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Report

Oleic Acid: Its Effects on Stratum Corneum in Relation to (Trans)Dermal Drug Delivery

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Calorimetric studies with porcine stratum corneum (SC) have shown that the lipid phase transitions associated with the intercellular bilayers are markedly affected by treatment with oleic acid. Specifically, the transition temperatures (T_m) and cooperativity are reduced, whereas no effect was observed on the endotherm associated with keratin denaturation, suggesting that oleic acid primarily affects the SC lipids. The decrease in the lipid-associated T_m's was further correlated with the amount of oleic acid taken up by the SC. Parallel experiments with silastic implied that the uptake is dependent on the thermodynamic activity of oleic acid in the vehicle itself. The *in vitro* transport of Piroxicam across human and hairless mouse skin (HMS) was significantly enhanced by oleic acid, as a function of the extent of oleic acid uptake, with an attendant change in T_m. These results emphasize the role of SC lipids in percutaneous absorption. Transport also depended on the donor concentration of ionized drug suggesting that the enhanced transport mechanism cannot be accounted for solely on the principles of the classical pH-partition hypothesis. Accordingly, a model of skin permeability enhancement involving solid-fluid phase separation within the SC lipids is proposed for oleic acid, consistent with the existing phospholipid literature. In conjunction with the use of oleic acid as an enhancer, very soluble hydrophilic salts were recognized as key factors in attaining maximum delivery. Oleic acid uptake, lipid ΔT_m, and enhanced drug flux were all found to correlate, exhibiting a bell-shaped curve as a function of the ethanol vehicle concentration. Therefore, uptake and/or DSC experiments are useful for formulating enhanced topical delivery systems.

KEY WORDS: enhanced topical delivery; oleic acid; hydrophilic permeants; formulation optimization; lipid phase-separation model.

INTRODUCTION

Approaches to improve skin absorption have included prodrugs, iontophoresis and barrier perturbation with penetration enhancers. Two of the first (trans)dermal penetration enhancers were DMSO (1) and Azone (2), but the list has grown substantially, including *N,N*-diethyltoluamide (3), the active ingredient in most mosquito repellants, and simple solvents like ethanol (4). Of significance to this paper has been the recognition that various *cis*-unsaturated fatty acids, like oleic, can be utilized as penetration enhancers.

The outer layer of the skin, *viz.*, the stratum corneum (SC), is generally recognized as the primary barrier to facile dermal or transdermal drug delivery. Although only 10 to 15% of the total SC mass is comprised of lipids, these lipids largely dictate the overall skin permeability properties. The human SC intercellular lipids consist mainly of cholesterol along with ceramides and free fatty acids, which are structurally organized into multilamellar bilayers (5). Porcine and hairless mouse SC are compositionally similar to that reported for man (6,7), and they have been widely employed as transport models of skin delivery. The high fraction of ceramides (~40%) and free fatty acids (~10%) with the almost-complete absence of phospholipids in these three mammalian species makes the SC constituently unique (8,9).

Free fatty acids (FFA) have been shown to induce a multiplicity of membrane-associated effects at the cellular level, including lymphocyte mitogenesis (10), the stabilization of erythrocytes (11), and alterations in membrane-bound enzyme function (12). The utility of selected FFA as topical penetration enhancers for pharmaceuticals has been recently revealed (13–15). The ability of certain FFA to increase permeability of the skin appears to be related to a selective perturbation of the intercellular lipid bilayers present in the SC (13,16). Golden *et al.* (13) have related this lipid perturbation by FFA to the enhanced permeability of coapplied drugs. Following the treatment of porcine SC with certain FFA, the transition temperatures for two lipid-associated endotherms were shifted to lower values. Increases in flux for salicylic acid across isolated porcine skin were subsequently observed and correlated with the before-mentioned decrease in the T_m for either of the two lipid-associated endotherms. The maximum effects (on flux and T_m) were achieved with *cis*-11-octadecenoic (vaccenic) and *cis*-9-octadecenoic (oleic) acids. In contrast, little or no changes in the T_m or flux were found following treatment with the

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corresponding saturated or *trans*-unsaturated FFA. The present work examines the perturbation of SC lipids by oleic acid, some pertinent implications for drug delivery, and a working hypothesis explaining its molecular mechanism of action.

MATERIALS AND METHODS

Isolation of Porcine Stratum Corneum

The full details of this procedure are described elsewhere (16). Following sacrifice, thoracic sections of full-thickness skin were removed, dermatomed to approximately 500 μm, and incubated with a 0.5% trypsin solution to separate the stratum corneum from the epidermis. These SC sheets were washed briefly with cold hexane to remove contaminating superficial lipids, rinsed with isotonic Sorensen's buffer, and air-dried on a wire-mesh screen. The SC samples were stored in a desiccator until needed for DSC or uptake studies.

Uptake Studies

Weighed pieces (~20 mg) of porcine SC or silastic (Dow-Corning, 0.005-in. thick, no fillers) were treated with 2 ml of the oleic acid:ethanol:H_2O vehicle for 2 hr. The vehicles contained 0.25% (v/v) oleic acid and were varied with respect to their ethanol concentration from 0 to 100%. During this incubation period, the vehicles were carefully stirred with small magnetic stirbars under ambient conditions in sealed vials. Roughly 0.1 μCi of ^{3}H-oleic acid was included in the vehicles to quantitate the amount taken up. Two hours was established as the appropriate length of time to be employed, in these experiments, by comparing samples treated for both 2 and 48 hr. Although different surface areas were not investigated, the equivalence of the 2- and 48-hr samples indicated that partitioning rather than adsorption was the principal phenomenon being investigated. Following incubation, the SC or silastic pieces were removed, reweighed, and rinsed with ethanol to remove any excess material clinging to the surface. These samples were then combined with 10 ml of scintillation cocktail and analyzed by a standard liquid scintillation procedure (as in the diffusion studies) to determine the amount of oleic acid incorporated.

Differential Scanning Calorimetry (DSC)

The details of this procedure have also been described elsewhere (13). Sections of SC (15 to 20 mg) were prehydrated in a humidity chamber at 95% RH to approximately 30% by weight, prior to treatment with the designated oleic acid vehicles. These vehicles were identical to those employed in the flux and uptake experiments. At least three SC samples for each treatment were evaluated and compared with more than 20 determinations for the untreated control. Only SC from pigs which exhibited transitions within ±1°C of the control value were utilized. After 2 hr of treatment, the SC was removed, rinsed with cold ethanol, reweighed, and scanned on an ultrasensitive MC-2 calorimeter (Microcal, Amherst, MA) from 35 to 110°C at 45°C/hr. This particular calorimeter has an inherent sensitivity of greater than 0.05 mcal/min, which translates to an effective sensitivity of better than 0.1 kcal/mol for lipids, and 1.0 kcal/mol for proteins. Since the lipid and protein transitions of SC fall within this range, this instrument is ideally suited for studying these phase changes. In addition, the instrument digitalizes the data allowing the determination of T_m to within 0.1°C. The enthalpy associated with the individual lipid phase transformations were determined by the instrument computer. In situations where the two lipid curves were partially overlapped, a deconvolution routine was utilized to estimate the individual contributions.

Flux Studies

The flux studies were performed with both hairless mouse and human skin. The human samples were obtained fresh from local hospitals following various surgical procedures. Prior to use, the full-thickness skin was dermatomed to a thickness of 350 μm, and stored in a sterile saline phosphate buffer (PBS). Transport experiments were usually initiated within 24 hr postsurgery. Hairless mouse skin (HMS) was obtained by excision from the abdominal or dorsal regions of the sacrificed animal and used without any further manipulation. The isolated skins (HMS or human) were mounted between two symmetrical Teflon diffusion half-cells containing small stir bars and placed in a 32°C water bath over magnetic stirrers. The surface area and volume for these cells were 0.2 cm^2 and 1.0 ml, respectively.

Piroxicam (4-hydroxy-2-methyl-*N*-2-pyridinyl-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide) was chosen as the permeant. Piroxicam is a unique NSAID with two ionizable sites (~pK_a's, 1.8 and 5.2 in H_2O), permitting it to exist in aqueous solutions as cationic, neutral, and anionic species depending on the pH. In order to maintain a constant thermodynamic activity in the applied donor phase, either saturated solutions or suspensions of Piroxicam buffered at the desired pH were employed. Approximately 0.3 μCi/ml of ^{14}C-labeled Piroxicam was equilibrated in each donor vehicle with unlabeled drug in order to measure the flux or solubility. The radiolabeled and unlabeled Piroxicam was of a high purity (>98%) as determined by HPLC. The apparent solubility of Piroxicam was determined by measuring the donor concentration in the vehicles at the conclusion of the flux experiments. In some cases, the solubility was also predetermined by overnight equilibration in a particular donor vehicle.

The receiver compartment contained Sorensen's isotonic buffer adjusted to a final pH of 7.38. Aliquots of 100 μl were periodically withdrawn from the receiver compartment, with replacement, to monitor the transport. The amount of drug in the receiver compartment was always negligible with respect to that in the donor chamber, such that sink conditions were effectively maintained throughout the experiment. Concentrations were measured by a conventional liquid scintillation technique. The donor and receiver solutions were also occasionally checked by HPLC. No evidence of metabolism or degradation was observed.

RESULTS AND DISCUSSION

Oleic Acid Uptake

The uptake of oleic acid by isolated porcine SC and

silastic membranes was measured from a series of ethanol:H_2O vehicles. As depicted in Fig. 1, the amount of oleic acid taken up by SC reached its peak value with the vehicle containing 40% ethanol. The uptake of oleic acid by silastic was similar, again with a maximum at 40% ethanol (Fig. 2), suggesting that the extent of uptake is governed by the thermodynamic activity of oleic acid in the applied vehicle. This inference is derived from the recognition that silastic is an inert, hydrophobic polymer of comparable lipophilicity to SC and that the physical properties of silastic are largely unaffected by most solvents or penetration enhances (17). In the silastic experiments, no significant changes in weight were observed (other than that due to the oleic acid), consistent with the idea that there are no solvent-induced membrane changes. While the same statement cannot be made for SC regarding exposure to 100% ethanol, the close semblance between the SC and silastic data would at least suggest that the amount of oleic acid taken up by the SC is not qualitatively affected by ethanol.

The bell shape of these curves can be partially explained by the fact that, above 40% ethanol, oleic acid is entirely solubilized by the vehicle. Consequently, increasing the ethanol percentage has the effect of reducing the effective distribution coefficient for oleic acid into the more lipophilic SC or silastic membranes. On the other hand, when the ethanol concentration is less than 40%, the vehicles are not homogeneous solutions. Without defining the number and physical properties of each existing phase, it is difficult to analyze the uptake results for these low ethanol vehicles. The uptake of oleic acid into SC exceeds that of silastic by a factor of almost 10. Correcting for the mass of lipid in normal SC (~10–15%) increases that calculation to roughly 2 orders of magnitude. Although the reason for this difference is not clear, it could indicate that some of the oleic acid is distributed in nonlipid components of the SC or that the bilayer is capable of significant volume expansion, in contrast to the rigid silastic polymer. In either case, the close similarity in the shape of the two uptake curves (i.e., SC and silastic), which correlate with flux and DSC data (see below), argues that the fraction of oleic acid in the bilayer, as a function of the applied ethanol concentration, is constant with respect to the total amount present in the SC.

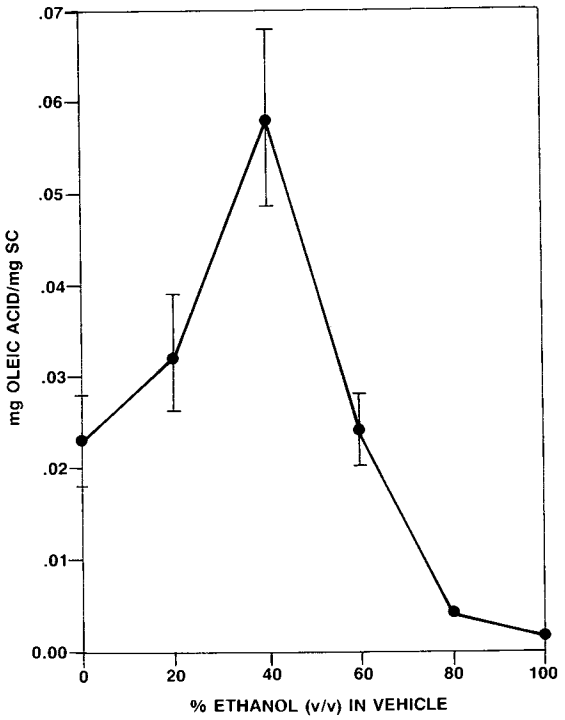

Fig. 1. The uptake of oleic acid by isolated porcine stratum corneum as a function of the vehicle ethanol concentration. All vehicles contained 0.25% (v/v) oleic acid. Error bars depict SE (n = 3).

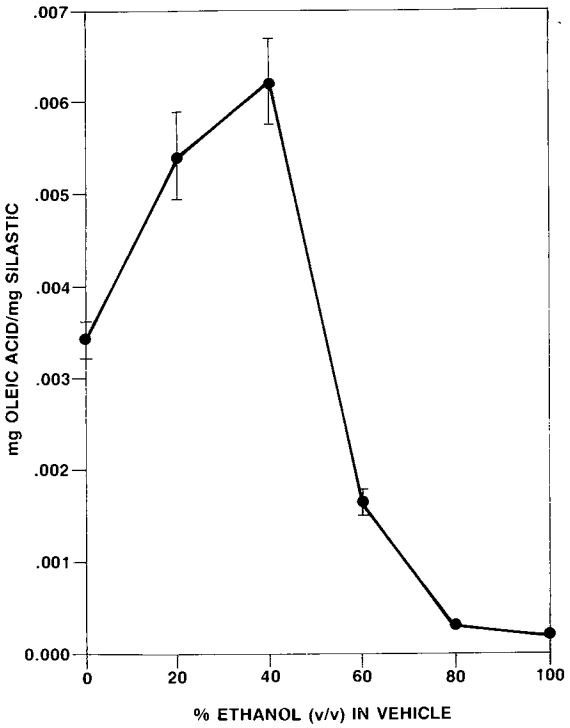

Fig. 2. The uptake of oleic acid by silastic membranes as a function of the vehicle ethanol concentration. All vehicles contained 0.25% (v/v) oleic acid. Error bars depict SE (n = 3).

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was employed to measure directly the effects of oleic acid on the thermal phase properties of SC. Untreated porcine SC undergoes three apparent phase transitions at about 60, 70, and 100°C (Fig. 3). The two lower endotherms are known to be the result of lipid-associated phase changes within the SC, while the transition at 100°C has been attributed to a denaturation of the α-keratin matrix within the corneocytes (18). Upon reheating, the two transitions at 60 and 70°C coalesce into a single endotherm at about 60°C, while the keratin peak is absent (18). The total enthalpy associated with the lipid peaks remains the same (i.e., ~0.7 cal/g SC) before and after reheating. While the diminution of the protein endotherm can be ascribed to denaturation, the reason for the irreversibility of the lipid peaks is not fully understood. One possible explanation for the concresence of the 60 and 70°C lipid endotherms may be a heat-labile association with a protein component of the SC, possibly the corneocyte envelope.

Vehicles of different ethanol:H_2O concentrations, all

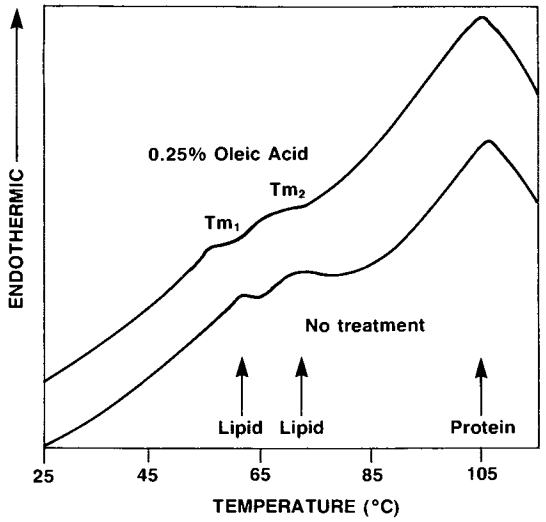

Fig. 3. Thermal transitions of porcine stratum corneum determined by differential scanning calorimetry. Compared are untreated samples and those treated with an ethanol:H_2O solution containing 0.25% (v/v) oleic acid.

containing 0.25% (v/v) oleic acid, were utilized to treat SC prior to evaluation in the calorimeter. As shown by the data in Fig. 3 and Table I, pretreatment of SC with oleic acid shifts the T_m of the first two endotherms to lower values with no apparent effect on the temperature of the third transition. The magnitude of this decrease in T_m is largest for the first lipid peak. This type of downward shift in T_m following the incorporation of *cis*-unsaturated FFA has been observed for phospholipid systems and explained on the basis of melting point depression (19). For the analogy of SC, the endogenous lipids would be the solvent and oleic acid would represent the solute. The net change in the first transition temperature (T_{m_1}) was calculated by subtracting its value from the untreated control. The plot shown in Fig. 4 indicates that T_{m_1} is most extensively reduced by exposure to the vehicles containing 40 and 50% ethanol. These are the same vehicles that delivered the greatest amount of oleic acid to the SC. In fact, the changes in T_{m_1} and the uptake are highly correlated ($r = 0.99$), indicating that oleic acid is markedly affecting the physical properties of SC lipids, suggesting that a constant fraction of this FFA is distributed into the lipid bilayer. The lack of effect on the 100°C transition implies that oleic acid perturbs primarily the lipids of the SC.

Table I. Effects of Oleic Acid on the Thermal Properties of Porcine Stratum Corneum

Vehicle treatment	ΔH (cal/g SC)[a]	T_m (°C)	Relative cooperativity ($T_f - T_i$) (°C)[b]
None ($n = 8$)	0.40 (0.07)[c]	60.4 (0.3)[c]	36 (1)[c]
	0.32 (0.03)	72.5 (0.02)	
40% ethanol:H_2O ($n = 4$)	0.25 (0.11)[c]	63.2 (0.9)[c]	28 (1)[c]
	0.26 (0.12)	73.6 (2.8)	
40% ethanol:H_2O, 0.25% oleic acid ($n = 4$)	0.27 (0.05)[c]	53.6 (1.1)[c]	44 (3)[c]
	0.18 (0.04)	69.8 (2.5)	

[a] Based on dry weight of stratum corneum.
[b] Temperature range over which both lipid transitions occur.
[c] Numbers in parentheses represent SE.

The enthalpy associated with the two lipid transitions is significantly decreased with oleic acid treatment (Table I). However, a similar reduction in enthalpy is also observed with the ethanol:H_2O control, indicating that the changes are not unique to oleic acid. Treatment of SC with oleic acid also increased the peak width of the overall lipid transitions relative to both controls. Assuming that a constant fraction of the oleic acid taken up by the SC is distributed in the intercellular lipids, this reduction of cooperativity suggests that the oleic acid present in the SC lipid bilayer is heterogeneously dispersed, and not highly clustered. Given the inertness and rigidity of the corneocyte envelope, it is also not likely that any oleic acid associated with the keratin or other nonlipid intracellular elements of the SC are indirectly responsible for the observed DSC changes.

Effect of Oleic Acid on Transport

The effect of oleic acid on the flux of Piroxicam as a function of the vehicle ethanol concentration is summarized in Tables II and III. A similar enhancement in transport is observed for both human and hairless mouse skin (HMS), albeit of significantly different magnitudes, reaching 200 fold

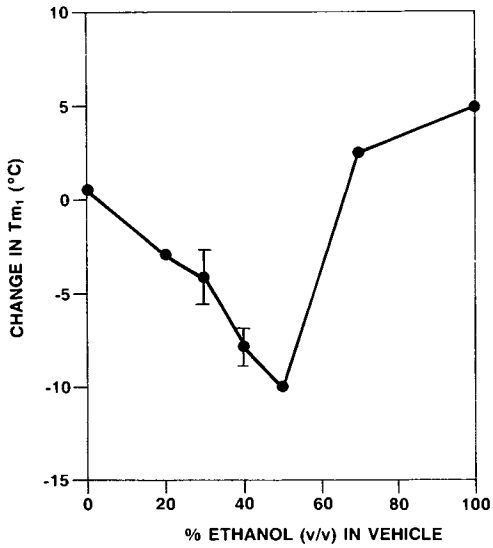

Fig. 4. The change in the transition temperature (T_{m_1}) for the second lipid endotherm (i.e., $T_{control}$–$T_{treated}$) in porcine stratum corneum as a function of the vehicle ethanol concentration. All vehicles contained 0.25% (v/v) oleic acid. Error bars depict SE ($n = 3$); for those points with no error bars, $n = 2$.

Table II. Piroxicam Flux Through Hairless Mouse Skin *in Vitro* with Various Ethanol:Buffer Vehicles (Each Containing 0.25% Oleic Acid) at 32°C

Ethanol (%, v/v)	C_d (mg/ml)[a]	K_p (cm/hr) × 10^2	Flux (μg/hr/cm²)	Extent of enhancement[b]
0	0.04	0.41 (.13)[c]	0.16	1.1
10	0.19	0.95 (.39)[c]	1.82	12.1
20	0.46	1.58 (1.38)	7.3	48.7
30	0.71	2.26 (.21)	15.9	106.0
40	1.19	2.55 (.61)	30.1	200.0
50	1.50	1.34[d]	20.0	132.0
100	1.20	0.12 (.05)	1.3	8.7

[a] Total concentration of drug saturated with respect to vehicle at pH 7.4.
[b] Flux relative to 100% buffer vehicle containing no oleic acid.
[c] Number in parentheses refers to the standard deviation.
[d] The average of only two replicates.

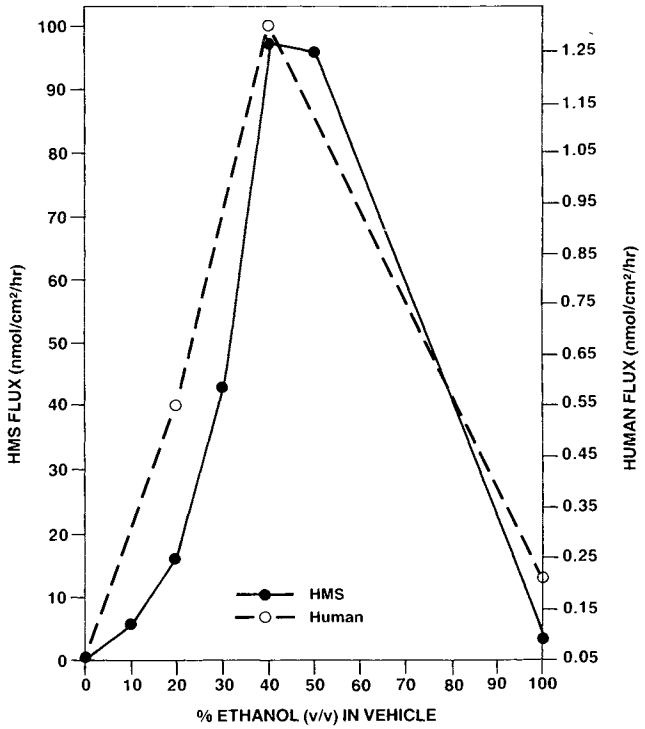

Fig. 5. The *in vitro* transport of Piroxicam across human and hair mouse skin as a function of the vehicle ethanol concentration. All vehicles contained 0.25% (v/v) oleic acid present as a penetration enhancer.

in the case of HMS. There is a marked flux "window" for the ethanol vehicle concentration (Fig. 5), which has also been observed for several other hydrophilic permeants tested in conjunction with either oleic acid or Azone (20). This parabolic flux behavior has also been reported in a recent publication describing the transdermal delivery of insulin from aqueous vehicles containing Azone and propylene glycol (21). Apparently, this window phenomenon for enhanced delivery applies to some large as well as polar molecules; however, it is unexpected on the basis of previous reports on oleic acid (14,15).

The plots shown in Fig. 4 (ΔT_m vs ethanol) and Fig. 5 (flux vs ethanol) are nearly mirror images of one another. Plotting the change in T_{m_1} (i.e., control–treated) versus the flux of Piroxicam across human and HMS does, indeed, yield a highly linear correlation (r = 0.95; Fig. 6) between flux and ΔT_m. A similar relationship was also observed for ΔT_m versus the flux of salicylic acid for a series of different

Table III. Piroxicam Flux Through Human Skin *in Vitro* with Various Ethanol:Buffer Vehicles (Each Containing 0.25% Oleic Acid) at 32°C

Ethanol (%, v/v)	Flux (μg/hr/cm²)[a]	Relative flux[b]
0	.02	0.3
20	.18	3.0
40	.43	7.2
100	.07	1.0

[a] Values are the average of two measurements which were within 20% of each other in all cases.
[b] Flux relative to 100% ethanol vehicle with 0.25% oleic acid.

FFA (13). Therefore, the enhanced transport of Piroxicam appears to be directly related to oleic acid perturbation of the SC lipids. While colligative properties may explain the lowering of the SC lipid T_m's by oleic acid, the relationship of this phenomenon to the mechanism of enhancing diffusion is not obvious, since at physiological temperatures (*viz.*, ~32°C), the SC lipids are largely unmelted even in the presence of oleic acid. Whatever the mechanism, it appears that the extent of enhancement may be predicted with the appropriate DSC experiments. Further, these correlations between flux, uptake, and ΔT_m indicate that the intercellular lipids are, indeed, a primary element of the microenvironment through which enhanced diffusion occurs.

Additional HMS experiments were conducted to ascertain the effect of pH on the enhanced transport of Piroxicam (Table IV). As expected, increases in the pH of the vehicle led to an increase in total Piroxicam solubility. Since each donor solution was saturated with respect to total drug concentration, the amount of unionized drug in the vehicle did not change appreciably over this pH range. However, the flux was significantly altered with changes in pH, varying from 3.8 to 26.7 μg/hr/cm². If permeation through the skin were only a function of neutral drug concentration (i.e., pH-partition hypothesis), changes in the vehicle pH should produce little or no effect on the transport. The diffusion of Piroxicam across untreated HMS was found to be independent of pH over the range of 4 to 5. The data obtained with oleic acid, though, indicate that the enhanced transport is dependent on the total Piroxicam concentration including the anion and neutral drug species. Oleic acid would appear

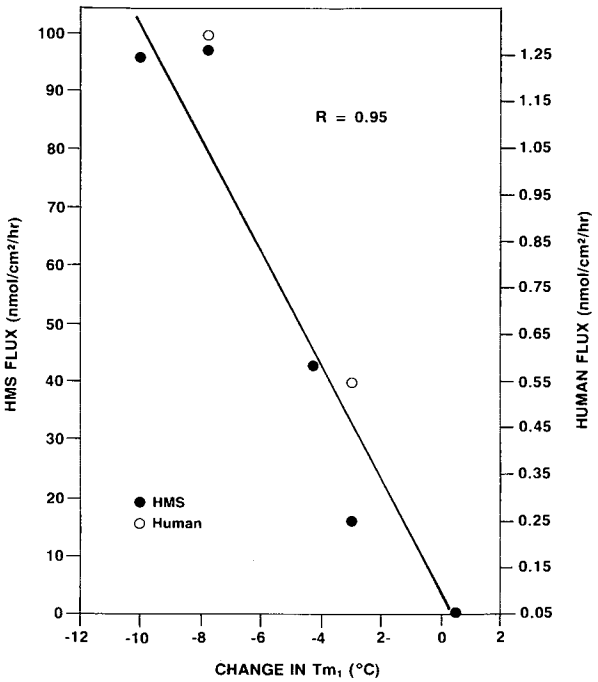

Fig. 6. Correlation (R = 0.95) of the change in T_{m_1} with the enhanced transport of Piroxicam across isolated human and hairless mouse skin.

in these studies to alter the properties of the SC such that the diffusion of ionized molecules is facilitated. The fact that both oleic acid and piroxicam are negatively charged at this pH, would also imply that the enhanced transport is not simply a matter of ion-pairing. Hence, the identification of highly soluble, hydrophilic salts in combination with oleic acid could yield dramatic improvements in absorption. This prognostication has, in fact, been tested for other ionizable drugs and found to be true (20).

Mechanism of Enhancement

The molecular mechanism remains to be determined by which oleic acid increases the transport of polar species. Recent X-ray diffraction and FTIR spectroscopic studies with hairless mouse skin have suggested that separate fluid and solid lipid phases coexist in untreated SC at normal physiological temperatures (22,23). The relevance of distinct phases to enhanced transport of polar or ionic species through skin can be inferred from the lipid biophysics literature. Unexpected high permeabilities for ions like Na^+ and K^+ in phospholipid liposomes have been reported at the lipid transition temperatures (24), where the gel and liquid crystalline phases would be in equilibrium. The increased permeability for these ions could be the result of diffusion across the phase boundaries or permeable interfacial "defects." Further, the data reported here for Piroxicam and elsewhere for other ionizable drugs (19) indicate that the enhanced flux through SC, in part, is dependent on the diffusion of ionized drug molecules. In view of the possible existence of separate fluid-solid domains in the SC and the analogies with facilitated ion diffusion through phospholipid vesicles at the T_m, penetration enhancement may result from similar interfacial "defects" in the SC lipids following treatment by oleic acid. Contrary to the increase in "fluidity" proposed by other authors (25), this mechanism accounts for the significant enhancement at physiological temperatures, well below the oleic acid-altered lipid transitions seen by DSC. The heterogeneous insertion of oleic acid throughout the bilayer would create numerous defects without necessarily fluidizing the endogenous SC lipids.

The effects of oleic acid on the thermal phase properties of the SC provide additional evidence for this phase separation hypothesis. As indicated previously, oleic acid selectively perturbs the inherent lipid structure of the SC reducing the transition temperatures and cooperativity associated with their phase properties. *Cis*-unsaturated fatty acids (*viz.*, oleic, linoleic) can cause gel-fluid phase immiscibility in phospholipid vesicles as measured by DSC and Raman spectroscopy (26,27). The results of these calorimetric studies (26) agree with the similar decrease in cooperativity and T_m obtained here for SC with oleic acid. Furthermore, these effects appear to be selective for *cis*-unsaturated fatty acids as they were not observed for saturated ($C \geq 12$) or *trans*-unsaturated acids. This behavior is consistent with the proposed model as transport studies have shown that the saturated ($C \geq 12$) or *trans*-unsaturated fatty acids have little, if any, effect on SC permeability (13).

The enhanced transport of polar or ionized molecules through phase-separated defects may require water to be associated with these interfacial regions. The presence of water in such regions has been inferred in work by Klausner *et al.* (19). With fluorescent probes, *cis*-unsaturated fatty acids (*viz.*, oleic, linoleic, and arachidonic) were found to partition preferentially into the fluid-separated regions of mixed-phase phospholipid vesicles and intact lymphocytes or isolated cell membranes. In contrast, the saturated or *trans*-unsaturated fatty acids (*viz.*, elaidic, stearic, and nonadecanoic) were found to distribute in the solid gel phase of these membranes or vesicles. Lifetime analysis of one fluorescent probe indicated that three distinct lipid domains may exist in these systems, which can be assigned to the gel, fluid, and interface regions of the bilayer. Subsequent fluorescent experiments with D_2O suggested that water is associated with this postulated interfacial area. Work by Anderson *et al.* (28) with a series of prodrugs of intermediate polarity has shown that the permselective properties of normal SC resembles that of a polar, hydrogen-bonding environment, unlike what would be expected for diffusion through a hydrocarbon phase. The association of water molecules with

Table IV. Effect of pH on the Solubility and Flux of Piroxicam for a 50% Ethanol Vehicle Containing 0.25% Oleic Acid: The Transport Studies Were Conducted *in Vitro* with Hairless Mouse Skin

pH	Solubility (mg/ml)	Flux (μg/hr/cm²)	Permeability × 10^3 (cm/hr)
4.2	1.1	3.8	3.4 (0.96)[a]
5.2	1.2	8.1	6.8 (3.63)[a]
6.7	2.3	26.7	11.5 (1.07)[a]

[a] Numbers in parentheses refer to the standard deviation.

inherent bilayer defects is consistent with the hypothesized phase separation model. Therefore, it follows that these defects may exist in normal skin as determinants of basal skin permeability. Recent work by Grubauer *et al.* (29) has demonstrated that TEWL is differentially affected by the type of lipids which are normally present in the skin. The results led the authors to conclude that the sphingolipids and free sterols are arranged in relatively cohesive lamellar structures, while the nonpolar lipids may be confined in a separate, more loosely bound SC intercellular compartments. Our results with oleic acid are at least consistent with the hypothesis that penetration enhancement may occur by a mechanism involving phase separation.

In summary, it appears that oleic acid is capable of selectively perturbing the lipids of the stratum corneum resulting in a net increase of Piroxicam flux. The effect is most likely specific for *cis*-unsaturated or short-chain ($C \leq 12$) FFA. The extent of the effect of oleic acid on lipid perturbation and flux is quantitatively related to the amount of FFA incorporated by the SC bilayer or to the shift in the endogenous lipid transition temperatures. Enhanced transport was found here to be dependent on the increasing concentration of ionized drug in the donor phase, suggesting that diffusion occurs by a mechanism different from the classical pH-partition hypothesis. This relationship of enhanced flux to the aqueous solubility offers the potential for improvements in drug delivery particularly, if highly soluble salts can be identified. While the effects of oleic acid on the SC will be identical regardless of the permeant, the mechanism of enhanced transport may be different for molecules of different polarities. The postulated mechanisms discussed here apply strictly to polar and moderately polar compounds. Finally, our data and the existing phospholipid literature are consistent with, but do not prove, a model of enhancement involving solid-fluid phase separation for the SC lipid domains, which is increased by oleic acid and selected other lipid perturbants.

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