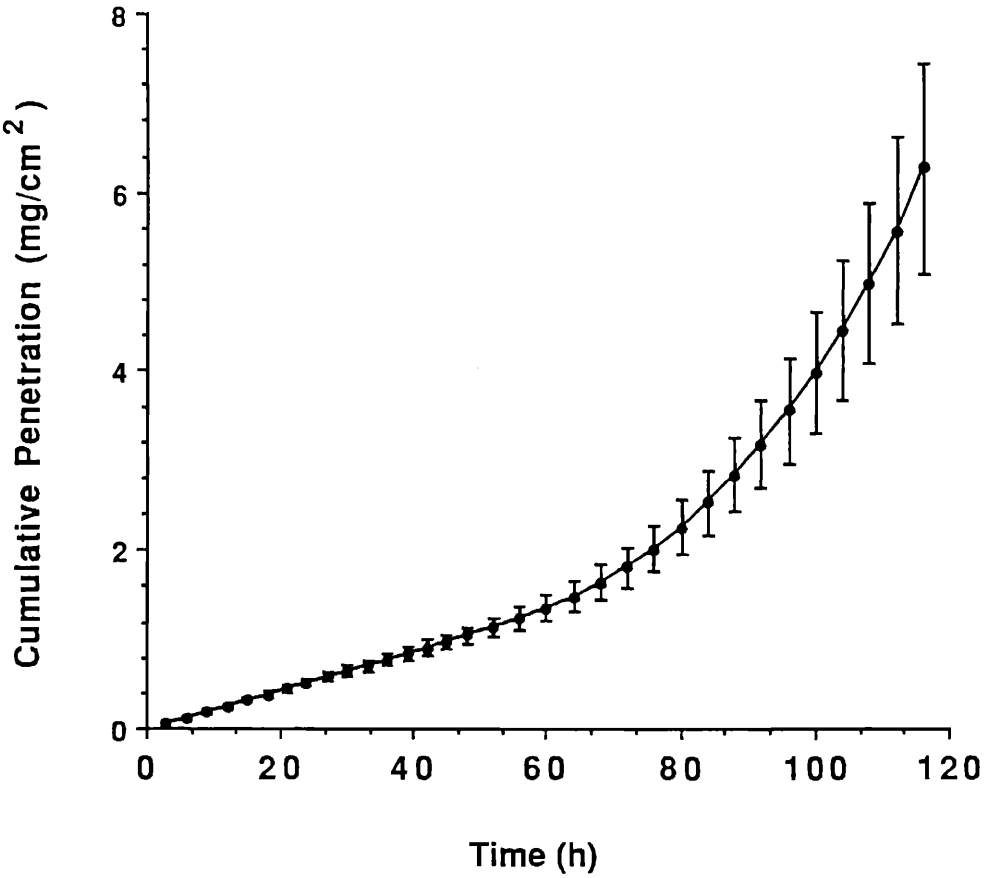

Figure 2. The cumulative amount of theophylline penetrating the excised skin sample versus time from infinite aqueous dosing.

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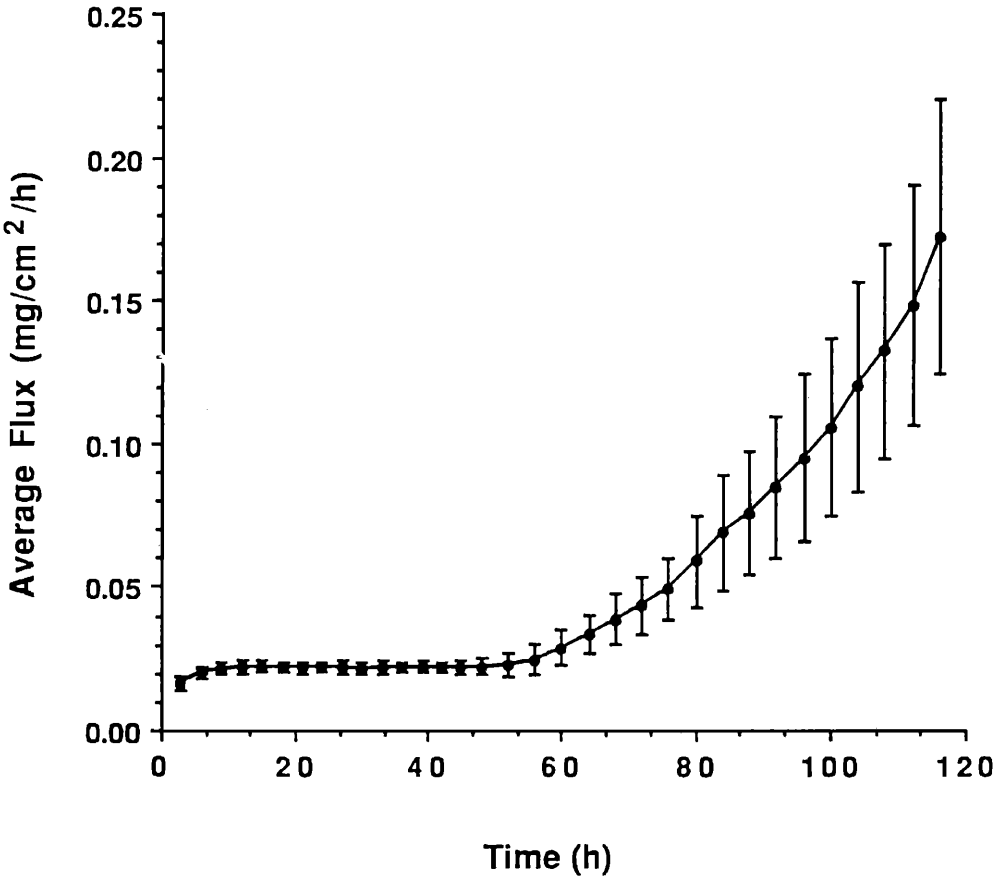

Figure 3. Flux versus time profile for theophylline from aqueous suspension.

The mobile phase consisted of methanol:water:HCl in the proportions 60:40:1 v/v at a flow rate of 1.0 ml/min. Donor solutions were prepared by adding an excess of permeant to each of the neat solvents employed. The suspensions were allowed to equilibrate for several days in a 37°C water bath with periodic shaking. Several systems were examined as a percent of saturation (w/w) and were not used until completely dissolved. Solubilities were determined in a previous study (5).

RESULTS AND DISCUSSION

Experiments were performed on excised fuzzy rat skin to determine inter- and intra-subject variability, stability to hydration, and influence of membrane thickness on flux and lag time. The variability in flux and lag time between animals was not significantly different from the corresponding inter-subject data (Table I). Values of steady-state flux and lag time were independent of skin thickness (pooled t-test, $\alpha = 0.05$) for samples dermatomed to 320 μm and 450 μm (Table II). The effect of continuous exposure to water on fuzzy rat skin integrity is shown in Figures 2 and 3. The cumulative plot (Figure 2) shows that a steady state is reached after a few hours. After about 50 hours,

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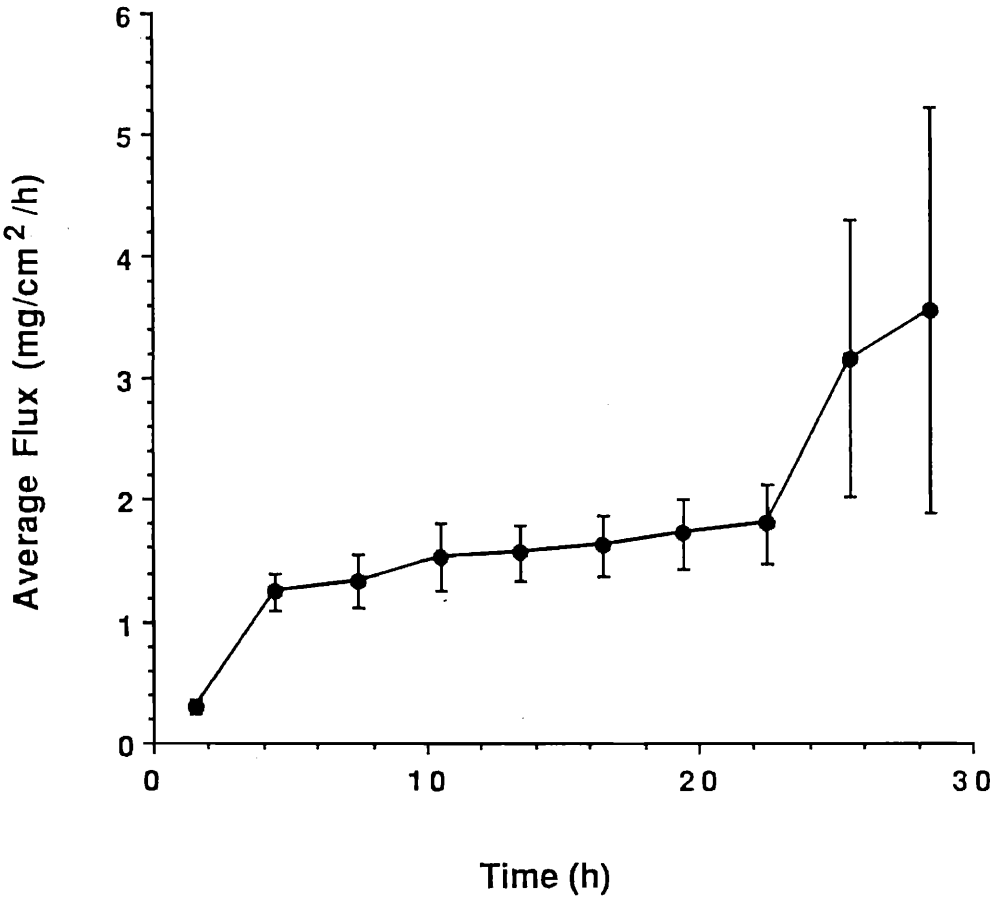

Figure 4. Flux versus time profile for theophylline from saturated methanol donors.

there is an increase in slope and variability, signalling membrane deterioration. The change in skin properties with time is shown even more graphically in the flux plot (Figure 3). Similar profiles were observed for nude mouse skin (8). Human skin is more stable than fuzzy rat skin to water-induced changes in permeability (9).

Solvents affected the ability of the skin to maintain its barrier properties. Methanol was found to be the most damaging of the solvents studied. While methylparaben flux from methanol was essentially constant from about 10 to 23 hours, rapid deterioration of the membrane's barrier function then occurred (Figure 4).

Several guidelines for the remaining experiments were established from these initial findings. The skin was dermatomed to 450 μm for the remainder of the experiments to maximize sample yield (approximately 12–15 dorsal skin samples per animal). A minimum of six replicates (three replicates from two separate animals run on different days) was measured for each treatment (solvent/solute system). In most cases four treatments (12 cells) were run simultaneously. The data for each treatment were pooled only after testing for significant differences. The experiments were run for about 24 hours, with continuous sample collection every two or three hours.

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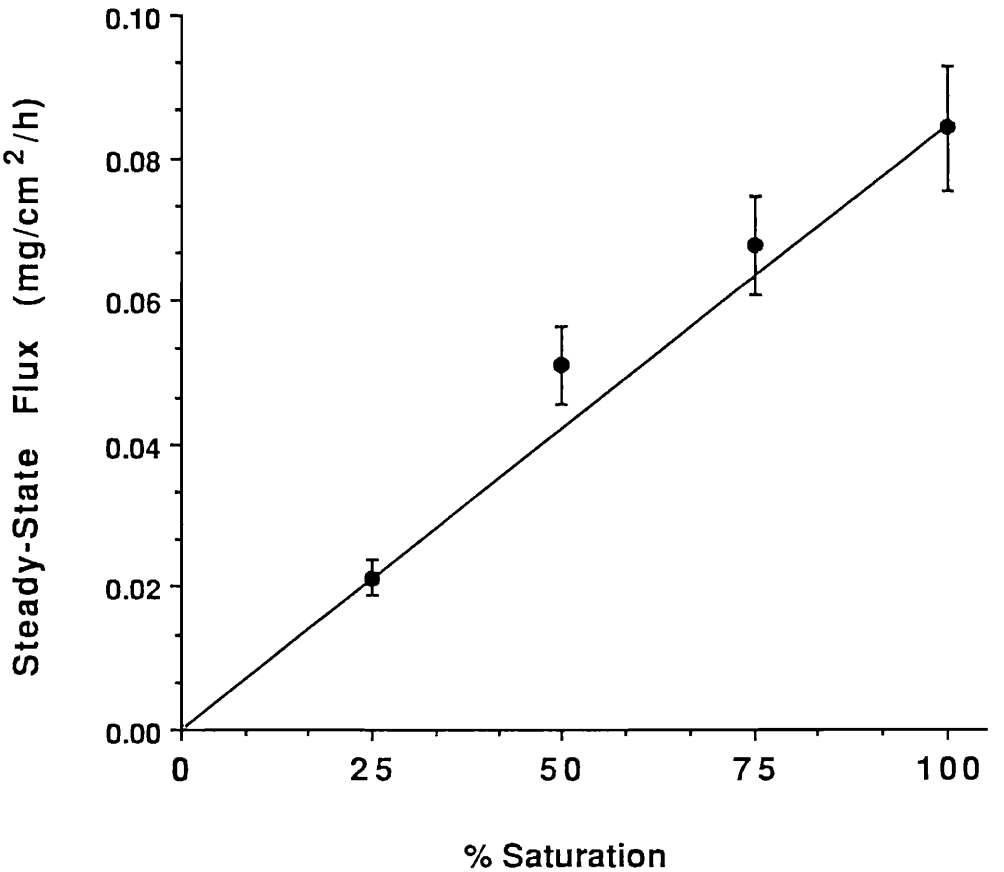

Figure 5. Methylparaben flux as a function of the percent saturation (activity) for propylene glycol treatment.

In previous work on permeation through polydimethylsiloxane membranes from interactive solvents (alcohols), it was shown that at high paraben concentrations flux remained constant or declined as concentration was increased further (5). This was a consequence of the decrease in alcohol activity, which resulted in an attenuation of solvent/membrane interaction. The same pattern was not observed with permeation through fuzzy rat skin. The flux profiles for methylparaben delivered from 1-propanol and propylene glycol donors were linearly related to solute activity (Figures 5, 6). This indicates that the interaction was not reduced by the decrease in solvent activity, as was found with the synthetic membrane.

Since flux is a linear function of concentration, it is necessary to utilize but a single concentration at which to make comparisons between solvents. One approach is to select a fixed concentration of permeant in all solvents. However, this method neglects the fact that solute activity will vary between solvents, thus biasing the results. A better approach is to compare data for saturated systems, in which the solute activity is constant and maximal. With this design, the flux from all of the systems should be

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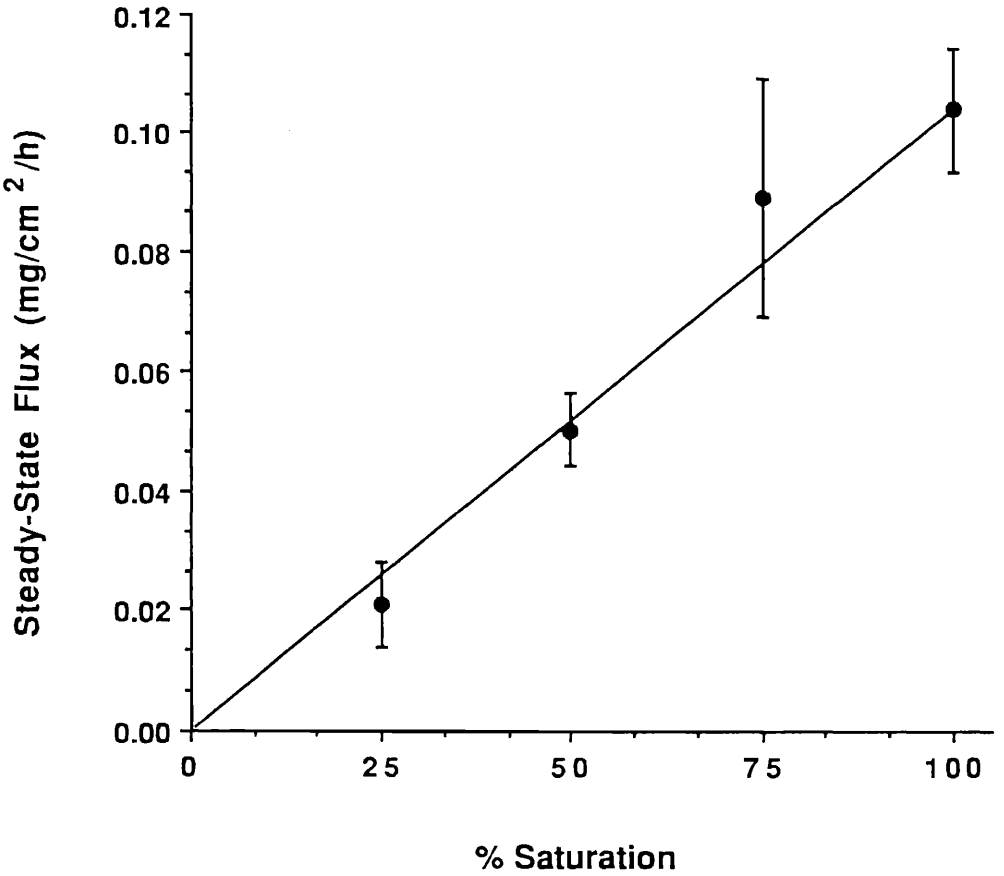

Figure 6. Methylparaben flux as a function of the percent saturation (activity) for 1-propanol treatment.

Table III
Theophylline Solubilities and Permeation Data

Solvent	Solubility @ 37°C (mg/cm^3)	n	Flux ($\mu mol/cm^2/h$) Mean	Flux ($\mu mol/cm^2/h$) 95% C.I.[a]
PEG 400		9	0.011	$0.011 \leq \mu \leq 0.012$
Glycerin		5	0.014	$0.012 \leq \mu \leq 0.016$
DMIS		6	0.029	$0.024 \leq \mu \leq 0.033$
Propylene glycol		6	0.044	$0.032 \leq \mu \leq 0.057$
Water	10[b]	12	0.13	$0.12 \leq \mu \leq 0.14$
1-Propanol	4.1	6	0.21	$0.17 \leq \mu \leq 0.25$
Ethanol	4.8	6	0.29	$0.27 \leq \mu \leq 0.32$
Methanol	8.4	6	0.65	$0.54 \leq \mu \leq 0.75$
Average lag time ± standard error (h)		56	1.5 ± 0.1	

[a] Confidence interval.
[b] Calculated using 8.07 mg/cm^3 at 32°C (reference 10).

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Table IV
Methylparaben Solubilities and Permeation Data

Solvent	Solubility @ 37°C (mg/cm^3)	n	Flux ($\mu mol/cm^2/h$) Mean	Flux ($\mu mol/cm^2/h$) 95% C.I.[a]
PEG 400	330	9	0.055	$0.049 \leq \mu \leq 0.061$
Glycerin		6	0.11	$0.094 \leq \mu \leq 0.12$
DMIS		6	0.16	$0.14 \leq \mu \leq 0.18$
Propylene glycol	260	6	0.55	$0.49 \leq \mu \leq 0.61$
Water	3.5	12	0.67	$0.60 \leq \mu \leq 0.73$
1-Propanol	360	6	0.68	$0.61 \leq \mu \leq 0.76$
Ethanol	380	6	1.36	$1.1 \leq \mu \leq 1.6$
Methanol	470	6	9.1	$7.7 \leq \mu \leq 10$
Average lag time ± standard error (h)		93	3.3 ± 0.1	

[a] Confidence interval.

equal in the absence of solvent-induced skin damage. Significant differences in flux are a measure of the effect of specific skin/solvent interactions.

Permeation data for saturated solutions of theophylline and methylparaben in each solvent are collected in Tables III and IV. The same data are graphically presented in Figure 7. Theophylline flux from suspension in propylene glycol was similar to a value reported by Sloan *et al.* (10) using hairless mouse skin (0.032 ± 0.005 $\mu mol/cm^2/h$) at 32°C. Limited flux data for propylparaben (propylene glycol flux: 0.55 $\mu mol/cm^2/h$ ± SE 0.05; dimethylisosorbide flux: 0.17 $\mu mol/cm^2/h$ ± SE 0.03) parallel data for corresponding methylparaben systems. The average coefficient of variation in flux values was 14% for methylparaben and 15% for theophylline. The large variation in measured lag times (average CV of 26%) limited their usefulness for quantitative assessment. Methylparaben flux was greater than that of theophylline from each donor solvent. In the absence of solvent-induced changes in membrane permeability, the flux values for each solute should have been constant. However, examination of the data in Tables III and IV reveals significant differences in the flux values between solvents for both solutes.

Permeant solubility per se is not a factor in the results. Theophylline solubility in water was not measured, but can be estimated as approximately 10 mg/cm^3 (10). Its flux from saturated solution in water is about half that from ethanol, although its solubility is about twice as great (Table III). On the other hand, theophylline solubility in methanol is intermediate between that of water and ethanol, yet skin penetration from methanol was highest. Similarly, methylparaben flux from 1-propanol is more than ten times greater than from polyethylene glycol 400, although solubility in these solvents is nearly identical (Table IV). Furthermore, methylparaben flux from water and propylene glycol are comparable despite a difference in solubility of nearly two orders of magnitude.

To quantitate the solvent interaction, the data were normalized to an arbitrary reference. Water was selected as the reference solvent since it allows comparison to most other skin permeation studies. The larger number of replicates for aqueous systems ($n = 12$) resulted in a smaller flux confidence interval. Flux ratios for the fuzzy rat skin are presented in Table V. The alcohols consistently increased solute flux up to a max-

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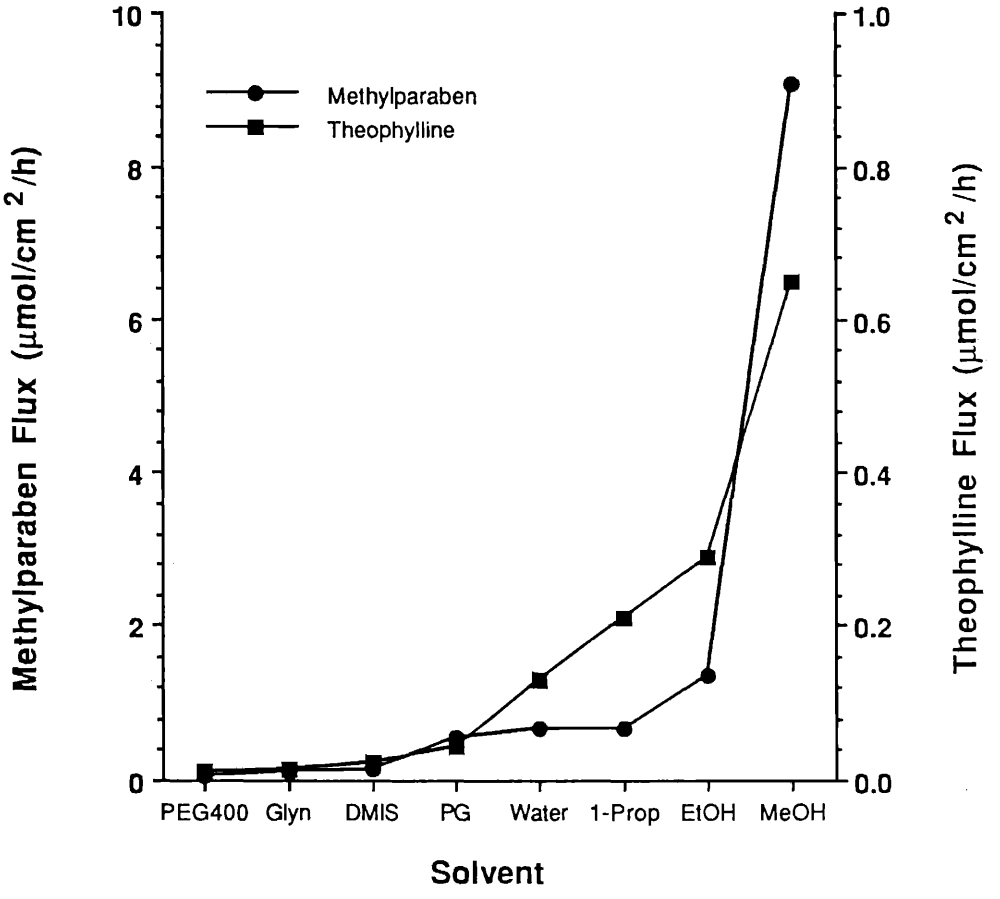

Figure 7. Average solute flux from saturated donors for each solvent.

Table V
Flux Ratio Data for Fuzzy Rat Skin

Solvent	Theophylline		Methylparaben	
	μ	95% C.I.[a]	μ	95% C.I.[a]
PEG 400	0.089	$0.079 \leq \mu \leq 0.10$	0.083	$0.070 \leq \mu \leq 0.096$
Glycerin	0.11	$0.093 \leq \mu \leq 0.13$	0.16	$0.13 \leq \mu \leq 0.19$
Dimethylisosorbide	0.22	$0.19 \leq \mu \leq 0.26$	0.25	$0.21 \leq \mu \leq 0.29$
Propylene glycol	0.35	$0.26 \leq \mu \leq 0.45$	0.83	$0.71 \leq \mu \leq 0.95$
Water	1.0		1.0	
1-Propanol	1.7	$1.4 \leq \mu \leq 2.0$	1.0	$0.85 \leq \mu \leq 1.2$
Ethanol	2.3	$2.0 \leq \mu \leq 2.6$	2.0	$1.6 \leq \mu \leq 2.4$
Methanol	5.2	$4.3 \leq \mu \leq 6.1$	14	$11 \leq \mu \leq 16$

[a] Confidence interval.

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imum of 14-fold for methanol. The degree of flux enhancement decreased with ethanol and further still with 1-propanol. Neat alcohols applied to human cadaver skin exhibited this same pattern (11). Methanol altered skin permeability about 3.5 times more than ethanol, while 1-propanol had little effect. Octanol greatly increased theophylline flux through hairless mouse skin in an irreversible manner (10).

Propylene glycol treatment resulted in a moderate decrease in flux relative to aqueous systems. The more polar solvents PEG 400 and glycerin had a negative effect on flux. Dimethylisosorbide, a glucitol derivative, previously described as a non-damaging solvent (12), produced flux ratios between those of propylene glycol and the other polyols. Flux from PEG 400 was slightly less than one tenth that from water (Table V). Zatz and Dalvi (13), employing hairless mouse skin, also found a tenfold difference in benzocaine flux between aqueous and PEG 400 suspensions. Flux ratios for PEG 400, dimethylisosorbide, and ethanol do not differ significantly between permeants (Table V). The flux ratios for glycerin, propylene glycol, and methanol were greater with methylparaben, while theophylline exhibited a higher flux ratio for 1-propanol treatment. Thus, there are some quantitative differences when comparing permeants, but there is qualitative agreement in flux ratio for each solute studied.

If skin were a simple, homogeneous membrane, we might expect the flux ratios shown in Table V to apply across the board to any solute. Consequently, it would be possible to anticipate the flux of any compound in a given solvent by knowing the flux ratio for the solvent and the results of a single penetration experiment from a reference solvent. However, this scenario is complicated by the fact that skin is a composite membrane resulting in multiple permeation pathways.

The rather wide range of flux ratios suggests that all of the solvents included in this study must be thought of as interactive; none are inert. As to the nature of the various interactions, we can only speculate without additional data. Among the possibilities are alteration of stratum corneum hydration, increase in the normal fluidity of the intercellular lipid layer, and extraction of lipid and/or other components. Methanol, of the alcohols studied, has the greatest effect on skin properties.

All of the solvents, with the exception of water itself, would be expected to pull water out of the stratum corneum. Dehydration may explain the rather low flux levels from PEG 400, glycerin, and dimethylisosorbide. There is evidence that normal alcohols can extract lipid material from the stratum corneum (14,15).

As with previous studies (13), there is no evidence that penetration from propylene glycol is greater than from water. Progressive hydroxylation of propane (from 1-propanol to propylene glycol to glycerin) results in a stepwise decrease in permeability.

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REFERENCES

(1) *Facts and Comparisons* (J. B. Lippincott Co., St. Louis, MO, 1988).
(2) B. J. Poulsen, in *Design of Topical Products: Biopharmaceutics, Drug Design,* E. J. Ariens, Ed (Academic Press, New York, 1973), Vol. 4, pp 149–192.

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From: SCC Media Library & Resource Center (library.scconline.org)

(3) B. Idson, Percutaneous absorption, *J. Pharm. Sci.*, **64**, 901–924, 1975.
(4) J. N. Twist and J. L. Zatz, Influence of solvents on paraben permeation through idealized skin model membranes, *J. Soc. Cosmet. Chem.*, **37**, 429–444, 1986.
(5) J. N. Twist and J. L. Zatz, Membrane-solvent-solute interaction in a model permeation system, *J. Pharm. Sci.*, 77, 536–540, 1988.
(6) J. N. Twist and J. L. Zatz, A model for alcohol-enhanced permeation through polydimethylsiloxane membranes, *J. Pharm. Sci.*, in press.
(7) S. DelTerzo, C. R. Behl, R. A. Nash, N. H. Bellantone, and A. W. Malik, Evaluation of the nude rat as a model: Effect of short term freezing and alkyl chain length on permeability of n-alkanols and water, *J. Soc. Cosmet. Chem.*, **37**, 297–307, 1986.
(8) E. Vander Merwe, C. Ackerman, and C. J. Van Wyk, Factors affecting the permeability of urea and water through nude mouse skin in-vitro. I. Temperature and time of hydration, *Int. J. Pharm.*, **44**, 71–74, 1988.
(9) J. R. Bond and B. W. Barry, Limitations of hairless mouse skin as a model for in-vitro permeation studies through human skin: Hydration damage, *J. Invest. Dermatol.*, **90**, 486–489, 1988.
(10) K. B. Sloan, S. A. Koch, K. G. Siver, F. P. Flowers, Use of solubility parameters of drug and vehicle to predict flux through skin, *J. Invest. Dermatol.*, **87**, 244–252, 1986.
(11) R. J. Scheuplein and I. H. Blank, Mechanism of percutaneous absorption. IV. Penetration of non-electrolytes (alcohols) from aqueous solutions and from pure liquids, *J. Invest. Dermatol.*, **60**, 286–296, 1973.
(12) B. W. Barry, D. Southwell, and R. Woodford, Optimization of bioavailability of topical steroids: Penetration enhancers under occlusion, *J. Invest. Dermatol.*, **82**, 49–54, 1984.
(13) J. L. Zatz and U. G. Dalvi, Evaluation of solvent-skin interaction in percutaneous absorption, *J. Soc. Cosmet. Chem.*, **34**, 327–334, 1983.
(14) K. Knutson, R. O. Potts, D. B. Guzek, G. M. Golden, J. E. McKie, W. J. Lambert, and W. I. Higuchi, Macro- and molecular physical-chemical considerations in understanding drug transport in the stratum corneum. *J. Control. Rel.*, **2**, 67–87, 1985.
(15) T. Kai, V. Mak, R. O. Potts, and R. H. Guy, Mechanism of skin penetration enhancement: Effect of n-alkanols on the permeability barrier. Presented at the 15th International Symposium on Controlled Release of Bioactive Materials, Abstract 124, Basel, Switzerland, 1988.

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