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Topical Application of Penetration Enhancers to the Skin of Nude Mice: a Histopathological Study

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Abstract—Eighteen potential penetration enhancers, some at concentrations that might be used for that purpose, have been examined to evaluate their irritancy potential on nude mouse skin. A biopsy technique was employed followed by histological examination. Up to 50% glycerol, 10% hydroxyethyl lactamide (HELA), 10% oleyl alcohol, 10% Solketal, 10% glycofurol, 100% tetrahydrofurfuryl alcohol (THFA) and 10% urea induced no discernible change in the histological appearance of the skin whereas 100% dimethyl sulphoxide (DMSO), 100% dimethyl formamide (DMF), 100% *N*-methyl-2-pyrrolidone, 10% Azone, 10% oleic acid, 10% methyl laurate, 10% benzyl alcohol and 10% glycerol formal caused severe skin irritation.

Penetration enhancers are agents that increase the permeability of the skin, which itself is a complex stratified barrier the elements within which are affected by different enhancers. Some alter the composition of the cell content (Montes et al 1967), while others affect the cohesiveness between cells and the composition of the intercellular material (Pinkus 1952) or have a direct effect on the cell membrane (Cooper 1984). Recent studies suggest that a major route of skin penetration is through the intercellular channels. The composition of these has been identified and shown to contain structured lipids which undergo a solid-liquid phase transition around 40°C (Elias et al 1983). It is possible that some penetration enhancers act to disrupt the structure of the intercellular lipids and lower the phase transition temperature, thereby increasing the permeability of skin to more polar compounds. To increase the rate of transfer of lipophilic compounds it is necessary to modify the partitioning characteristics at the stratum corneum-viable tissue interface. This may be possible by combining a penetration enhancer with a cosolvent. Some agents can establish a reservoir in the stratum corneum, which may facilitate the diffusion of a drug (Feldman & Maibach 1974), and others, when penetrating the epidermis, may carry the drug through by acting as a solvent. Many of these agents may act by a combination of the various effects on the skin whilst others may be involved in a direct chemical insult on the skin. The exact mechanism by which most penetration enhancers work, however, is largely unknown.

Investigations of percutaneous absorption include the use of solvents which accelerate the penetration of drugs (Jacob et al 1964; Stoughton & Fritsch 1964; Maibach & Feldmann 1976; Mollgaard & Hoelgaard 1983; Barry et al 1984). However, few commercial products employ such accelerants. This is largely due to the lack of understanding of their mechanism of action and the degree of reversibility of the effects. Also their dermal and systemic toxicities have not been established.

The changes in skin produced by single or repeated applications of many commonly used raw materials contained in medicines and cosmetics have been widely studied (Cejka et al 1970; Delacretaz et al 1971; Ingram & Grasso 1975; Rantuccio et al 1979; Motoyoski 1983); but little effort has been made so far to establish the short and long term effects of penetration enhancers on the skin.

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Visual patch test reactions to many penetration enhancers have been reported in the literature (Barry et al 1984), but few investigators have used biopsies to assess skin reactions (Wright & Winer 1966; Nater et al 1977). Visual assessments usually rely mainly on dermal changes and may, therefore, give an incomplete picture. Histological assessment on the other hand enables epidermal changes also to be taken into account.

The purpose of the present study was to measure the skin irritation and/or skin damage produced by application of potential penetration enhancers on the skin of nude mice. Although not an ideal model for human skin, the aim was to use the tissue to establish the relative dermal tolerance of the penetration enhancers which may allow a more accurate and rational choice of enhancers for inclusion in commercial formulations.

Materials and Methods

A variety of penetration enhancers was selected to provide a representative group which may be expected to act by the different mechanisms postulated above. Dimethyl sulphoxide (DMSO), dimethyl formamide (DMF), propylene glycol, glycerol, *N*-methyl-2-pyrrolidone (NM Pyrr), *N,N*-diethyl-*m*-toluamide (DEET), oleic acid, oleyl alcohol, tetrahydrofurfuryl alcohol, glyceryl triacetate and urea were reagent grade and used as received (BDH Chemicals). Azone (Nelson Research), benzyl alcohol BP (Albright & Wilson), glycofurol (Hoffman La Roche), hydroxyethyl lactamide (HELA) methyl laurate (Sigma), Solketal and glycerol formal (Aldrich) were all used as received.

Most compounds were tested as 10% w/v solutions in purified water, but some were tested over a concentration range at which they have been found to be effective absorption enhancers. The test preparations were also gelled with 1% w/w neutralized carbomer (Carbopol 940—neutralized with sodium hydroxide) when possible, to enable

uniform dispersion of insoluble components. The 1% w/w neutralized carbomer gel was also tested as a control.

Male nude mice MF 1 h, source—Olac (1976) Ltd, an allelic variant of white mice, 4 weeks old and weighing 10 to 22 g (average weight 15 g) were used. The animals were not pretreated in any way before the experiment.

The test compounds were filled into a polyvinyl chloride (PVC) cup coated with polyvinylidine chloride (PVDC) of surface area of 0·8 cm^2 and volume 0·3 cm^3. One cup was fastened to the dorsal side of the animal using Blenderm surgical tape. A few drops of Superglue were used to secure the edge of the cup to the skin. Three animals were exposed to each preparation and were maintained three in a cage with food and water freely available throughout the experiment. The materials were kept in contact with the skin for 24 h.

Table 1. Histological assessment method.

Epidermal changes	
A. Epidermal thickening	
2 × normal in places	1
2 × normal generally	2
2–3 × normal in places	3
2–3 × normal generally	4
More than 3 × normal	5
B. Increase in the cell layers of the stratum granulosum	
by 1 cell layer	1
by 2 cell layers	2
by 3 cell layers or more	3
C. Hyperkeratosis	
Mainly loose	1
Mainly severe	2
Half loose half compact	3
Compact	4
Compact severe	5
D. Spongiosis	
Slight	1
Extensive	2
Microvesicle formation	3
Bullae formation	4
E. Intracellular oedema	1
F. Destruction of the epidermis	
Superficial	15
1/4 of sectioned area	18
1/2 of sectioned area	20
3/4 of sectioned area	25
Whole of sectioned area	30
G. Hyperaemia	
Slight (increased blood cell concentration in part of epidermis)	5
Moderate (increased blood cell conentration throughout epidermis	10
Extensive (continuous mass of blood cells in part of epidermis)	15
Dermal changes	
H. Increase in the density and thickness of the collagen bundles	
Slight (an increase in places)	1
Slight to moderate (an increase almost thoughout)	2
Moderate (an increase throughout)	3
Moderate to severe (bundles appear as continuous mass in places)	4
Severe (bundles appear as a continuous mass throughout)	5
I. Fractured collagen	
Slight (fractured in places)	1
Moderate (more than half the layer fractured)	2
Severe (fractured throughout)	3
J. Infiltration of the dermis	
Slight in the upper-most layer (an increase in cell numbers just detectable)	1
Slight diffuse (an increase in cell numbers just detectable)	2
Moderate in the upper-most layer (cell number almost double)	3
Moderate diffuse (cell number almost double)	4
Severe in the upper-most layer (cell numbers more than double)	5
Severe diffuse (cell numbers more than double)	6

Immediately after the mice were killed and, unless material had leaked from the cup during 24 h, when the results were not analysed, specimens of the exposed areas and of an adjacent untreated skin area were taken for histological examination. The skin pieces were pinned flat and immediately fixed in neutral formalin for at least 48 h, dehydrated through a graded series of alcohols, treated with antemedia and then embedded in paraffin. Sections of 5 μm thickness were cut from each sample and stained with haematoxylin-eosin for microscope observation. Three sections were selected randomly and examined using a scoring system modified from Ingram & Grasso (1975) (Table 1). The final score was the modal score from at least three animals.

Results and Discussion

The results from the experimental skin penetration

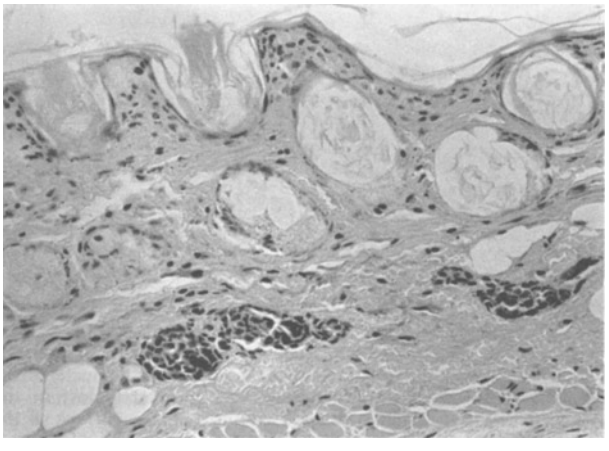

FIG. 1. Nude mouse skin: untreated back is adjacent to the treated area showing thin stratum corneum and stratum malpighi. H&E × 12·5.

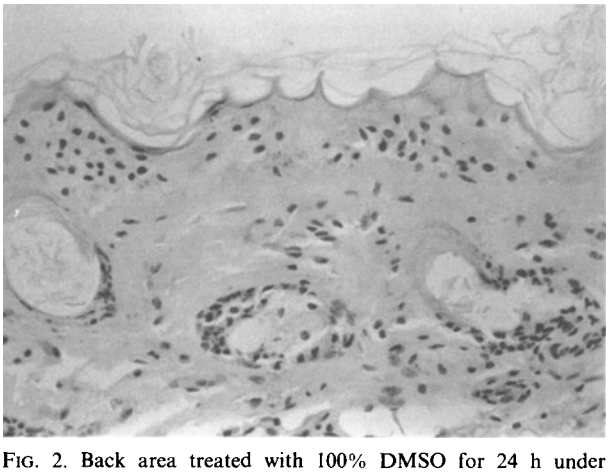

FIG. 2. Back area treated with 100% DMSO for 24 h under occlusion. The section shows hyperkeratosis marked acanthosis and disorganization of the prickle cell layer, intra-epidermal vesiculation and severe cell infiltration throughout the dermis and into the epidermis. H&E × 12·5.

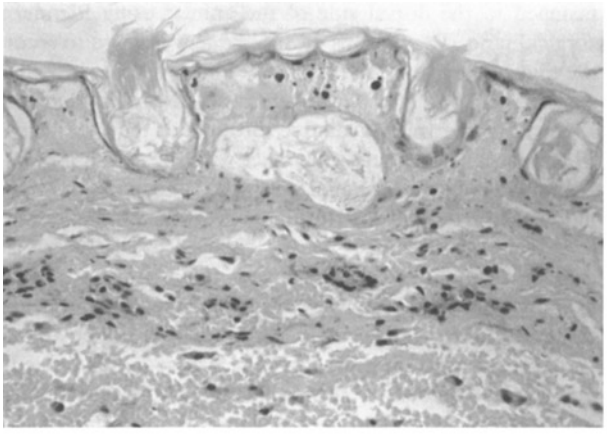

FIG. 3. Back area treated with 100% DMF for 24 h under occlusion. The section shows considerable loss of the cells nuclei in the epidermis, slight hyperkeratosis, gross alterations of the collagen fibres and oedema in the dermis together with some cell infiltration all indicating severe disorganization of the skin structures. H&E × 12·5.

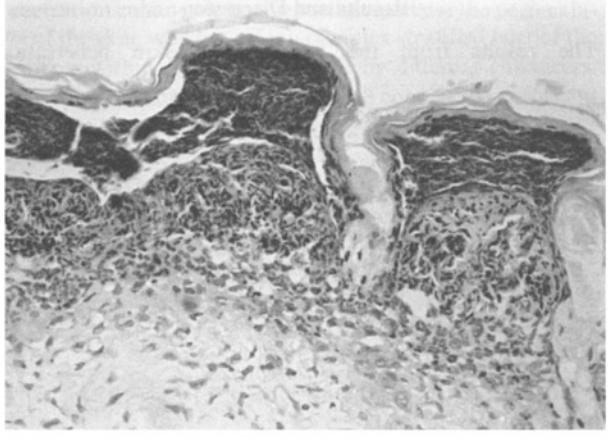

FIG. 4. Back area treated with 100% *N*-methyl-2-pyrrolidone. The photomicrograph demonstrates that the outline of the nuclei of the cells and the cell membranes had been completely lost in the epidermis and show large vesicles erupting on the surface of the epidermis. The collagen bundles in the dermis have become thicker and more dense, and have formed into a continuous mass in places. Ulcers and slight cell infiltration are also seen in the dermis. H&E × 12·5.

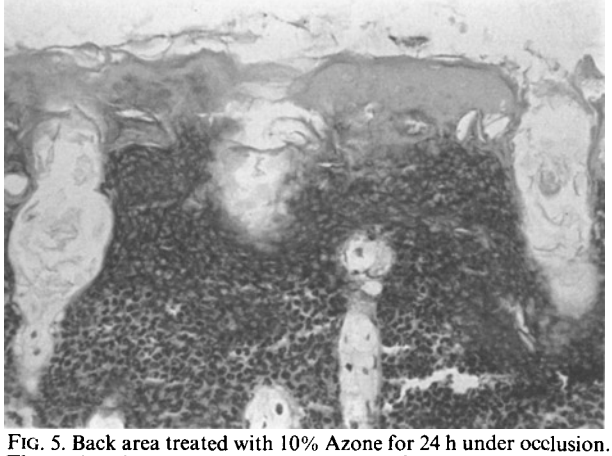

FIG. 5. Back area treated with 10% Azone for 24 h under occlusion. The section shows a condensed thick layer of stratum corneum over a crust in which severe hyperaemia may be seen. Extensive cell infiltration particularly in the upper part of the dermis can also be observed. H&E × 12·5.

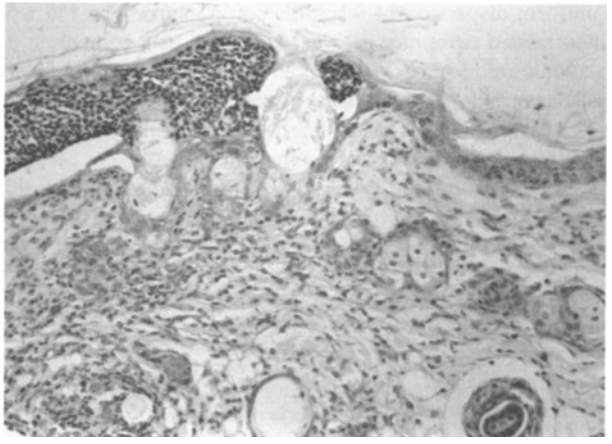

FIG. 6. Back area treated with 10% oleic acid for 24 h under occlusion. The section shows compact hyperkeratinized stratum corneum with ulcerative eruptions, hyperplasia and hyperaemia throughout the epidermis and oedema, alterations of the collagen fibres, and inflammation in dermis all indicating severe trauma. H&E × 12·5.

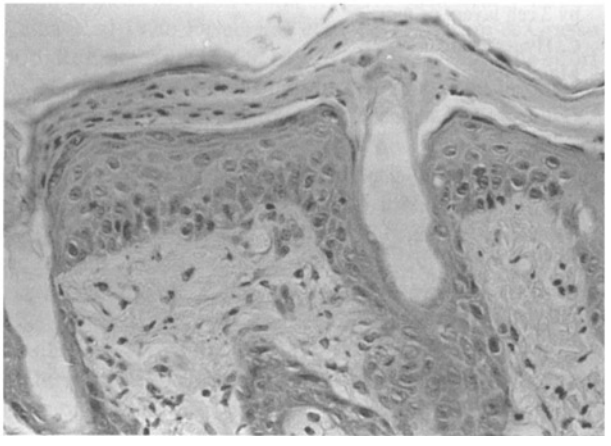

FIG. 7. Back area treated with 10% methyl laurate for 24 h under occlusion. The section shows partial compact hyperkeratosis with ulcerative eruptions, hyperplasia and some hyperaemia in the epidermis. Vesicles in the epidermis and dermis, hardening of the collagen bundles, and also oedema and inflammation in the dermis. H&E × 12·5.

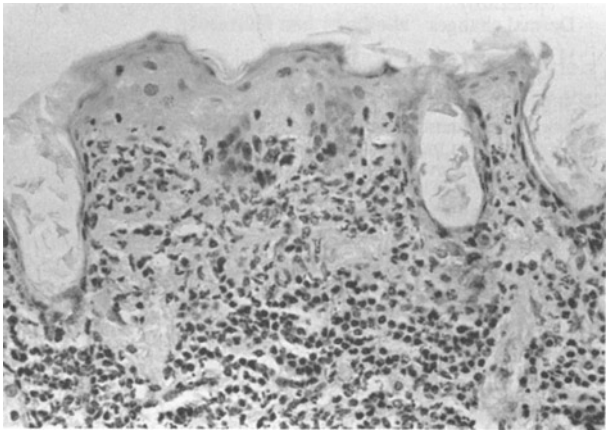

FIG. 8. Back area treated with 10% benzyl alcohol for 24 h under occlusion. The section shows severe compact hyperkeratosis, acanthosis, spongiosis, intracellular oedema and some ulcerative eruptions in the epidermis. The collagen bundles in the dermis appear slightly fractured, and there is some cell infiltration in the area. H&E × 12·5.

Table 2. Relative irritancy derived from score in Table 1 of various concentrations of the test compounds on the skin of nude mice.

Enhancer	Concentration (%)					
	1	10	25	50	75	100
DMSO	—	—	—	3	12	21
DMF	—	—	—	—	—	37
Propylene glycol	—	0	5	11	—	—
Glycerol	—	0	0	0	—	—
N M Pyrr	—	10	—	—	—	37
Azone	9	45	—	—	—	—
DEET	—	11	—	—	—	12
HELA	—	6	—	—	—	—
Oleic acid	—	51	—	—	—	—
Oleyl alcohol	—	6	—	—	—	—
Methyl laurate	—	29	—	—	—	—
Benzyl alcohol	—	22	—	—	—	—
THFA	—	—	—	—	—	10
Glyceryl triacetate	—	5	—	—	—	12
Solketal	—	0	—	—	—	—
Glycofurol	—	4	—	—	—	—
Glycerol formal	—	28	—	—	—	—
Urea	—	0	—	—	—	—

enhancers are summarized in Table 2. The histological score was obtained by adding the sum of the scores for the epidermal features (A–E) to the hyperaemia score (G) and the dermal score (H–J). Where destruction of the epidermis had taken place, the score for this alone (F) was combined with the hyperaemia score (G) and the dermis score (H–J). The histology scores provided a means of comparing the inflammatory effects of the different penetration enhancers. Preparations which scored from 0 to 10 were regarded as not causing undue reactions in the nude mouse skin. Preparations which scored from 11 to 20 caused skin reactions, which alone were not sufficiently extensive to exclude their potential use. Preparations which scored above 21 were considered to cause unacceptably severe damage.

Skin treated with 1% Carbopol gel for 24 h under occlusion showed that the stratum corneum had become loose compared with the untreated control, otherwise this section was no different from the untreated section. Seven of the enhancers: glycerol, HELA, oleyl alcohol, Solketal, glycofurol, THFA and urea, did not cause any significant change in the histology over 24 h at the concentrations tested. When 50% propylene glycol was applied, hypertrophy, dermal inflammation and proliferation stimuli were seen, indicating that at this high concentration an irritant effect may be expected. DEET and glyceryl triacetate also only produced a mild effect on the skin over the concentration range used. The eight remaining enhancers each provided an unacceptable irritant reaction at one of the concentrations tested.

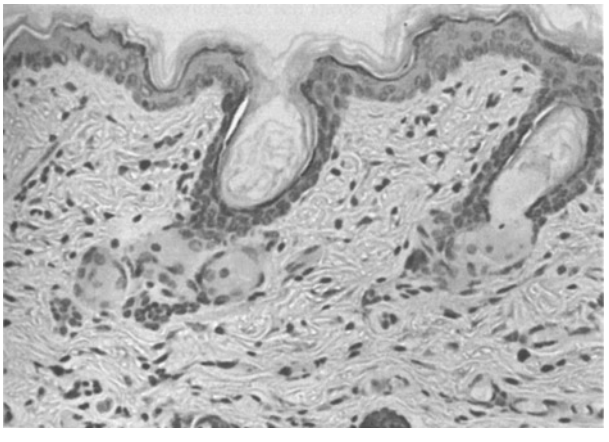

FIG. 9. Back area treated with 10% glycerol formal for 24 h under occlusion. The section shows extensive destruction of the epidermis together with hyperkeratosis, acanthosis and spongiosis. Cell infiltration of the dermis can also be seen. H&E × 12·5.

The histopathological findings for those enhancers are exemplified in Figs 1 to 9. The microscopic appearance of the treated skin was, in general, as follows: in the epidermis, acanthosis accompanied with swelling of the cells or proliferation of the basal cells and hyperkeratination; in the dermis, oedema cell infiltration and alteration of the collagen bundles. With the severe irritants such as glycerol formal, methyl laurate, oleic acid, Azone, *N*-methyl-pyrrolidone and DMF, extensive destruction of the epidermis together with ulcerative eruptions and hyperaemia was seen while the dermis appeared severely thickened and compact after most treatments.

The findings for DMSO, DMF and Azone were of particular interest. The results indicated that DMSO may not be as innocuous on the skin as suggested by Steinberg (1967) and Arno et al (1967). Sections treated with 50, 75 and 100% DMSO showed an increase in the histopathological changes, the 100% DMSO also causing an unacceptably high degree of irritancy. Our results are in good agreement with findings by Wright & Winer (1966). DMF was tested undiluted, and found to completely disrupt the normal structure, thus proving to be far more toxic to nude mouse skin than DMSO.

In our study, Azone was also found to be unacceptably irritant even though Stoughton (1982) reported that it did not cause any local irritations or sensitization on human skin.

Animal models are often used to assess percutaneous penetration and to ascertain how this process may be modified by enhancers. While it is often difficult to extrapolate the results to predict absorption rates in man, rough correlations are possible. Also, it is useful to have an indication of the 'damage potential' of enhancers, however, the animal results must be treated with caution, since some substances which enhance absorption in animals may do so as a result of histological damage which may be absent or less severe in humans.

The results of the present work provided no evidence of erythema, oedema or dryness of the skin (the usual visual signs of skin irritation) nor was there any indication of the chronic effects of treatment with the penetration enhancers examined, but there were indications of the kind of effects and the severity of the pathological changes that could be encountered. The study indicated that most penetration enhancers induced some inflammatory effects on the skin and that those enhancers described in the literature as being the most effective also caused the most severe irritation to nude mouse skin.

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