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# A biodegradable transdermal penetration enhancer based on *N*-(2-hydroxyethyl)-2-pyrrolidone I. Synthesis and characterization

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## Summary

Penetration enhancers represent a popular method of increasing drug flux through the skin for local or systemic activity. Unfortunately, it is thought that the local irritation commonly seen with penetration enhancers is directly related to the penetration enhancement ability of the enhancer. A potential method of avoiding irritation while maintaining enhancement is to utilize a 'soft' enhancer which is metabolized to inert components in the viable epidermis after achieving the desired effect in the dead cells of the stratum corneum. In the present report, model fatty acid esters of *N*-(2-hydroxyethyl)-2-pyrrolidone were synthesized in order to test this approach. It was found that a 2 order of magnitude increase in permeability for hydrocortisone through mouse skin could be achieved in vitro with these enhancers. The ester linkage was readily cleaved by hydrolytic enzymes in plasma and skin homogenates, while having relatively good solution stability at neutral and slightly acidic pH. Finally, these agents appear to have much less irritation potential than traditional penetration enhancers. Thus, this novel class of enhancers has a high potential for increasing drug flux without irritation in transdermal drug delivery.

## Introduction

The value of utilizing agents to increase the permeability of drugs through the stratum corneum has been recognized since the 1960's. Early penetration enhancers were primarily aprotic solvents such as dimethyl sulfoxide (DMSO) (Stoughton and Fritsch, 1964; Munro and Stoughton, 1965). However, these agents were typically effective only at high concentrations (> 50%) (Scheuplein and Ross, 1970; Kurihara-Bergstrom et al., 1986; Barry, 1987). The high dose and reported irritancy and toxicity of these agents have precluded their clinical use (Swanston et al., 1982; Woodford, 1987). Interest in transdermal penetration enhancers was renewed in the 1980s with reports of more lipophilic agents

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such as 1-dodecylazacycloheptan-2-one (Azone®) and *n*-decyl-methylsulfoxide that are effective at concentrations well below 15% (Oertel, 1977; Cooper, 1982; Stoughton, 1982; Barry, 1987; Shannon et al., 1985; Woodford, 1987). Simple fatty acids and alcohols have also been shown to be effective penetration enhancers at low concentrations when used in combination with propylene glycol (Cooper, 1984; Barry, 1987; Aungst, 1989).

Initial reports have suggested that newer penetration enhancers function with little or no irritation (Stoughton, 1982; Vaidyanathan et al., 1987). However, more recent studies suggest that there is a significant potential for irritation (Aungst, 1989), and that the extent of irritation may be related directly with the extent of permeation enhancement (Okamoto et al., 1988; Lashmar et al., 1989). Skin irritation caused by transdermal delivery systems is recognized as one of the critical constraints of transdermal drug delivery (Monkhouse and Huq, 1988).

The 'soft' drug approach is a potential way of avoiding undesirable side effects (Bodor, 1977). A biodegradable (soft) drug is synthesized so that it may elicit an effect and then break down to inactive and nontoxic fragments. A transdermal penetration enhancer would seem to be an ideal goal for this approach. The stratum corneum, the outermost layer of the skin and the target tissue for penetration enhancers, is metabolically inactive. However, enzymatic activity, hydrolytic in particular, has been recognized in the remaining layers of the skin (Ando et al., 1977; Elias et al., 1979; Yu et al., 1979; Ghosh and Mitra, 1990). Thus, a compound resembling a known penetration enhancer with a hydrolytically labile linkage would appear to be the ideal penetration enhancer. This approach has recently been used by several investigators (Wong et al., 1988, 1989a,b; Catz and Friend, 1989; Chukwumerije et al., 1989). In the present report, a novel class of penetration enhancers based on fatty acid esters of *N*-(2-hydroxyethyl)-2-pyrrolidone (HEP) is introduced. The synthetic, physical-chemical, biochemical, histopathological, and preliminary permeation enhancement properties of two model penetration enhancers of this class are reported.

## Experimental

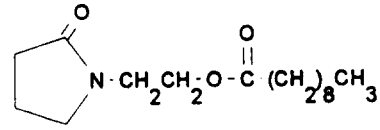
### *Chemicals*

*N*-(2-Hydroxyethyl)-2-pyrrolidone (HEP, Morton Thiokol, Danvers, MA), decanoyl chloride (Aldrich, Milwaukee, WI), oleoyl chloride (Sigma, St. Louis, MO), pyridine (Sigma), [$^3$H]hydrocortisone (NET 185, New England Nuclear, Boston, MA), and 1-dodecylazacycloheptan-2-one (Azone®, The Upjohn Company, Kalamazoo, MI) were used as received.

### *Synthesis*

*Decanoic acid, 2-(2-oxo-1-pyrrolidinyl)ethyl ester (HEP-Dec) (Scheme 1).* A solution of 4.56 g (0.035 mol) of HEP in 20 ml of tetrahydrofuran (THF) was added dropwise with stirring to a solution of 6.73 g (0.035 mol) of decanoyl chloride in 20 ml of THF at 0°C. The solutions were kept under nitrogen gas prior to and following the addition. Pyridine (2.77 g, 0.035 mol) was then added dropwise with continued stirring, and the solution was allowed to come to room temperature. Reverse-phase HPLC (acetonitrile : water (80 : 20), 1.5 ml/min, 210 and 254 nm detection) showed the formation of a new peak with a retention time of approx. 9 min, the area of which plateaued in under 1 h. Following the filtration of the pyridine salt, the THF was removed using a rotary evaporator under reduced pressure. Water was added to hydrolyze any remaining acid chloride, and the mixture was extracted with an equal volume of hexane. The hexane layer was extracted an additional time with 0.1 M HCl. The hexane was removed using a rotary evaporator, yielding a viscous liquid.

Fractions from preparative RP-HPLC of the above liquid (60 : 40 acetonitrile : water, 25 ml/




Scheme 1. Structure of the decanoic acid ester of *N*-(2-hydroxyethyl)-2-pyrrolidone (HEP-Dec).

min) were evaporated to remove the acetonitrile, and extracted three times with hexane. The hexane was dried with anhydrous magnesium sulfate and removed with a rotary evaporator, yielding the desired ester. Gas chromatography with flame ionization detection found no HEP or decanoic acid.

$^1$H-NMR ($CDCl_3$): $\delta$ 0.90 (3H, t, $CH_3$); 1.26 (12H, s, broad, $CH_3$-$\mathbf{[CH_2]_6}$); 1.61 (2H, t, $CH_3$-$[CH_2]_6$-$\mathbf{CH_2}$); 2.06 (2H, m, N-$CH_2$-$\mathbf{CH_2}$-$CH_2$-CO); 2.31 (2H, t, $COCH_2$); 2.39 (2H, t, $COCH_2$); 3.47 (2H, t, N-$CH_2$); 3.54 (2H, t, N-$CH_2$); 4.21 (2H, t, O-$CH_2$).

Anal.: Calc. for $C_{16}H_{29}NO_3$: C, 68.81; H, 10.32; N, 4.94. Found: C, 68.46; H, 10.64; N, 4.77. MS: 283; requires 283.

*Oleic acid, 2-(2-oxo-1-pyrrolidinyl)ethyl ester (HEP-Ol).* The reaction vessel was purged with nitrogen and then flamed to remove any water. A solution containing 1.313 g (0.017 mol) of pyridine and 6.432 g (0.051 mol) of HEP in 25 ml of THF was added to a solution of 5 g (0.017 mol) oleoyl chloride in 25 ml of THF. The mixture was stirred at room temperature for 15 min. The THF was removed by rotary evaporation under reduced pressure, and 20 ml of hexane was added to the reaction mixture, which was then extracted with 50 ml of deionized water. The extract was dried over anhydrous sodium sulfate and evaporated. Fractions from preparative normal-phase HPLC of the above liquid (60:40 THF:hexane, 15 ml/min) were evaporated to remove the solvent, yielding the desired ester. Analytical normal-phase HPLC (60:40 THF:hexane, 1 ml/min, 240 nm detection, retention time of approx. 9 min) and GC/MS analysis demonstrated greater than 95% purity.

$^1$H-NMR ($CDCl_3$): $\delta$ 0.88 (3H, t, $CH_3$); 1.27–1.30 (20H, d, broad, $CH_3$-$\mathbf{[CH_2]_6}$-$CH_2$-CH=CH-$CH_2$-$\mathbf{[CH_2]_4}$); 1.61 (2H, t, $[CH_2]_4$-$\mathbf{CH_2}$-$CH_2$-CO); 2.03 (6H, m, N-$CH_2$-$\mathbf{CH_2}$-$CH_2$-CO and $\mathbf{CH_2}$-CH=CH-$\mathbf{CH_2}$); 2.31 (2H, t, $COCH_2$); 2.38 (2H, t, $COCH_2$); 3.46 (2H, t, N-$CH_2$);3.54 (2H, t, N-$CH_2$); 4.21 (2H, t, O-$CH_2$); 5.34 (2H, t, CH=CH).

Anal.: Calc. for $C_{24}H_{43}NO_3$: C, 73.24; H, 11.01; N, 3.56. Found: C, 73.16; H, 11.03; N, 3.46. MS: 393; requires 393.

*Solid phase extraction*

Solid phase extraction was used to prepare samples for GC analysis. Bond Elut (Analytichem International, Harbor City, CA) octadecylsilane disposable columns (1 $cm^3$, 40 $\mu$m) were used with a Vac Elut system. The columns were activated with 1 ml of acetonitrile and rinsed with 1 ml of water. A known volume of an aqueous sample (0.5–1 ml) was slowly forced through the column with an applied vacuum (5 lb $inch^{-2}$). Following a 1 ml rinse with water, the sample was eluted with 5 ml of 100% acetonitrile. Finally, the internal standard was added to the eluent. The extraction procedure was validated by measuring HEP-Dec recovery from 1 ml samples of various concentration (10–100 $\mu$g/ml). The mean recovery was 106($\pm$19)%, and no concentration dependent trend was observed.

*$^1$H-NMR*

$^1$H-NMR spectra were run in $CDCl_3$ at 300 MHz on a Bruker AM 300. Chemical shifts are expressed in ppm down field from internal tetramethylsilane.

*Gas chromatography*

A Hewlett-Packard 5890A gas chromatograph (Palo Alto, CA) equipped with a 5970 Series Mass Selective Detector, 7673A Automatic Sampler, and an Ultra 2 (Hewlett-Packard) capillary column (12.5 m, i.d. 0.25 mm, 0.33 $\mu$m) were utilized. The operating conditions included an injector temperature of 235°C, oven temperature of 125°C, transfer line temperature of 275°C, and a helium flow rate of 0.7 ml/min. For the decanoate ester, the temperature gradient program used was an initial temperature of 125°C, increasing at a rate of 30°C/min to a final temperature of 230°C (retention time of approx. 9 min). For the oleate ester, the temperature gradient program used was an initial temperature of 155°C, increasing at a rate of 40°C/min to a final temperature of 310°C (retention time of approx. 8 min).

Standard solutions of HEP-Dec were prepared at concentrations of $3.5 \times 10^{-5}$, $1.1 \times 10^{-4}$, $1.4 \times 10^{-4}$, $1.8 \times 10^{-4}$, and $2.5 \times 10^{-4}$ M. The standards were prepared with a 9:1 solution of ethyl

acetate/hexane that contained the internal standard, Azone, at a concentration of $1.1 \times 10^{-4}$ M. These solutions were refrigerated and remained stable for the duration of the study. The standard curves were linear over the above concentration range. For quantitation, 60, 98, 111, and 126 $m/z$ were monitored.

*High-pressure liquid chromatography*

Analytical reverse-phase high-pressure liquid chromatography (RP-HPLC) utilized a Whatman (Maidstone, England) Partisil 10 ODS-3 column (25 cm, i.d. 4.6 mm) and guard column, Beckman 110A pump (Fullerton, CA), Waters Associates WISP 710B autosampler (Milford,MA), Milton Roy/LDC SM4000 UV detector, and a Nelson Analytical 760 Interface with Series 2600 software. Preparative RP-HPLC utilized the above stationary phase in a Whatman Magnum 20 column (50 cm, 22 mm i.d.), a Rainin Rabbit HP pump (Woburn, MA), a Rheodyne 10 injector with a 2 ml loop (Cotati, CA), a Tracor 970A UV detector (Austin, TX), a Perkin Elmer LCI 100 integrator (Norwalk, CT), and an LKB 2212 Helirac fraction collector (Bromma, Sweden). Analytical and preparative normal-phase HPLC runs were also performed with the above systems, using Whatman 10 $\mu$m silica as the stationary phase.

*Solubility studies*

Excess HEP-Dec was added to 0.9% saline in 1.5 ml microcentrifuge tubes kept at either 25 or 37°C using an Eppendorf (Hamburg, West Germany) 5320 Thermostat (done in triplicate). Samples were intermittently mixed (Eppendorf 5432 mixer), centrifuged for 5 min at 12000 rpm (Eppendorf 5415 microcentrifuge), and sampled for GC analysis.

*Stability studies*

The solution stability of HEP-Dec was determined as a function of pH at 37°C (Lauda RM 20 water bath, Brinkmann Instruments, Westbury, NY) using an initial concentration of 150 $\mu$g/ml. All buffer solutions were prepared from deionized water and ionic strength was adjusted to 0.15 M using NaCl. Approx. 50% of the ionic strength contribution came from the buffer species, as calculated by the program BUFCALC (Lambert, 1990). Samples were withdrawn at appropriate intervals, and rate constants and confidence intervals were determined from slopes of first-order kinetic plots. The pH of the solutions was measured at the conclusion of the studies, and found to be within 0.18 pH units of the initial pH in all cases.

*Plasma and skin homogenate studies*

Citrated human whole blood was centrifuged (Fisher Centrific, Springfield, NJ) for 10 min at 10000 rpm, and the plasma collected. Mouse plasma was generated in a similar manner using heparinized whole blood and a microcentrifuge. Female hairless mice (SKH-HR-1) were received from the Skin Cancer Hospital, Temple University, Philadelphia, PA. Mouse abdominal skin was excised and any underlying fat removed with scissors. Approx. 1 g of fresh skin was homogenized in 15 ml of a pH 7.4 phosphate buffer for several minutes using a tissue homogenizer (Tekmar Tissumizer, Cincinnati, OH). The homogenate was centrifuged and the supernatant fluid removed. The homogenate was cooled in an ice bath during the preparation. Where indicated, plasma and homogenate solutions were diluted with a $5 \times 10^{-3}$ M phosphate buffer, pH 7.4 ($I = 0.15$). Solutions were equilibrated to 37°C before the start of the study. The initial HEP-Dec concentration was 150 $\mu$g/ml. Samples were withdrawn over approx. 30 min for GC analysis.

*Skin irritation studies*

Transepidermal water loss (TEWL), as a quantitative marker of skin irritation, was measured using a ServoMed EP-1C evaporimeter as described previously (Scott et al., 1982; Blichmann and Serup, 1987). Male Sprague-Dawley rats (Upj:TUC(SD)spf) were used for the irritation study. Animals were randomized to treatments on the basis of basal readings to minimize differences between group mean basal values. The test substances were applied (200 $\mu$l of a 63 mM solution) to four rats per group once daily for 3 days from either ethanol or a 80:20 propylene glycol:ethanol mixture. Test materials were applied to a defined 1 inch$^2$ site on the mid-dorsum,

on 3 successive days. TEWL at the site was determined, under sedation, both before treatment began and on the day after the last treatment (day 4). The treatment response was the difference between the basal and the posttreatment reading for each individual. Clinical signs of irritation were scored daily (Draize et al., 1944).

After the final TEWL readings were taken the animals were euthanized by $CO_2$ asphyxiation and samples of treated skin were taken for histology. Tissue samples were fixed in 10% neutral buffer formalin, processed for routine light microscopy, sectioned and stained with hematoxylin and eosin. Two sections from near the center of the treated site were examined for each animal.

*Permeability studies*

The in vitro permeability coefficient of [$^3$H]hydrocortisone through full thickness hairless mouse skin was determined at 37°C using a modification of a previously described method (Lambert et al., 1989). The modification involved using normal saline as the donor and receiving solutions, and pretreating the stratum corneum side of the skin by applying 100 $\mu$l of neat enhancer to a 4 cm$^2$ area ($n = 2$).

## Results and Discussion

*Design of soft penetration enhancers*

A remarkable number of compounds have been reported to increase the permeation of drugs through the skin (Barry, 1987). At first glance, the shear number and variety would discourage most investigators from attempting a rational approach to generate an optimal enhancer. However, we believe that it is possible to categorize most penetration enhancers into three classes; those that act primarily as solvents and hydrogen bond acceptors (e.g., DMSO, DMAC, DMF), simple fatty acids and alcohols, and weak surfactants containing a moderately sized head group. The first group is of little interest due to irritation, potential toxicity, and the high concentration required. Fatty acids and alcohols function well, but appear to require the presence of propylene glycol in order to enhance permeation (Cooper, 1984; Barry, 1987; Aungst, 1989). The synergism with propylene glycol is not clear, complicating a rational formulation approach with these agents. In addition, one is forced to use two potentially irritating agents. The final group of agents is exemplified by Azone®, which has a 7-membered lactam ring and a dodecyl chain.

The approach used in the present study was to synthesize compounds which mimic Azone, yet can be readily hydrolyzed in vivo. An ester linkage would appear to be an obvious choice as a biodegradable functionality due to the ubiquitous nature of esterases. What is not obvious is the question of whether the linkage can be incorporated while still maintaining activity. Furthermore, should the alkyl portion utilize a fatty acid or alcohol? The answer to the first question is a primary objective of the present study. The latter question appears to be one of convenience and possibly local toxicity.

A number of choices were made in an attempt to choose the optimal structure. The first consideration was the ring size. Okamoto et al. (1988) and Mirejovsky and Takruri (1986) have shown that a ring size of 5–7 makes little difference in enhancement for derivatized lactams and cyclic amines, respectively. Thus, *N*-(2-hydroxyethyl)-2-pyrrolidone, a relatively inexpensive and nontoxic alcohol containing a 5-membered lactam ring was chosen. The second consideration was the fatty acid. A number of investigators have noted that an enhancer chain length of approx. 8–12 appears to yield the optimal enhancement (Takruri, 1986; Akitoshi et al., 1988; Okamoto et al., 1988; Tsuzuki et al., 1988; Chukwumerije et al., 1989; Mirejovsky and Aungst, 1989; Wong et al., 1989b). Thus, the decanoic acid ester of HEP was chosen. The oleic acid ester of HEP was also included in order to evaluate the effect of unsaturation in the alkyl chain. Unsaturated acyl chains can modify the 'fluidity' of lipid bilayers, albeit in a complicated manner (Stubbs and Smith, 1984). Aungst (1989) has reported that unsaturated fatty acids are more effective penetration enhancers than their corresponding saturated isomers.

*Physical-chemical properties*

In order to maximize the concentration of the penetration enhancers in the kinetic studies, it

was necessary to determine the aqueous solubilities. Knowledge of the solubility is also of interest for eventual formulation development of the penetration enhancers. Estimates of the solubilities of HEP-Dec and HEP-Ol were made using the Yalkowsky-Valvani equation for organic liquid nonelectrolytes (Yalkowsky et al., 1988)

$$\log S = c_1 \log K + c_2 \quad (1)$$

where $S$ is the solubility (molar), $K$ denotes the octanol-water partition coefficient, and $c_1$ and $c_2$ equal $-1$ and 0.8, respectively. The logarithms of the partition coefficients for HEP-Dec and HEP-Ol were estimated using the program CLOGP (Medchem Software, Version 3.4, Pomana College, Claremont, CA) and were found to be 4.03 and 7.19, respectively. Based on these values, the solubility at 25°C was estimated to be 0.167 and $1.60 \times 10^{-4}$ mg/ml, respectively.

The solubility of HEP-Dec was determined at 25 and 37°C (see Table 1). Two different amounts of the enhancer were added to separate tubes to ensure that an excess was present. There appears to be little or no temperature dependence for the solubility. The solubility value for HEP-Dec predicted by the Yalkowsky-Valvani equation is within 28% of the measured value. This is an excellent agreement considering the fact this value is based on an estimated partition coefficient.

The rate constants of HEP-Dec degradation are shown as a function of pH in Fig. 1. Linear first-order plots were obtained at every pH and the reactions were carried out through at least one half-life, with the exception of pH 4–6. This may explain the larger confidence intervals obtained for the rate constants in this range. The half-life at pH 6 is approx. 150 days. The shape of the pH-stability plot is typical of the U-shaped curves obtained for the hydrolysis of esters, which can be described by

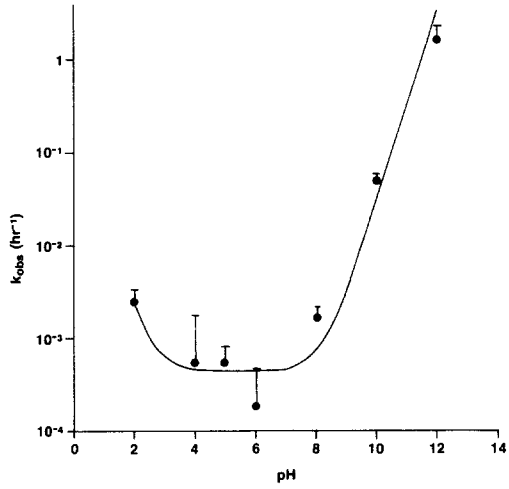



Fig. 1. pH-stability of HEP-Dec at 37°C (ionic strength of 0.15). The line represents the theoretical fit described by Eqn 2, and the bars denote the upper 95% confidence intervals.

$$k_{obs} = k_{H^+} a_{H^+} + k_{H_2O} + k_{OH^-} a_{OH^-} \quad (2)$$

where $a_{H^+}$, $a_{OH^-}$, $k_{H^+}$, $k_{OH^-}$, and $k_{H_2O}$ refer to the hydrogen ion activity, hydroxide ion activity, the second-order rate constants for specific acid and specific base catalysis, and the pseudo-first-order rate constant for hydrolysis, respectively. A regression fit (PCNONLIN, Statistical Consultants, Inc., Lexington, KY) of the data in Fig. 1 calculated $k_{H^+}$, $k_{OH^-}$, and $k_{H_2O}$ to be 0.216 (0.189) $M^{-1}$ $h^{-1}$, 342 (168) $M^{-1}$ $h^{-1}$, and 0.000449 (0.000182) $h^{-1}$, respectively (standard error in parentheses). The degradation rate observed at neutral pH and 37°C is dramatically slower than that seen in the presence of esterases (see below). Nonetheless, some attention must be paid to enhancer stability if these enhancers are to be formulated in aqueous based formulations.

TABLE 1

*Aqueous solubility of HEP-Dec at 25 and 37°C*

| Temperature (°C) | Solubility ($\mu$g/ml) | SD | $n$ |
|---|---|---|---|
| 25 [a] | 205 | 36 | 4 |
| 25 [b] | 199 | 17 | 3 |
| 37 [a] | 194 | 6 | 3 |
| 37 [a] | 211 | 25 | 3 |

[a] 400 $\mu$g of HEP-Dec added to the microcentrifuge tube.
[b] 800 $\mu$g of HEP-Dec added to the microcentrifuge tube.

*Enzymatic lability*

Preliminary studies of the enzymatic hydrolysis of HEP-Dec found the compound to be rapidly

TABLE 2

*Data for the enzymatic hydrolysis of HEP-Dec at 37°C*

| Sample | Time (min) | Concentration ($\mu$g/ml) | SD [a] |
|---|---|---|---|
| 10% mouse plasma | 0 | 116 | 5 |
| | 5 | 42.7 | 7.4 |
| | 10 | 30.4 | 0.9 |
| 20% mouse plasma | 0 | 73.2 | |
| | 10 | 30.1 | |
| 10% human plasma | 0 | 157 | 20 |
| | 4.5 | 68.3 | 16.2 |
| | 9.5 | 36.5 | 3.2 |
| | 14.5 | 30.0 | 0.6 |
| 20% human plasma | 0 | 150 | 6.5 |
| | 5 | 41.2 | 3.5 |
| | 10 | 30.5 | 0.4 |
| | 15 | 29.0 | 0.1 |
| | 20 | 28.4 | 0.1 |

[a] Performed in duplicate.

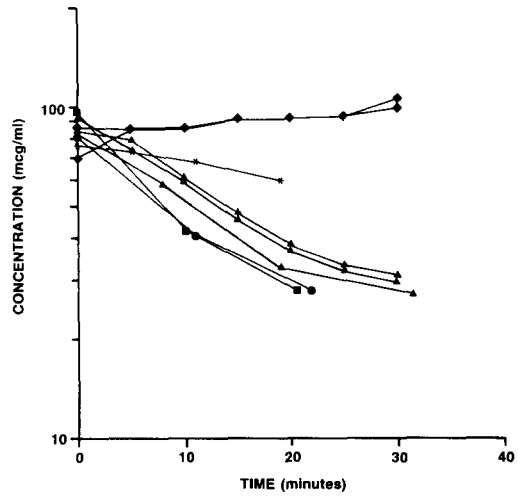



Fig. 2. Hydrolysis of HEP-Dec in various mouse skin homogenate dilutions (37°C). (●) 100%; (■) 50%; (▲) 25%; (∗) 12.5%; (♦) pH 7.4 buffer.

hydrolyzed in mouse plasma and skin homogenates. Thus, samples diluted with phosphate buffer were included in the present study in an attempt to slow the rate of hydrolysis and allow the determination of rate constants. HEP-Dec concentrations in various human and mouse plasma samples as a function of time are shown in Table 2. In less than 10 min, the concentration dropped to approximately one fifth of the initial value in all of the plasma samples. This is particularly apparent in the mouse plasma, where a significant drop in concentration is observed before the initial time point could be taken. The rate of hydrolysis appears to slow in the latter time points (> 50% degradation), possibly due to inhibition by the hydrolysis products.

Similar results were observed with the skin homogenate samples (Fig. 2). The first-order kinetic plots also appear to be nonlinear, with the apparent rate constant decreasing as the concentration of HEP-Dec decreases. Thus, no rate constants were determined. The 100 and 50% homogenate solutions appear to hydrolyze the enhancer at a similar rate. An apparent decrease in rate is observed with the 25 and 12.5% solutions. No hydrolysis is observed with a pH 7.4 phosphate buffer control.

TABLE 3

*Post-treatment TEWL values*

| Treatment | TEWL (mg cm$^{-2}$ h$^{-1}$) (SE) | | $p$ [a] | |
|---|---|---|---|---|
| | ETOH | PG:ETOH | ETOH | PG:ETOH |
| Vehicle | 0.20 (0.04) | 0.94 (0.01) | – | – |
| HEP | 0.13 (0.01) | 1.08 (0.05) | 0.6932 | 0.5593 |
| HEP-Dec | 0.22 (0.02) | 1.20 (0.04) | 0.9301 | 0.2940 |
| HEP-Ol | 0.86 (0.08) | 1.11 (0.16) | 0.0001 | 0.4941 |
| Decanoic acid | 0.25 (0.02) | 1.16 (0.04) | 0.7588 | 0.3776 |
| Azone | 0.98 (0.33) | 2.16 (0.24) | 0.0001 | 0.0001 |
| Oleyl alcohol | 0.44 (0.07) | 2.31 (0.40) | 0.1668 | 0.0001 |

[a] $p$ for comparison of TEWL with each enhancer vs the corresponding vehicle.

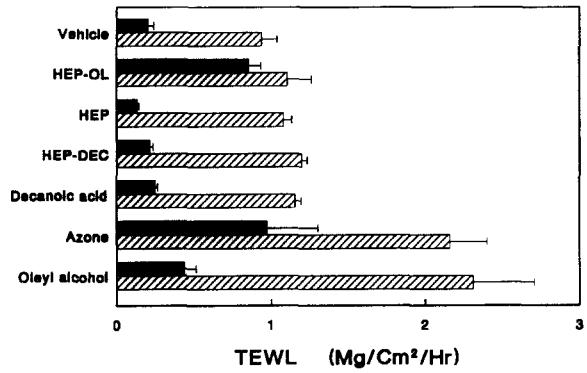



Fig. 3. Penetration enhancer effect on TEWL as a function of vehicle composition. Solid bars represent ethanol solutions and hatched bars represent propylene glycol/ethanol solutions (80:20). The error bars represent S.E. and $n = 4$.

*Changes in TEWL, clinical irritation and histopathology*

Changes in average TEWL, after three once daily treatments with 63 mM of several enhancers, each evaluated in either ethanol or propylene glycol:ethanol vehicles, are listed in Table 3. The $p$ values (two-sided) were obtained from a one-way analysis of variance with the enhancer vs the corresponding vehicle control. The average basal TEWL in comparable SD rats is 0.25 mg $cm^{-2}$ $h^{-1}$ ($\pm 0.008$, $N = 63$).

To reveal trends the same data are shown graphically in Fig. 3. Appreciable differences were seen in the specific activity of the materials, as a function of the vehicle used to apply them. We view ethanol as distributive vehicle, which is sufficiently volatile under non-occlusive conditions that it plays essentially no role in solute transport. Thus, any influence on TEWL seen with a material applied in ethanol reflects the intrinsic barrier diffusive and perturbational potential of that compound. By contrast, propylene glycol (PG) has a relatively low vapor pressure, persists on the skin surface and readily penetrates the stratum corneum, and thus, can act as co-solvent. This phenomenon is responsible for the potentiation effect seen with some, but not all solutes. Why the propylene glycol effect is not generally true is unknown.

Clear differences were seen among the materials, both with respect to enhancement with an ethanol vehicle (intrinsic action) and the degree to which the effect is potentiated by PG (facilitated action). HEP, HEP-Dec and decanoic acid all have minimal activity at 63 mM, in ethanol and likewise show little potentiation by propylene glycol. None of these agents were effective enhancers of TEWL under these assay conditions.

TABLE 4

*Structural changes in the epidermis accompanying topical application of skin penetration enhancers, in vivo*

| Treatment | Vehicle | Stratum Corneum | Granular layer | Basal-Spinous | Dermis |
|---|---|---|---|---|---|
| Ethanol (EtOH) | same | normal | normal | normal | normal |
| PG:EtOH | same | normal | normal | normal | normal |
| HEPD | EtOH | normal | normal | normal | normal |
| HEP | PG:EtOH | C + FPK | R/A | normal | normal |
| HEP-Dec | EtOH | normal | normal | normal | normal |
| HEP-Dec | PG:EtOH | C + FPK | R/A | normal | normal |
| HEP-Ol | EtOH | C + FPK | R/A | ↑ mitosis | normal |
| HEP-Ol | PG:EtOH | C + FPK | R/A | ↑ mitosis | normal |
| Decanoic acid | EtOH | normal | normal | normal | normal |
| Decanoic acid | PG:EtOH | C + FPK | R/A | ↑ mitosis | normal |
| Azone | EtOH | C + FPK | R/A | ↑ mitosis | normal |
| Azone | PG:EtOH | C + FPK + IA | R/A | ↑ mitosis | ↑ leukocytes |
| Oleyl alcohol | EtOH | FC | normal | normal | normal |
| Oleyl alcohol | PG:EtOH | C + FPK | R/A | ↑ mitosis | ↑ leukocytes |

C, compact; FC, focal compaction; FPK, focal parakeratosis; IA, intracorneal abscesses; R/A, reduced or absent.

Under the same assay conditions, HEP-Ol, Azone and oleyl alcohol all were active in ethanol. However, in the presence of propylene glycol both Azone and oleyl alcohol showed potentiation and HEP-Ol did not. The fact that the effect remains consistent for HEP-Ol under different vehicle conditions may be an advantage in obtaining consistent enhancement in combination with various excipients, while it is obvious that enhancers such as oleyl alcohol and Azone are sensitive to other components of the vehicle.

Clinical irritation was not observed with animals receiving HEP, HEP-Dec, HEP-Ol or decanoic acid in either vehicle. Two of four animals receiving Azone in ethanol and four of four receiving Azone in PG-ethanol had erythema by day 4. All three animals receiving oleyl alcohol in PG-ethanol showed erythema by day 4.

The structural changes in the epidermis following enhancer treatment were initially seen in the staining properties and organization of the stratum corneum (SC). Changes were also seen in the viable epidermis and in some cases the dermis (Table 4). Ethanol had little detectable effect on the epidermis, while propylene glycol caused mild stimulation as evidenced by an increased thickness of the cellular layers and subtle change in the staining properties of SC. The normal SC resists penetration of histologic dyes and thus, staining is usually restricted to the innermost layers in contact with the stratum granulosum. In the case of PG, individual squames had greater affinity for acidic stain components and a gradation in resistance to staining was not apparent. In addition to changes in the staining properties, the overall organization of the SC was altered. In tissue processed for conventional light microscopy, the strong lipid solvents used create the artifactual separation of individual squames in the normal SC leading to the loose, 'basket-weave' pattern seen in standard photographs of well preserved specimens. The most characteristic change in the appearance of the SC which parallels the increase in TEWL was a progressive compaction due to increased adhesiveness of individual squames. The increased cohesion of the cells persists, even in the presence of strong lipid solvents, which suggests that the lipids are altered or are not present. Simultaneously, the viable layers showed evidence of metabolic activation, cell volume increases, nuclei were enlarged, and an increased number of mitoses were present in the basal layer. As TEWL increases further, the epidermis appears to adapt to the increase in water flux by increasing the rate of cell division, and transit, which accounts for the disappearance of the granular cell zone. The cells reach this zone before having had time to reach this level of maturation. This also accounts for the gradual transformation of the SC from a highly differentiated, chemically resistent, diffusion barrier in which cellular elements are surrounded by specialized lipids, into a partially differentiated, water permeable, lipid depleted membrane comprised of chemically, permeable adherent cells.

*Correlation between TEWL and histomorphology*

The correlation between TEWL and skin structural events is complex and beyond the scope of this communication. Based on the data in Fig. 3 and Table 4, and other unpublished results of one of the authors (JMH), one can conclude that changes in water flux are generally observed with signs of toxic injury to the epidermis and dermal inflammation. The reverse is not always true (i.e., TEWL may increase without damage or toxicity and in the rat). Overall, TEWL did not directly correlate with irritation, either clinically or microscopically. The significance of this is that TEWL cannot be used as an exclusive measure of irritation. An example of this in the current study was Azone, which at 63 mM, caused injury to the epidermis in both ethanol and propylene glycol/ethanol vehicles, while the degree of increase in TEWL was not dissimilar from agents which showed essentially no evidence of toxicity at the same molar concentration.

It is true that mathematically there are likely to be parallel polar and lipophilic pathways (exclusive of trivial factors such as pilosebaceous and eccrine, shunts) through the stratum corneum (Scheuplein and Blank, 1971; Lambert, 1988) although none have been visualized experimentally. If such physical pathways actually exist, polar and lipid compounds would presumably permeate via different routes and a given enhancer will often

increase the permeability of various drugs to a different extent (Lambert et al., 1989), possibly by preferentially affecting a particular pathway. Thus, any increase in TEWL (which reflects alteration of the polar path) will not necessarily indicate a greater flux for all solutes. It is, however, likely that the more polar the solute the better TEWL will predict changes in overall flux.

*Permeation enhancement*

The ability of HEP-Dec to enhance the permeability of hydrocortisone was investigated in hairless mouse skin. Hydrocortisone was utilized since it is a compound of moderate polarity (log octanol-water partition coefficient of approx. 1.6; Hansch and Leo, 1979). The transport of hydrocortisone through human and mouse skin is known to be rate limited by the stratum corneum, and the permeability coefficient in mouse skin may be increased 100-fold by pretreatment with Azone (Smith, 1982; Lambert et al., 1989). In the present study, excised skin was pretreated with either HEP-Dec, Azone, or a saline control. Plots of cumulative amount permeated vs time are shown in Fig. 4. The permeability coefficients calculated from these plots are $3.08(0.856) \times 10^{-5}$, $1.76 \times 10^{-5}$, and $6.17 \times 10^{-8}$ cm/s, respectively (standard deviation given in parentheses for the HEP-Dec data). Thus, both Azone and HEP-Dec increase the permeability of hydrocortisone by well over 2 orders of magnitude. The permeability coefficient of hydrocortisone through stripped mouse skin (stratum corneum removed) is approx. $2 \times 10^{-5}$ cm/s (Lambert et al., 1989). This suggests that the permeation of hydrocortisone is no longer limited by the stratum corneum following the above penetration enhancer treatments.

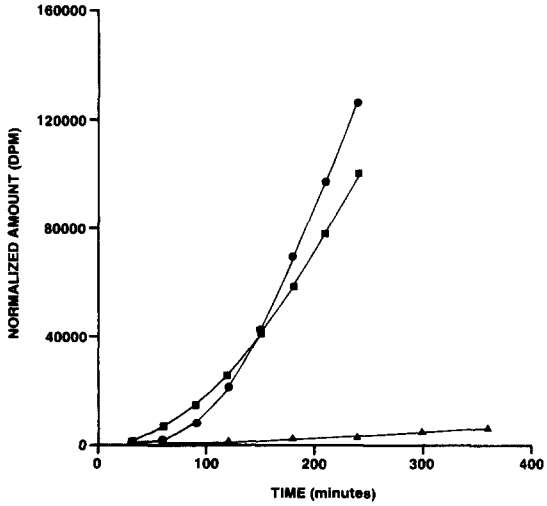



Fig. 4. Typical plots of amount of hydrocortisone permeating through hairless mouse skin at 37°C. The amounts are normalized with respect to the concentration of hydrocortisone in the donor side. (■) Azone treated; (●) HEP-Dec treated; (▲) control.

While the above treatments probably represent an over application of the penetration enhancers, they do provide strong evidence for the enhancement potential of these compounds. Recent experiments have investigated the enhancement of drug flux by HEP-Dec from more pharmaceutically relevant topical formulations. These results will be described in a subsequent report.

## Conclusions

The primary objective of the present study was to investigate whether it is possible to synthesize a biodegradable penetration enhancer with the appropriate properties for transdermal use. In addition to acting as an enhancer, the compound should be relatively solution stable, enzymatically labile, and well tolerated at concentrations which are effective in both passive and active vehicles. Fatty acid esters of HEP appear to meet this objective. HEP-Dec has been shown to be at least as active as the recognized penetration enhancer Azone, by increasing the in vitro permeability of a model permeant through mouse skin by nearly 500-fold. It has moderate solution stability in neutral to slightly acidic pH. Stability is anticipated to be much better in nonaqueous topical formulations since hydrolysis is anticipated to be the major degradation pathway. Enzyme catalyzed hydrolysis to the parent alcohol and fatty acid should be rapid in the viable epidermis, based on both skin homogenate and plasma kinetic studies. Finally, it appears that HEP, HEP-Dec and HEP-Ol exhibit much less irritation than traditional enhancers like Azone and oleyl alco-

hol. Thus, this novel class of penetration enhancers show much promise.

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