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# The percutaneous absorption of *m*-azidopyrimethamine: A soft antifolate for topical use

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## Summary

The in vitro transdermal transport of the soft dihydrofolate reductase inhibitor *m*-azidopyrimethamine (MZP), a potential antipsoriatic drug, has been studied from a range of aqueous propylene glycol vehicles. The determination of solubilities in the various vehicles used and the measurement of skin-vehicle and lipid-vehicle partition coefficients were also undertaken. Although permeation rates were slow, measurable fluxes across hairless mouse skin were observed which were dependent upon the composition of the vehicle. In nonbuffered aqueous propylene glycol the flux decreased with increasing propylene glycol while in buffered systems it increased as the pH was lowered. Here, the basic diaminopyrimidine is more fully protonated and suggests the involvement of the ionic form in transport. Oleic acid was the only member of a series of enhancers of percutaneous transport to significantly increase the flux of MZP. This effect is probably mediated by both ion-pair facilitation and by direct modification of epidermal barrier properties.

## Introduction

The 5-fold elevation of dihydrofolate reductase levels in psoriatic epidermis (Grignani et al., 1967) offers a rationale for the use of antifolate therapy in this disease. Their use to suppress the epidermal proliferation which is characteristic of psoriasis began with aminopterin (Gubner et al., 1951; Rees et al., 1955; Rees and Bennett, 1959). Shortly after, methotrexate supplanted aminopterin as a less toxic and more manageable regimen (Edmundson and Guy, 1958) and this drug is still an important treatment option in psoriasis. Non-classical, lipophilic antifolates have also been investigated (Roth and Cheng, 1982; Werbel, 1984) with pyrimethamine showing clinical activity (DiBella et al., 1977; Weinstein et al., 1981) but no advantage over methotrexate was perceived. Initial attempts to reduce systemic toxicity by topical application of methotrexate were unsuccessful (Fry and McMinn, 1967; Comaish and Juhlin, 1969; Stewart et al., 1972; Weinstein et al., 1981; Bjerring et al., 1986). Little penetration of the polar drug through the stratum corneum was apparent (McCullough et al., 1976) although in vitro studies allowed optimum conditions to be proposed (Ball et al., 1982; Vaidyanathan et al., 1985). Ester prodrugs were used in a strategy to improve topical availability (Weinstein and McCullough, 1975; McCullough et al., 1976) but only about 2% of the applied dose was absorbed. The in vitro use of

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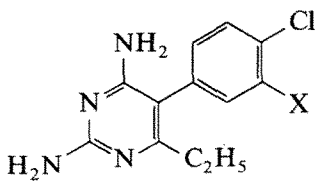
decyl methyl sulphoxide, an enhancer of percutaneous absorption, was also found to improve delivery but this could not be extended into the clinical situation. The use of Azone as an enhancer has, however, proved effective and clinical antipsoriatic efficacy has been demonstrated (McCullough and Weinstein, 1988; Weinstein et al., 1989).

To extend these studies we have investigated the in vitro topical delivery of the antiproliferative agent *m*-azidopyrimethamine (**1**). This is a lipophilic antifolate designed as a soft drug (Stevens et al., 1987) to overcome the problems of the extended biological half life of some 9 days exhibited by pyrimethamine (**3**). On metabolic reduction the azide is converted to an amino residue yielding *m*-aminopyrimethamine (**2**), a compound almost totally devoid of antifolate activity (Bliss et al., 1987). Such a compound might be expected to demonstrate topical absorption by virtue of its lipophilic nature but, due to its metabolic inactivation, it should limit its activity to the cutaneous tissue without the risk of systemic toxicity (Kamali et al., 1988; Baker et al., 1989). Aqueous propylene glycol mixtures were chosen for investigations as this cosolvent is widely used for topical pharmaceutical products (Cooper, 1984) with the cosolvent effectively increasing the skin penetration of various topical drugs (Lorenzetti, 1979).




(**1**) X = $N_3$ *m*-Azidopyrimethamine (MZP)
(**2**) X = $NH_2$ *m*-Aminopyrimethamine (MAP)
(**3**) X = H Pyrimethamine

## Experimental

*Chromatography*

HPLC analyses were undertaken using a system constructed from an Altex 100A dual-piston reciprocating solvent-metering pump and a reversed-phase stainless-steel Shandon-type column (10 cm × 4.6 mm i.d.) packed with Hypersil-ODS (5 μm). Samples were introduced by means of a Rheodyne 7125 injection valve, fitted with a 20 μl loop, and detection was accomplished with a Pye LC3 variable wavelength UV detector, fitted with an 8 μl flow cell, and operated at a wavelength of 250 nm with a sensitivity of 0.04–0.08 AUFS. The mobile phase consisted of aqueous acetonitrile (35%), containing diethylamine (0.1% v/v) adjusted to a pH of 2.5–3.0 with orthophosphoric acid. Typically, MZP and MAP were analysed in the 0.5–5.0 μg $ml^{-1}$ range with pyrimethamine (3 μg $ml^{-1}$ as internal standard).

*Preparation of skin and diffusion cells*

Male hairless mice (type MF1, Olac Ltd), 2–6 months old and 20–25 g in weight, were killed by cervical dislocation. The dorsal skin was carefully dissected and placed in ice-cold saline (0.9% w/v) and any adhering fat and subcutaneous tissue were removed from the dermal surface. The skin was mounted, epidermis uppermost, in a glass Franz-type diffusion cell (Franz, 1975) comprising an upper donor chamber and a lower receptor chamber, capacity 20–30 ml, secured with a spring clamp. The cell was jacketed to maintain the temperature at 32 °C by means of a recirculating water pump (Churchill). A side-arm sampling port, normally sealed, facilitated sampling of the receptor solution and a perspex lid was fitted to the donor compartment to minimise evaporation. The receptor solution was stirred magnetically with a teflon-coated stirring bar. The available area for diffusion between the two compartments was 2.4 $cm^2$.

*Preparation of test vehicles*

Test vehicles comprised both non-buffered (5–100% propylene glycol in water) and buffered aqueous propylene glycol mixtures. Suspensions in the non-buffered vehicles were prepared by stirring MZP free base (20 mg) in vehicle (10 ml) for 48 h in a sealed container protected from light. The pH of these suspensions was in the range 7.1–8.5. Suspensions were prepared in McIlvaine (Elving et al., 1956) or Britton-Robinson (Mongay

and Cerda, 1974) buffers (pH 4.4–8.97) containing propylene glycol (10% w/v). Addition of propylene glycol (PG) and MZP altered the pH of the aqueous buffers, usually to a slightly more alkaline value and pH values quoted are those of the final suspension. Penetration enhancer pretreatment solutions were prepared by mixing the enhancer to the required concentration (% v/v or % w/v) in an appropriate solvent. Enhancers used were 5% Azone in propylene glycol, 5% Azone with 0.1% Tween 20 in normal saline, 5% oleic acid (OA) in propylene glycol, 15% decyl methyl sulphoxide (DCMS) in propylene glycol and 5% DCMS in pH 5.0 buffer. Control experiments using propylene glycol, Tween 20 in saline and buffer as pretreatment regimes were also carried out. Suspensions of caffeine in buffered aqueous propylene glycol (10%) were prepared in the same manner.

*Permeation procedure*

The receptor chamber was filled with 50% aqueous ethanol (20–30 ml), previously de-gassed by sonication to prevent the accumulation of air bubbles at the skin-receptor fluid interface, and the skin was left to equilibrate with the receptor solution for 12 h. Any air bubbles which had accumulated at the top of the receptor chamber were removed via the sampling port by carefully tipping the cell assembly. The vehicle under study (1 ml) was introduced into the donor chamber and samples of the receptor solution (1 ml) were removed at various time intervals up to 48 h after addition of the donor phase. At each point the sample was replaced with a fresh aliquot of the receptor solution and samples were assayed by HPLC. The cumulative mass of drug transported was calculated according to:

$$M_t[n] = V_r \cdot C[n] + V_s \cdot \sum_{m=1}^{n-1} \{C[m]\}$$

where $M_t[n]$ is the current, cumulative mass transported across the membrane at time $t$, $C[n]$ is the current concentration in the receptor medium and $\Sigma\{C[m]\}$ is the summed total of the previous measured concentrations $\{m = 1 \text{ to } (n-1)\}$. $V_r$ is the volume of the receptor medium and $V_s$ is the volume of sample removed for analysis.

Pretreatment with putative enhancers was effected by applying the solution (1 ml) to the epidermal surface for 12 h. The pretreatment fluid was then removed, the skin surface was washed briefly with distilled water, and the donor phase of MZP suspension (1 ml) in propylene glycol (10%) in buffer (pH 5.0) was added. Studies with caffeine were performed similarly but with phosphate-buffered saline at pH 7.4 (NaCl, 8 g; KCl, 0.2 g; $KH_2PO_4$, 0.2 g; $Na_2HPO_4 \cdot 2H_2O$, 1.44 g in 1 l) as the receptor solution. The skins were left in contact with the buffer for 12 h to allow UV-absorbing materials to leach from the skin. Samples were collected, suitably diluted, and assayed for caffeine by UV spectroscopy at 273 nm against a buffer blank. Permeation studies were performed in triplicate, and all systems were protected from light for the duration of the study. Flux was taken as the slope of the linear part of the $M_t$ vs $t$ plot (typically between 12 and 36 h) and lag times were estimated by extrapolation. Permeability coefficients ($K_p$) were calculated by dividing the flux by the solubility of the permeant in the test vehicle.

*Solubility and partition determinations*

Solubilities were determined by stirring excess MZP (~ 20 mg) with the appropriate solvent or vehicle (10 ml) in a stoppered glass sample tube at 22 ± 2°C for at least 48 h. The suspension was left to stand for 6 h and an aliquot of the supernatant fluid was withdrawn and filtered through a 0.2 μm membrane filter (Millipore GS). The first 0.5 ml of filtrate was discarded, a second aliquot was diluted with methanol until drug content was within the range 0–5 μg $ml^{-1}$, and this dilution was assayed by HPLC.

Solutions of MZP were prepared in the vehicles described above [propylene glycol (10%) in McIlvaine buffer (pH 4.4–8.0)] or propylene glycol-distilled water mixtures (5–100% propylene glycol) at concentrations approximately half that of saturation. Aliquots (1 ml) of either isopropyl myristate (IPM) or a 0.1 M solution of oleic acid in IPM (IPM-OA) were added to 5 ml quantities of the MZP solutions in glass sample tubes. Parti-

tion into skin was measured by adding the buffered MZP solutions (5 ml) to sample tubes containing accurately weighed portions (~ 200 mg) of whole hairless mouse skin (from which adhering subcutaneous fat and other tissue had been removed). Duplicate tubes containing only the MZP solutions were included as controls to assess any loss due to uptake by glass. The tubes were gently shaken in a water bath at 37 °C for 72 h and were then left to stand for a further 24 h at 37 °C. Samples of the aqueous layer were taken, appropriately diluted with methanol, and were assayed by HPLC. The partition coefficient ($P$) between the two phases was calculated using:

$$P = \left[V_{aq}(C_0 - C_t)\right] / \left[V_{org} C_t\right]$$

where $C_0$ and $C_t$ are the concentrations in the aqueous phase initially and after partitioning respectively, and $V_{aq}$ and $V_{org}$ are the volumes of the aqueous and organic phases, respectively. The densities of the MZP solutions were determined using 25 ml density bottles so that weight ratios could be used for partition coefficient determinations for the skin samples. Triplicate determinations of the partition coefficient were made for each MZP solution in IPM, IPM with oleic acid or skin. pH values refer to the apparent pH after solution of the propylene glycol and MZP in the buffer, and were essentially the same as the pH values measured for the MZP suspensions used in the permeation studies.

## Results and Discussion

The solubility of MZP was determined in propylene glycol-water and propylene glycol-buffer mixtures and the mean saturated solubilities are recorded in Tables 1 and 2. The solubility of MZP increases exponentially with increasing propylene glycol concentration and is modelled by the equation:

$$\ln(S_f) = -5.778 + 7.175f \quad (r = 0.996)$$

where $S_f$ is the solubility of MZP in the mixed solvent system of cosolvent and $f$ is the volume fraction of the cosolvent. The intercept [$\ln(S_0)$] is the solubility of the drug in water and provides a value of 0.0031 $\mu$mol ml$^{-1}$ which corresponds to that measured experimentally. The slope ($\alpha$, 7.175) is a constant characteristic of the system.

TABLE 1

*Effect of propylene glycol concentration on the solubility of MZP in aqueous propylene glycol vehicles (figures in parentheses are standard errors of the mean)*

| Propylene glycol (%) | Solubility ($\mu$mol ml$^{-1}$) |
|---|---|
| 0 | 0.0031 (0.0015) |
| 10 | 0.0042 (0.0014) |
| 20 | 0.0057 (0.0011) |
| 30 | 0.0193 (0.0008) |
| 40 | 0.0445 (0.0036) |
| 50 | 0.1950 (0.0161) |
| 60 | 0.2690 (0.0353) |
| 70 | 0.4130 (0.0133) |
| 80 | 0.8050 (0.0092) |
| 90 | 1.4900 (0.0294) |

The solubility of MZP increases with decreasing pH in the buffered propylene glycol (10%) systems. The solubility in this, essentially aqueous, mixture is determined by the extent of ionisation of the molecule. MZP is a weak base (p$K_a$, 7.19; Stevens et al., 1987) and the more hydrophilic protonated species is thus favoured at lower pH values. MZP was found to be stable in the solvent

TABLE 2

*Effect of pH on the solubility of MZP in a series of 10% propylene glycol suspensions buffered with McIlvaine buffers (figures in parentheses are standard errors of the mean)*

| Initial pH | Final pH | Solubility ($\mu$mol ml$^{-1}$) | Ionisation (%) |
|---|---|---|---|
| 4.37 | 4.51 | 4.114 (0.112) | 99.79 |
| 4.98 | 5.07 | 1.511 (0.487) | 99.25 |
| 5.38 | 5.46 | 0.605 (0.153) | 98.17 |
| 6.05 | 6.12 | 0.210 (0.096) | 92.16 |
| 6.45 | 6.53 | 0.102 (0.048) | 82.05 |
| 7.04 | 7.13 | 0.0349 (0.020) | 53.45 |
| 7.53 | 7.60 | 0.0132 (0.0077) | 28.01 |
| 8.00 | 8.07 | 0.0095 (0.0072) | 11.65 |
| 8.82 [a] | 8.40 | 0.0032 (0.0018) | 5.81 |
| 9.64 [a] | 9.10 | 0.0026 (0.0022) | 1.22 |

[a] Britton Robinson buffers.

TABLE 3

*Partition data for MZP between propylene glycol (10%) in McIlvaine buffered solutions and isopropyl myristate, 0.1 M oleic acid in isopropyl myristate and hairless mouse skin (figures in parentheses are standard errors of the mean)*

| pH | Isopropyl myristate | Isopropyl myristate-oleic acid | Skin |
|---|---|---|---|
| 3.93 | [a] | 0.45 (0.10) | 1.98 [c] |
| 4.51 | [a] | 1.86 (0.34) | 3.11 (1.42) |
| 5.46 | 0.53 (0.03) | 19.53 (0.65) | 11.60 (1.25) |
| 6.12 | 2.07 (0.08) | 42.32 (6.53) | 17.41 (2.88) |
| 6.53 | 4.11 (0.33) | 53.72 (1.21) | 19.70 (1.51) |
| 7.13 | 12.22 (2.35) | [b] | 27.16 (0.07) |
| 7.60 | 19.88 (3.045) | [b] | 32.26 (0.07) |
| 8.40 | 25.80 (6.18) | [b] | 37.68 (2.91) |

[a] Partition into IPM not detectable.
[b] No detectable MZP in aqueous phase.
[c] $n = 1$.

systems with no degradation being detected during the course of these experiments.

Partition coefficients for MZP were determined between IPM, 0.1 M oleic acid in IPM or hairless mouse skin and 10% (v/v) propylene glycol in McIlvaine buffers (pH 4.4 to 8.0) and between propylene glycol-distilled water mixtures and IPM. The results are presented in Tables 3 and 4. As indicated by the solubility data, the affinity of MZP for the aqueous phase increases with the proportion of propylene glycol and caused a reduction in the partition coefficient. This trend in partition coefficient is also observed in the buffered systems where the fraction of the more water-soluble cation increases with a fall in pH. Although IPM has been proposed as a possible single-component model for skin lipids (Poulsen et al., 1968) the partition coefficients between the buffered MZP solutions and IPM and those between these solutions and skin do not correlate too well. The trends in partition data between the phases studied are, however, the same. The inclusion of oleic acid in IPM increases the partition into the lipophilic phase significantly across the pH range studied. At pH values 3.93 and 4.51 partition from the aqueous phase into IPM is undetectable. At pH 7.13, 7.60 and 8.40 partition into the oleic acid-IPM phase appears to be complete. At higher pH values the oleic acid will be predominantly ionised and be largely partitioned into the aqueous phase. At lower pH values ion-pairing between the MZP cation and oleic acid may thus contribute to the increased partition in the IPM-OA phase, whilst at higher pH values a general solubilisation enhancement in the lipophilic phase may occur.

TABLE 4

*Partition data for MZP between propylene glycol (PG)-distilled water mixtures and isopropyl myristate (figures in parentheses are standard errors of the mean)*

| Propylene glycol (%) | Partition coefficient |
|---|---|
| 0 | 55.69 (2.76) |
| 5 | 40.69 (4.31) |
| 10 | 20.59 (3.09) |
| 25 | 12.11 (2.26) |
| 40 | 5.25 (0.38) |
| 50 | 2.01 (0.29) |
| 60 | 0.88 (0.02) |
| 70 | 0.57 (0.03) |
| 80 | 0.29 (0.02) |
| 90 | 0.16 [a] |
| 100 | 0.06 [a] |

[a] $n = 1$.

In assessing the transport of MZP across hairless mouse skin it was found that distilled water, phosphate buffered saline or 50% propylene glycol as the receptor solution did not enable any permeation of MZP from an unbuffered 50% propylene glycol suspension to be detected. Various investigators have employed 50% ethanol as a receptor fluid for in vitro percutaneous penetration experiments (Akhter et al., 1984; Scott et al., 1986; Touitou and Fabin, 1988) and this receptor fluid was also found to be satisfactory in this case. The solubility of MZP in 50% ethanol is 2.3 mg $ml^{-1}$. All MZP concentrations determined in the receptor solutions during the course of permeation experiments were less than 10% of this value and thus sink conditions were approximated throughout these runs.

Permeation profiles were characterised by an initial lag phase which was followed after about 24 h by a steady-state period which was maintained for a further 12 h (Fig. 1). The non-linearity after this time suggests time-dependent changes in the permeation of the compound across the skin, pos-

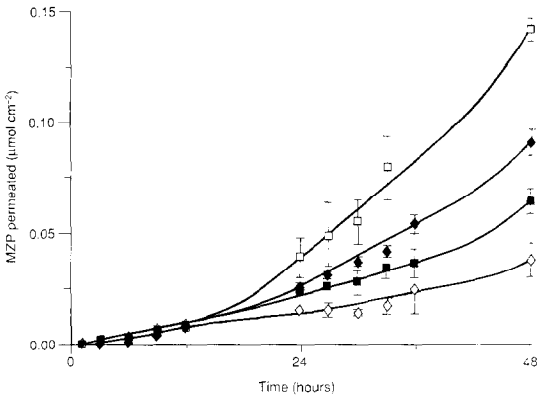



Fig 1. Effect of propylene glycol concentration on the permeation of MZP from suspension through hairless mouse skin [(□) water; (◆) 25%; (■) 50%; (◇) 100% propylene glycol] (points are mean values and error bars are ±SE).

sibly involving vehicle-induced changes in the diffusional resistance of the barrier. It is also difficult to conduct experiments for much longer than 24 h with hairless mouse skin and the observed increase in flux may also be due to the fragility of the membrane and a reduction in skin integrity. Turi et al. (1979) have found that propylene glycol readily permeates skin with a typical flux of about 1 mg $h^{-1}$ $cm^{-2}$ in hairless mouse skin.

The values of flux, permeability coefficient and lag-time for MZP from a series of unbuffered propylene glycol suspensions are presented in Table 5. Lag-times are large and, for the cosolvent vehicles, appear to increase with cosolvent concentration. The product of vehicle solubility and partition coefficient into IPM ($S \cdot P$) is constant with a mean value of 0.2241 (±0.02045) μmol $ml^{-1}$. If this behaviour were to be repeated with skin a constant flux would be expected, the activity coefficient of saturated solutions being unity (Sloan et al., 1986). As the flux values and permeability coefficients decrease with increasing concentrations of propylene glycol, the vehicle must be altering the barrier properties of the skin. This may, perhaps, be due to reduced hydration as the more concentrated vehicles are applied. The logarithm of the permeability constant is linearly related to the percentage of propylene glycol in the vehicle by:

$$\log K_p = 0.0265 - 0.0384[\mathrm{PG}(\%)] \quad (r = 1.0)$$

and the flux, more unexpectedly, is linearly related to the square root of the saturated solubility in the vehicle ($S^{1/2}$) by:

$$J = 0.0014 + 0.0167 S^{1/2} \quad (r = 0.99)$$

The dependence of skin permeability on vehicle composition, and hence drug partition, can be confirmed by a plot of the logarithm of the IPM-aqueous propylene glycol partition coefficient vs the logarithm of the permeability coefficient for MZP through hairless mouse skin. The linear relationship is:

$$\log K_p = 1.283 \log(P) - 2.214 \quad (r = 0.99)$$

The effect of varying pH from 4.51 to 9.10 on the penetration of MZP through a hairless mouse skin model is presented in Table 6 where the values of flux, permeability coefficient and lag-time for MZP from the series of buffered propylene glycol suspensions are recorded. Typical permeation profiles are shown in Fig. 2. The lag-time does not appear to vary with the pH of the applied donor phase while the flux decreases and the permeability coefficients decrease with increasing pH. These data suggest that both the ionised and unionised forms of MZP penetrate the skin.

TABLE 5

*Permeation data for MZP across hairless mouse skin from a series of unbuffered propylene glycol suspensions (Figures in parentheses are standard errors of the mean)*

| Propylene glycol (%) | Flux (μmol $cm^{-2}$ $h^{-1}$) | $K_p$ (cm $h^{-1}$) | Lag time (h) |
|---|---|---|---|
| 0 | 0.0040 (0.009) | 1.290 (0.290) | 12.8 (5.5) |
| 5 | 0.0028 (0.0001) | 0.667 (0.024) | 4.5 (1.6) |
| 10 | 0.0032 (0.006) | 0.561 (0.105) | 8.6 (0.9) |
| 25 | 0.0018 (0.0001) | 0.093 (0.0052) | 8.3 (0.4) |
| 50 | 0.0016 (0.0001) | 0.0082 (0.0005) | 11.5 (2.0) |
| 100 | 0.0010 (0.0004) | 0.0002 (0.0001) | 13.2 (4.5) |

TABLE 6

*Permeation data for MZP from suspension in a series of McIlvaine buffered 10% propylene glycol suspensions across hairless mouse skin (figures in parentheses are standard errors of the mean)*

| pH [a] | Flux ($\mu$mol cm$^{-2}$ h$^{-1}$) | $K_p$ (cm h$^{-1}$) | Lag time (h) |
|---|---|---|---|
| 4.51 | 0.0116 (0.0013) | 0.0028 (0.0003) | 8.3 (0.6) |
| 5.07 | 0.0066 (0.0010) | 0.0044 (0.0007) | 10.4 (1.0) |
| 5.46 | 0.0045 (0.0006) | 0.0074 (0.0010) | 6.4 (0.6) |
| 6.12 | 0.0029 (0.0009) | 0.0138 (0.0043) | 7.8 (2.0) |
| 6.53 | 0.0027 (0.0001) | 0.0266 (0.0010) | 6.7 (1.9) |
| 7.13 | 0.0013 (0.0001) | 0.0372 (0.0029) | 3.0 (1.6) |
| 7.60 | 0.0014 (0.0003) | 0.1060 (0.0230) | 7.8 (3.8) |
| 8.07 | 0.0023 (0.0003) | 0.2420 (0.0320) | 7.9 (0.4) |
| 8.40 [b] | 0.0017 (0.0001) | 0.5310 (0.0310) | 8.9 (1.8) |
| 9.10 [b] | 0.0016 (0.0002) | 0.6150 (0.0770) | 6.3 (0.5) |

[a] Apparent pH after addition of MZP.
[b] Britton Robinson buffers.

The observed partition coefficients, as a function of pH may be modelled by:

$$P_{obs}/\alpha = P_i + (1 - \alpha) \cdot P_u/\alpha$$

where $P_{obs}$ are the observed partition coefficients at various fractions ionised ($\alpha$) and $P_i$, $P_u$ are the partition coefficients of the ionised and unionised species, respectively (Irwin and Li Wan Po, 1979). When applied to the skin partition data this provides values of 16.72 ($\pm$0.45) for $P_u$ and 69.90 ($\pm$13.45) for $P_i$ ($n = 5$; $r = 0.999$) confirming the greater involvement of the ionised component. This is also probably the reason for the variation in permeability coefficients ($K_p = D \cdot P/h$) displayed in Table 6. These data are composite values determined by the diffusion and partition coefficients of both the protonated and unionised free base.

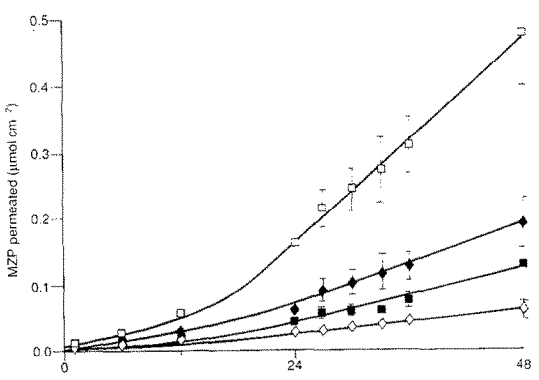



Fig. 2. Effect of pH of a 10% aqueous propylene glycol vehicle on the permeation of MZP from suspension through hairless mouse skin [(□) pH 4.51; (◆) pH 5.46; (■) pH 6.53; (◇) pH 7.60] (points are mean values and error bars are $\pm$SE).

To expose possible permeability changes due to skin damage caused by pH variation a series of pretreatment experiments were performed. Skins from hairless mice were pretreated for a period of 12 h with McIlvaine buffers at pH 4.37 and 7.57 and were then washed with distilled water. Transport of MZP from suspension in 10% propylene glycol (pH 7.60) through these samples provided a mean flux of 0.0019 $\mu$mol cm$^{-2}$ h$^{-1}$ for the pH 4.37 vehicle and 0.0025 $\mu$mol cm$^{-2}$ h$^{-1}$ at pH 7.57 ($n = 3$). These values, although higher, are not significantly different from those obtained from a pH 7.60 vehicle without the buffer pretreatment regime (0.0014 $\mu$mol cm$^{-2}$ h$^{-1}$) and therefore suggests that little skin damage is taking place at these pH values. The small rise in flux exhibited following pretreatments can probably be explained as the result of an increase in hydration of the skin specimen caused by the pretreatment regime.

It is possible that MZP permeates as an ion-pair with an anion present in the skin membrane. Sodium hexanesulphonate (5 molar equivalents) was included in the donor phase of an MZP suspension in 10% propylene glycol in order to a determine whether a lipophilic counterion would increase the flux of the cationic MZP species. The final pH of the donor phase was 5.84. The mean flux obtained was 0.0043 $\mu$mol cm$^{-2}$ h$^{-1}$. This was not significantly different from that obtained in the absence of the counterion (0.0045 $\mu$mol cm$^{-2}$ h$^{-1}$) at pH 5.46 and suggests no role for hexanesulphonate in this system. Protonated MZP may yet ion-pair with an in situ anion within the stratum corneum and exhibit facilitated transport as described for both anionic (Barker and Hadgraft, 1981) and cationic (Green and Hadgraft, 1987) permeants. The pH gradient in human skin ranges from 4.5 to 5.5 in the outermost layers to near 7.0 in the dermis (Katz and Poulsen, 1971).

Fatty acids, which are a component of skin lipids, will be largely ionised at these pH values while MZP will be significantly protonated within this range. The possibility exists therefore that facilitated transport, via vectors as yet unidentified, may still occur during percutaneous transport of MZP.

The effect of penetration enhancers on the transport of MZP through hairless mouse skin was also studied. During the course of this work reservations concerning the applicability of this skin model to study penetration enhancement of relevance to the human situation have been expressed (Bond and Barry, 1988a,b). However, its use in this instance is justified to enable comparison with earlier data to be made. Oleic acid was used as a solution in propylene glycol and Azone and DCMS were applied in both aqueous and propylene glycol-based solutions. Oleic acid in propylene glycol, Azone in propylene glycol and as an emulsion in 0.1% (w/v) Tween 20 in normal saline, and DCMS as a propylene glycol and aqueous solution have been used extensively by Barry and coworkers (Barry and Bennett, 1987; Goodman and Barry, 1986, 1988). The penetration enhancers were applied in a pretreatment regime of 12 h duration, followed by delivery of MZP from a 10% propylene glycol (pH 5.07) suspension. Control pretreatments comprised a solution of Tween 20 in normal saline, 10% propylene glycol in McIlvaine buffer (pH 4.98) and this buffer alone. Propylene glycol was used as a control for enhancer solutions based on this solvent and also to test for enhancement effects due to the solvent itself. Pretreatment periods to assess the effect of penetration enhancers have been used by others (Sherertz et al., 1987; Bond and Barry, 1988b) to allow the effects of the enhancers to be identified and isolated from other vehicle effects. There may also be clinical relevance (Kaidbey, 1976).

The flux values obtained from these experiments are presented in Table 7. Only oleic acid (5%) in propylene glycol significantly altered the permeation profile compared to that of the untreated control experiment. This material has been proposed as an effective penetration enhancer for lipophilic drugs (Cooper, 1984) and is an effective penetration enhancer for such molecules as 17-$\beta$-oestradiol, triamcinolone acetonide, trifluorothymidine (Loftsson, 1989), acyclovir (Cooper et al., 1985), 5-fluorouracil (Bond and Barry, 1988b), theophylline (Sherertz et al., 1987), naloxone (Aungst et al., 1986) and tetrahydrocannabinol (Touitou and Fabin, 1988). Its mode of action has been proposed to be that of a disruption of the ordered straight chain lipids in the stratum corneum caused by the kinked alkyl chain of the cis-octadec-9-enoic acid molecule which leads to an increase in lipid fluidity and a decrease in the resistance to permeation by the barrier. Oleic acid has also been shown to facilitate the permeation of cationic $\beta$-blockers across a lipoidal membrane (Green and Hadgraft, 1987) and it may exert its enhancing action through fluidisation of the intercellular skin lipids and also by ion-pair facilitation (Green et al., 1988). This ion-pairing effect can be seen for MZP in the partition data presented in Table 3 for oleic acid (0.1 M) in IPM where

TABLE 7

*Permeation data for MZP across hairless mouse skin from a suspension vehicle (10% propylene glycol in pH 4.98 McIlvaine buffer) following a 12 h pretreatment period with penetration enhancers (figures in parentheses are standard errors of the mean)*

| Pretreatment | Flux ($\mu$mol cm$^{-2}$ h$^{-1}$) | $K_p$ (cm h$^{-1}$) | Flux ratio [c] |
|---|---|---|---|
| Azone 5% in PG | 0.0055 (0.0014) | 0.0036 (0.0009) | 1.4 |
| Azone 5% in TS [a] | 0.00070 (0.0029) | 0.0046 (0.0019) | 1.4 |
| Oleic Acid 5% in PG | 0.0657 (0.0094) | 0.0435 (0.0062) | 17.3 |
| DCMS 15% in PG | 0.0044 (0.0003) | 0.0029 (0.0002) | 1.2 |
| DCMS 5% in buffer [b] | 0.0099 (0.0010) | 0.0065 (0.0007) | 1.3 |
| TS | 0.0049 (0.0025) | 0.0032 (0.0004) | |
| PG | 0.0038 (0.0003) | 0.0025 (0.0002) | |
| PG 10% in buffer | 0.0043 (0.0009) | 0.0028 (0.0006) | 0.6 |
| Buffer | 0.0015 (0.0050) | 0.0050 (0.0010) | |
| No pretreatment | 0.0066 (0.0010) | 0.0044 (0.0007) | |

[a] Tween 20 (0.1% v/v) in normal saline.
[b] McIlvaine buffer pH 4.98.
[c] Ratio of flux through skin pretreated with penetration enhancer to that pretreated with appropriate control (TS, PG or buffer).

partitioning into the lipid phase at all pH values is enhanced compared to the IPM phase alone. This suggests that MZP does, indeed, exhibit facilitated transport and that the enhancement observed here is probably due to both lipid modification and ion-pairing as observed earlier by Hadgraft and co-workers for naphazoline (Green et al., 1988). When oleic acid was incorporated into the propylene glycol used to prepare the MZP suspension, flux levels were not significantly higher than those obtained from a vehicle without the oleic acid. The flux values were $0.0072 \pm 0.0023$ (SE) $\mu$mol cm$^{-2}$ h$^{-1}$ for the vehicle including the oleic acid and $0.0066 \pm 0.0010$ (SE) $\mu$mol cm$^{-2}$ h$^{-1}$ for an equivalent vehicle without oleic acid. This parallels the earlier result afforded by hexanesulphonate and suggests that saturation of the membrane with carrier is essential for facilitated transport of MZP.

To isolate the roles of ion-pairing and lipid-modification in the enhancement afforded by oleic acid, the weak base caffeine was used as the permeant. Caffeine ($pK_a$, 0.6; Florence and Attwood, 1989) will be unionised at the pH values of interest and therefore facilitated transport due to ion-pairing should be eliminated. Caffeine suspensions in propylene glycol (10%) in McIlvaine buffers at pH 5.0, 6.4 and 8.0 were used as donor phases. Pretreatment experiments involved a 12 h exposure to either oleic acid (5%) in propylene glycol, propylene glycol in McIlvaine buffers pH 5.0, followed by permeation of caffeine from a suspension in propylene glycol (10%) in McIlvaine buffers (pH 5.0). The permeation profiles are presented in Fig. 3 and steady-state flux values (24–48 h) are summarised in Table 8. As expected, the pH variation did not affect the transport of caffeine across the hairless mouse skin. In contrast, pretreatment with oleic acid (5%) in propylene glycol resulted in a rapid increase in flux in the early stages of the experiment to $446.84 \pm 30.41$ (SE) $\mu$mol cm$^{-2}$ h$^{-1}$ over the first 6 h of the experiment. This reverts to a value approximately equivalent to that obtained without pretreatment after about 12 h which is some 6 times less than the initial flux. This indicates that the observed penetration enhancement, which is probably due to lipid fluidisation, appears to be reversible and probably corresponds to depletion of the oleic acid depot in the skin membrane. These data confirm the duality of the oleic acid enhancement, and show that both ion-pairing and direct modification of cutaneous barrier properties mediate the transport of MZP.

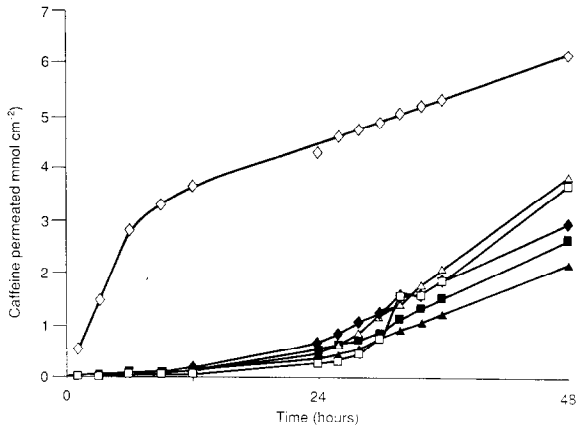



Fig. 3. Permeation profiles for caffeine from suspension through hairless mouse skin [without pretreatment: vehicles consist of buffered propylene glycol (10%) at: (▲) pH 5.0; (△) pH 6.4; (◆) pH 8.0; with pretreatment: vehicle consists of buffered propylene glycol (10%) at pH 5.0 with pretreatments of: (◇) oleic acid (5%) in propylene glycol; (■) propylene glycol; (□) buffered propylene glycol (10%) at pH 5.0].

TABLE 8

*Flux data for caffeine across hairless mouse skin from buffered (McIlvaine) 10% propylene glycol suspensions and from 10% propylene glycol suspensions (pH 5.0) following pretreatment regimens (figures in parentheses are standard errors of the mean)*

| Vehicle | Flux ($\mu$mol cm$^{-2}$ h$^{-1}$) | |
|---|---|---|
| Propylene glycol (10%) pH 5.0 | 80.49 | (13.33) |
| Propylene glycol (10%) pH 6.4 | 145.53 | (12.47) |
| Propylene glycol (10%) pH 8.0 | 97.79 | (12.40) |
| Oleic acid (5%) in PG [a] | 446.84 [b] | (30.41) |
| | 73.77 | (9.49) |
| Propylene glycol [a] | 93.77 | (15.99) |
| Propylene glycol (10%) pH 5.0 [a] | 152.41 | (4.53) |

[a] Pretreatment.
[b] Initial value from first 6 h.

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