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# Lipid-protein-partitioning (LPP) theory of skin enhancer activity: finite dose technique

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## Summary

We have examined the effectiveness of pretreating human epidermis with a range of accelerants on the permeation of model drugs 5-fluorouracil (5FU) and oestradiol (ES). To complement previous steady-state investigations with these materials, we have utilised a finite dose technique with drug deposited as a dried film. We used accelerants Azone and decylmethyl sulphoxide in both propylene glycol (PG) and water vehicles, oleic acid (OA) in PG, and PG. Following accelerant pretreatments, drug permeation was monitored for 4 days.

All PG-based accelerants and PG promoted 5FU penetration, 2% Azone in PG by 80-fold and PG by 12-fold (24-h results quoted). Water and aqueous-based accelerants were relatively ineffective, 3% Azone with 0.1% Tween 20 in saline producing only a 3.7-fold increase. A similar trend occurred with ES; 5% OA in PG was the most effective pretreatment, yielding a 35-fold increase, and PG produced a 9-fold effect. The aqueous-based enhancers were ineffective.

With the finite dose technique, PG pretreatment increased drug penetration, contrasting with its ineffectiveness in our previous steady-state work. The glycol may solvate the tissue when it is not fully hydrated, competing with drug for hydrogen-bonding sites. Additionally, PG may aid more drug to partition into the skin. The accelerants themselves, which probably disrupt the lipid bilayers, were more effective with PG rather than with water vehicles. As PG may solvate horny cells, this suggests that both drugs may permeate the stratum corneum transcellularly to some extent.

The three features of skin penetration enhancer activity (Lipid interaction, Protein alteration and Partitioning phenomena) represent the essential aspects of the LPP theory.

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*Abbreviations:* DCMS: decylmethyl sulphoxide, DSC: differential scanning calorimetry, ES: oestradiol, 5FU: 5-fluorouracil, OA: oleic acid, PG: propylene glycol, T/S: 0.1% Tween 20 in normal saline.

## Introduction

For many years, investigators have examined the effectiveness of penetration enhancers (accelerants or promoters) in increasing the absorption of drugs through the stratum corneum. Until fairly recently, little information has been available as to how enhancers interact with the components of the horny layer, thus reducing its barrier function.

Recent studies (Goodman and Barry, 1989) in our laboratories utilising differential scanning calorimetry (DSC) have increased our understanding of accelerant action. To supplement the DSC findings, we have investigated further the effect of enhancers on the stratum corneum using permeation studies. We have already reported some of this data (Goodman and Barry, 1988), obtained using an infinite dose technique (i.e. steady-state drug diffusion). This paper records a similar study, but using a finite dose design (a dry drug film). The results from the two investigations are compared and they provide additional understanding of accelerant action.

As in our previous permeation work, we have investigated the effectiveness of penetration enhancers Azone, oleic acid (OA), decylmethyl sulphoxide (DCMS) and propylene glycol (PG) on the skin permeation of model drugs 5-fluorouracil (5FU) and oestradiol (ES). In addition, we used both PG and aqueous vehicles for Azone and DCMS pretreatments. 5FU and ES have been extensively used for percutaneous absorption studies as models of a polar and relatively non-polar drug, respectively. The accelerants selected are effective at low concentrations and are the subject of recent literature interest.

The experimental design compares the permeation of a drug delivered from a solvent-deposited dried film through whole human epidermis both untreated and pretreated with promoters. With this approach, steady-state diffusion rates cannot easily be measured, but the amount of drug penetrating at set times can be determined. Physicochemical conditions are allowed to change, as they would in normal clinical application, and there is no excessive tissue hydration. Accelerant effectiveness can be measured by comparing drug penetration through treated and untreated tissue.

## Materials and Methods

*Chemicals*

5FU and OA (> 99% pure) were supplied by Sigma Chemical Co., Poole, U.K. ES and PG (both > 99% pure) were obtained from BDH Chemicals, Liverpool, U.K. 1-dodecylazacycloheptan-2-one (Azone), pharmaceutical grade, was a gift from Nelson Fine Chemicals, Irvine, CA, U.S.A. DCMS was a gift from Proctor and Gamble, Newcastle, U.K. Tween 20 was obtained from W. Ranson and Sons Ltd., Hitchin, U.K. and Optiphase X from LKB Scintillation Products, Cambridge, U.K. Radiolabelled 5FU (6-$^3$H) and ES(2, 4, 6, 7-$^3$H), both > 97% purity were supplied by Amersham International, Amersham, U.K.

*Epidermal membranes*

Human abdominal cadaver skin was obtained at post-mortem and stored at −24°C. Excess fat was removed before immersing the samples in water at 60°C for 45 s, after which the epidermis may be peeled off. Epidermal membrane was chosen to prevent any resistance to permeation arising from the aqueous dermis.

*Diffusion experiments*

Epidermal membranes were partially hydrated by floating them on water for 16 h before mounting them in stainless steel diffusion cells (diffusional area 0.126 cm$^2$). 24 cells were attached to an automated diffusion apparatus (Akhter et al., 1984); the small cell size allows all 24 to be set up from a single epidermal specimen thus aiding comparisons. Degassed receptor fluid (water containing 0.002% sodium azide to prevent bacterial growth) was pumped continuously through the cells at 2 ml h$^{-1}$. The receptor fluid temperature was 32°C, which produced a skin surface temperature of 30°C. Donor compartments of the cells were exposed to constant room conditions of 22°C and 60% relative humidity. The cells were left untreated for 60 h to allow the skin to equilibrate.

Batches of 3–5 cells were then treated for 12 h with one of the following penetration enhancers; 2% (w/w) Azone in PG, PG, 3.0% (w/v) Azone with 0.1% (w/v) Tween 20 in normal saline (T/S), T/S, 5% (w/w) OA in PG, 15% (w/w) DCMS in PG and 4% (w/v) DCMS in water. The 12-h treatment period was chosen because 3% Azone in T/S does not appear to be effective if applied for shorter periods (Sugibayashi et al., 1984). These accelerant concentrations have been found previ-

ously to be effective in our laboratories (Barry and Bennett, 1987; Goodman and Barry, 1988) and by others (Sekura and Scala, 1972; Sugibayashi et al., 1984; Touitou and Abed, 1985; Touitou, 1988). The donor volumes were 10 $\mu$l for the PG-based accelerants and 150 $\mu$l for the aqueous ones. (These volumes cover the skin throughout the treatment period, the cells containing aqueous enhancers used glass cover-slips to prevent evaporation of water.) A batch of 5 cells was also left untreated to act as a control during each diffusion run. This aids comparison between diffusion runs (i.e. different tissue specimens), because any enhancements seen can be compared directly to a control figure.

After 12 h, the accelerant mixtures were removed and donor compartments were rinsed with water and dried with a paper tissue. All membranes were then treated with a solution of radiolabelled drug in a volatile solvent. For ES, 30 $\mu$l of a 1.67% w/w solution in acetone was applied; the solvent quickly evaporated to leave a dry film of 0.5 mg drug on the skin surface. This procedure is consistent with previous work using steroids conducted in this department, enabling comparisons to be made (Barry and Bennett, 1987). However, 5FU is not sufficiently soluble in acetone to duplicate these conditions. Instead, 100 $\mu$l of a 0.3% (w/w) solution in acetone/ethanol (50 : 50, v/v) was applied; a dry film of 0.3 mg 5FU was left after the solvent had evaporated, aided by a stream of warm air. The skin surface was once more exposed to room conditions of 22°C and 60% relative humidity.

The permeation of drug was monitored by collecting receptor fluid samples every hour for the first 10 h and thereafter every 2 h for 4 days. Samples were mixed with 10 ml Optiphase X and counted on a Tri-Carb 460C scintillation counter.

*Calculation of results*

For each diffusion cell, the cumulative amount of compound penetrating per unit area was plotted against time. Drug diffusion rate was calculated by computer program and also plotted against time. The effectiveness of penetration enhancers can be determined by comparing the amount of drug permeating accelerant pretreated skin to that for untreated skin. This was defined as the enhancement ratio (ER):

$$ER = \frac{\text{(Amount of drug penetrating accelerant treated skin in time } t)}{\text{(Amount of drug penetrating untreated skin in time } t)}$$

where $t$ is the time from initial drug-skin contact.

## Results and Discussion

Our methodology uses accelerants to pretreat human epidermis, thus allowing a single drug formulation to be applied to treated and untreated tissue. Diffusional cells of small cross-sectional area enable 24 cells to be set up using epidermis from one source. The current work contrasts with our previous steady-state investigations (which used the same drugs and enhancers); with the latter studies, physicochemical parameters such as the permeability coefficient may easily be measured. However, the technique utilised fully hydrated tissue with saturated drug solutions. With this approach, conditions for drug permeation are already enhanced, thus accelerant effects may be masked. The present work uses a finite dose of drug and partially hydrated tissue exposed to controlled room conditions – this approach more closely mimics the in vivo situation of the clinic.

*5FU permeation*

Figure 1 details the amount of 5FU penetrating PG-based accelerant-treated and untreated skin from a dried acetone/ethanol deposited film after 24 and 96 h. The results are given as percentage of initial dose penetrated and ERs. The corresponding aqueous-based accelerant results are presented in Fig. 2. Comparison of the results in Figs. 1 and 2 reveals that the penetration enhancing effects of the various pretreatments lessens with time – for instance, the ER following 2% Azone in PG treatment is 82 after 24 h but 36.6 after 96 h. It is for this reason that the results are given at 24 h because this is the time over which the enhancer

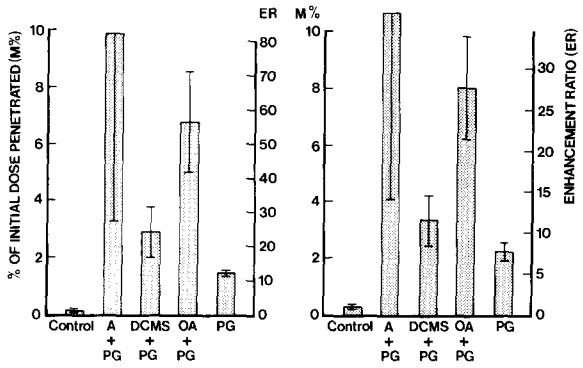


Fig. 1. Histograms showing the amount of 5-fluorouracil permeating PG-based accelerant-treated and untreated human epidermis from a 0.3 mg dried film, after 24 (left) and 96 (right) h. Results expressed as % of initial dose penetrated ± standard deviation, and enhancement ratio (ER).

effects are most prevalent and 96 h as this is the end of the diffusion run.

The Figs. 1 and 2 results reveal a clear division between the aqueous-based and PG-based accelerant pretreatments. The former group was at best only moderately effective; after 24 h 3% Azone in T/S and 4% DCMS in water yielded ER values of 3.7 and 2.5, and T/S and water itself did not significantly enhance drug penetration. (3% Azone in T/S has previously been reported to be effective in promoting 5FU penetration through hairless rat skin (Sugibayashi et al., 1984).) In contrast, all PG-based accelerants were much more

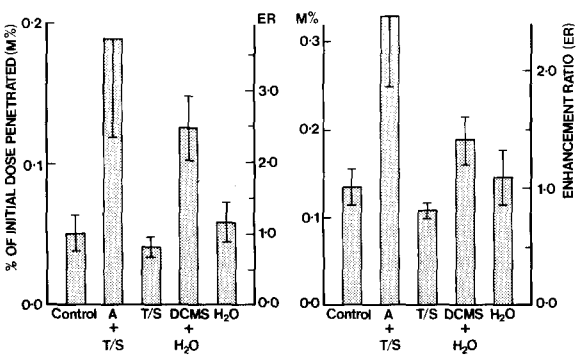


Fig. 2. Histograms showing the amount of 5-fluorouracil permeating aqueous-based accelerant-treated and untreated human epidermis from a 0.3 mg dried film, after 24 (left) and 96 (right) h. Results expressed as % of initial dose penetrated ± standard deviation, and enhancement ratio (ER).

effective with 2% Azone in PG and 5% OA in PG giving ERs of 82 and 56, respectively, after 24 h. Pretreatment with PG itself increased 5FU penetration 12-fold over 24 h – clearly, the glycol acted as a penetration enhancer in this experimental design, and may have contributed significantly to the enhancing ability of the Azone and OA/PG combinations. It is not certain whether the dramatic enhancement seen here with Azone would be repeated in vivo. However, experimental conditions do mimic the in vivo situation to an extent and Azone has been shown to be effective in vivo (Spruance et al., 1984; Ogiso et al., 1987). Additionally, a recent study by Mirejovsky and Takruri (1986) has demonstrated a good in vivo/in vitro comparison for Azone effectiveness, under certain experimental conditions.

Some of the above results were similar to those obtained in our previous steady-state study with the same drugs and enhancer pretreatments. (This previous investigation utilised fully hydrated tissue with saturated aqueous solutions of drug in the donor compartments of the cells.) For instance, 2% Azone in PG increased 5FU penetration 90-fold in the steady-state work, whereas 3% Azone in T/S was again only moderately effective. However, in that study PG pretreatment had no apparent effect on drug diffusion but there conditions for drug diffusion were already enhanced, i.e. saturated solutions applied to fully hydrated skin. These comparisons emphasize the importance of experimental design in assessing the effectiveness of penetration enhancers.

Typical plots of cumulative penetration of 5FU versus time are illustrated in Figs. 3 and 4. (For clarity, only a few data points are included on the plots – they also run up to 60 h only as little further change occurs thereafter.) Rate data, derived from computer differentiation, are also plotted. Again, there is a considerable difference between the aqueous accelerant pretreatments (Fig. 4) and the PG-based pretreatments (Fig. 3). Plots from the former group and the control are very similar. Here, the maximum rate is attained after approximately 1 h. This early maximum is unlikely to result from diffusion of drug through the bulk of the tissue; in our steady-state work, a lag time of around 10 h was typically found for 5FU.

A more likely explanation for the initial rapid rate is that it arises from shunt route diffusion. A small fraction of the original acetone/ethanol drug solution could penetrate the appendages before evaporation of the solvent. Thereafter any material deposited directly over the appendages would also penetrate rapidly. However, this rate should quickly become insignificant, owing to effective depletion i.e. lateral drug diffusion in the dry film would be negligible.

After the 1 h maximum (control and aqueous accelerant pretreatments), the rate falls continuously – thus the onset of drug diffusing through the bulk of the tissue is not apparent. A possible explanation for the absence of a pulse effect is that this is obscured by the falling shunt route diffusion rate. After initial drug solution/skin contact, a saturated solution of drug forms as the solvent evaporates, during which time partitioning of 5FU into the top layers of the stratum corneum will be maximal. After the surface dry film has formed this top layer drug reservoir diffuses through the membrane. However, as the rate falls continuously over 96 h it is likely that this reservoir becomes depleted; the falling rate indicates that the drug only slowly dissolves from the deposited film. Such behaviour has been observed previously (Akhter and Barry, 1985; Barry and Bennett, 1987).

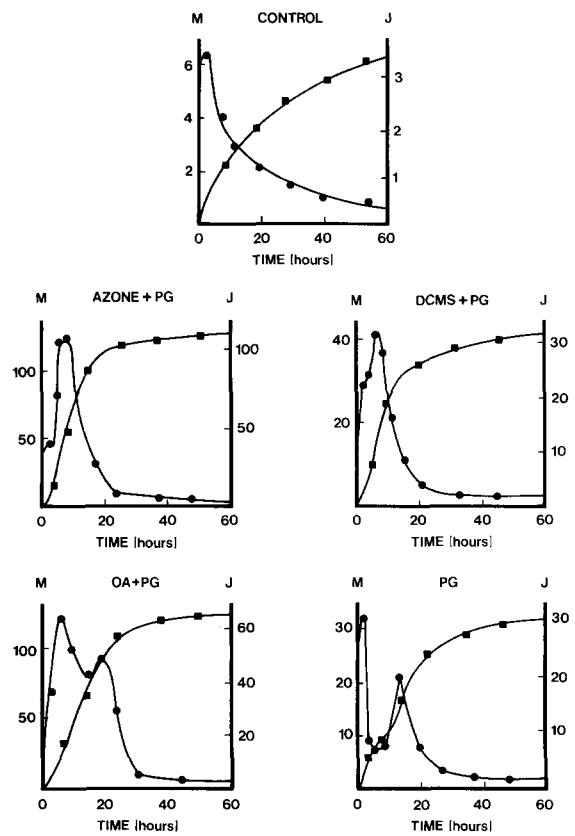


Fig. 3. Typical cumulative (squares; M, CPM $cm^{-2}$) and penetration rate (circles; J, CPM $cm^{-2}$ $h^{-1}$) profiles for 5-fluorouracil permeating untreated and PG-based accelerant-treated human epidermis from a 0.3 mg dried film.

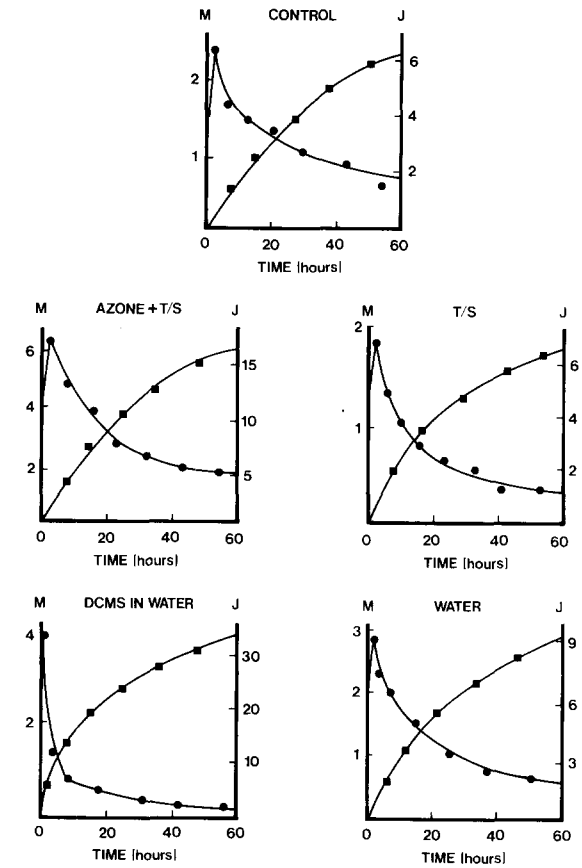


Fig. 4. Typical cumulative (squares; M, CPM $cm^{-2}$) and penetration rate (circles; J, CPM $cm^{-2}$ $h^{-1}$) profiles for 5-fluorouracil permeating untreated and aqueous-based accelerant-treated human epidermis from a 0.3 mg dried film.

In contrast, all the PG-based accelerant pretreatments produced considerably different rate profiles (see Fig. 3). Figure 3 helps to explain why the ER values drop with time as most of the drug penetrates skin in the first 24 h. Diffusion rates fall away continuously thereafter – this decrease probably represents depletion of the top layer drug reservoir, as discussed below for PG. The PG plot shows a maximum at 1.5 h after initial drug–skin contact, indicating similar behaviour to the control so this peak probably arises from shunt route diffusion. However, the PG rate plot indicates a second peak at around 13–14 h; this behaviour is reproducible, the averaged peak maximum occurring after $16.8 \pm 3.8$ h. The diffusion rate profile for 5FU permeating skin pretreated with 2% Azone in PG also shows a peak at 1.5 h but a substantial higher maximum occurs at 7.5 h (average for 5 cells, $6.9 \pm 1.5$ h). This second peak does not develop in the 3% Azone in T/S plot, i.e. the aqueous treatment. Pretreatment with 15% DCMS in PG yielded a rate plot with just one maximum, at $4.4 \pm 1.4$ h. A similar maximum ($4.5 \pm 1.4$ h) was observed following 5% OA in PG pretreatment but here a second maximum, at 16.6 $\pm$ 5.5 h, also resulted.

The outstanding features of the above results are the efficacies of the PG-based accelerants compared with the aqueous ones, and the subsequent appearance of the diffusion rate curves (Fig. 3). In particular, the increased 5FU penetration following PG pretreatment was somewhat unexpected, as no enhancement was seen in our previous steady-state work (Goodman and Barry, 1988). There may be a number of factors involved in the apparent effectiveness of PG in the present work. Firstly, treating the tissue with the glycol for 12 h may allow significant quantities of PG to enter the stratum corneum. This, in turn, could aid the partitioning of 5FU into the membrane, i.e. an increased stratum corneum/solvent partition coefficient results, compared to the control. Additionally, more of the drug solvent itself (acetone/ethanol) will preferentially partition into the skin before it evaporates, further enhancing the partition coefficient. This will then itself evaporate quite quickly and is unlikely to influence drug permeation further. However, 5FU is more soluble in water than in PG (12 mg $ml^{-1}$ vs. 2.2 mg $ml^{-1}$ at 25 °C (Touitou and Abed, 1985)). The ineffectiveness of the water pretreatment (Fig. 2) would suggest therefore that partitioning is not the only factor involved in the PG results.

A further explanation may be provided by our recent DSC study (Goodman, 1986; Goodman and Barry, 1989). This demonstrated that PG does interact with the stratum corneum components – principally, the $\alpha$-keratin conformational transition observed at around 95 °C becomes smaller and broader with increasing glycol concentration. There is also a very slight effect on the two major lipid transitions, i.e. PG may produce a marginal increase in lipid fluidity. In effect, PG may 'solvate' the tissue – it may form hydrogen bonds with polar groups on keratin chains and displace bound protein water. Thus, PG could aid 5FU penetration by competing with drug for the hydrogen bonding sites in the tissue. Therefore, the glycol may aid more drug to partition into the skin initially compared to the control. This deposit is then able to diffuse through the skin more easily than for the control sample, because of the tissue solvation effect – it eventually appears as the second peak (Fig. 3). This peak, representing material diffusing through the bulk of the tissue, is not seen in the control plot; for this instance it is not substantial enough to be differentiated from the falling 'shunt route' peak. It is likely that more 5FU will be held up in the tissue by hydrogen bonding.

A further consideration is that PG itself is likely to diffuse through the horny layer, and so begin to deplete. Therefore, it is possible that the rate of 5FU penetration falls from the second rate peak (16.8 h) as a result of PG depletion. However, there is some evidence to suggest that this is not the case. Møllgaard and Hoelgaard (1983a), after applying a finite dose of PG to human skin, found that significant quantities of the applied dose did not appear in the receptor for 20 h. A similar result was reported by Wotton et al. (1985); thus significant depletion of PG in the tissue after 16.8 h may be unlikely. More likely is that the initial drug 'reservoir' in the first few layers (following solvent evaporation) depletes, and the permeation process becomes dissolution rate-

controlled by the surface film hence the second peaks falls. Some authors have suggested that PG may enhance drug permeation by acting as a carrier, i.e. the drug is transported in a dissolved state across the membrane. However, such mechanisms would imply that the intact horny layer contains porous channels, large enough to allow mass flow of solvent. Current ideas as to the structure of the horny layer contradict this hypothesis.

In our previous steady-state work (Goodman and Barry, 1988), we observed no apparent accelerating effect with PG. However, it was likely that with that method the stratum corneum-vehicle partition coefficient was higher than in the finite dose approach because the skin was fully hydrated. 5FU is more soluble in water than in PG, so in the fully hydrated situation, PG is unlikely to enhance the partition coefficient. Also the glycol may not solvate the tissue significantly more than it is already under fully hydrated conditions. These results may help to explain the literature conflict regarding the ability of PG to promote percutaneous absorption. It is possible that the effect of water (which itself is an effective accelerant) may mask the enhancing ability of PG, dependent on experimental design.

The small 1.5-h peak in the diffusion rate profile for 5FU permeating skin pretreated with 2% Azone in PG probably arises from shunt diffusion. The considerably larger 7-h peak is absent in the plot for 3% Azone in T/S thus emphasizing again the importance of PG. Azone is most effective when used with a polar solvent – indeed this accelerant substantially increases the percutaneous delivery of PG (Touitou and Abed, 1985). Thus on application of 5FU to skin pretreated with 2% Azone in PG, a considerably higher concentration of PG (and more acetone/ethanol) will be present in the tissue than in the situation of pretreatment with PG only. This difference will probably enable substantially higher levels of 5FU to enter the top layers of the stratum corneum before all the donor solvent evaporates. The presence of Azone and the greater quantity of the glycol in the horny layer (i.e. increased tissue solvation) could also allow these higher top level concentrations to permeate the skin faster than in the PG-only treatment. Hence, overall more 5FU will enter the tissue with the Azone treatment, and the drug will permeate (and so deplete) faster. Indeed, after the 7-h peak the rate falls quickly, probably becoming dissolution rate-limited.

The single peak (4.4 h) in the 5FU rate profile following pretreatment with 15% DCMS in PG probably obscures the shunt route peak to some extent. A similar diffusion-promoting mechanism as for 2% Azone in PG is likely, namely higher levels of PG in the tissue compared to PG only, leading to increased drug partitioning and a greater flux. However, DCMS was not as effective as Azone as the latter possibly allows greater drug mobility within the skin, allowing a larger top layer reservoir to build up. In addition, Azone may permit more PG to enter the tissue than in the case of DCMS. The first of the two peaks in the rate profile for 5% OA in PG occurred at 4.5 h – as with DCMS, this peak probably obscures the shunt route peak. However, why there should be two major peaks in the OA pretreatment rate profile (which are reproducible) but only one for Azone and DCMS is unclear.

The ineffectiveness of water pretreatment was perhaps surprising. It is a better solvent for 5FU than is PG (i.e. the drug stratum corneum-solvent partition coefficient should be greater following water treatment), and water itself is an effective accelerant. However, water can be lost through evaporation whereas PG will only diffuse relatively slowly through the tissue, thus PG is likely to solvate the skin components longer than water. In addition, our recent DSC results (Goodman and Barry, 1989) indicated that PG may interact with stratum corneum protein more than does water, and may therefore more reduce drug–protein interactions – for instance, PG could compete for hydrogen bonding sites. Hence a temporary increase in tissue hydration may have less influence than the more prolonged presence of PG within the skin. PG may not have been effective in the steady-state study as there the skin was fully hydrated constantly.

The reason for the small 5FU enhancements obtained from aqueous Azone and DCMS may be similar to those already outlined above. Water may be quickly lost from the tissue by evapora-

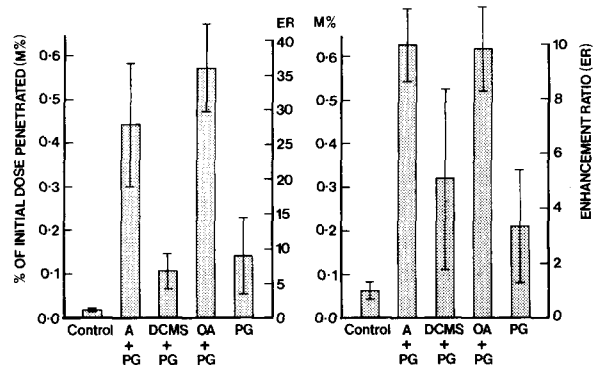


Fig. 5. Histograms showing the amount of oestradiol permeating PG-based accelerant-treated and untreated human epidermis from a 0.5 mg dried film, after 24 (left) and 96 (right) h. Results expressed as % of initial dose penetrated ± standard deviation, and enhancement ratio (ER).

tion, particularly from the top layers (PG would remain longer). Thus although the accelerants themselves may be present in the skin, drug–tissue interactions may still be significant and 5FU may bind to protein.

*ES permeation*

As for 5FU, the amount of ES penetrating untreated and accelerant-pretreated skin (from an acetone-deposited film) was determined after 24 and 96 h. As before, the results are presented as % of initial dose penetrated and ERs; Figs. 5 and 6 show the effects of PG-based and aqueous-based accelerant pretreatments. The results for the

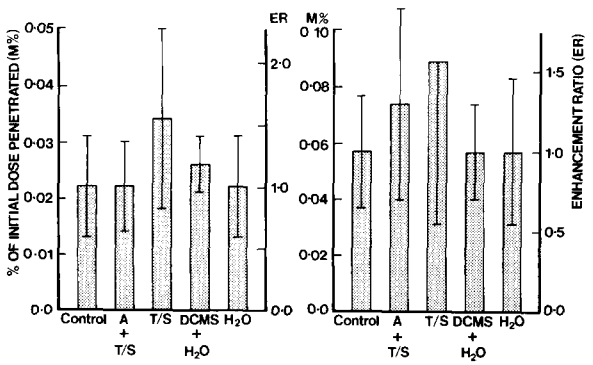


Fig. 6. Histograms showing the amount of oestradiol permeating aqueous-based accelerant-treated and untreated human epidermis from 0.5 mg dried film, after 24 (left) and 96 (right) h. Results expressed as % of initial dose penetrated ± standard deviation, and enhancement ratio (ER).

amount of ES penetrating untreated skin (0.060% of a 0.5 mg film, averaged from the two control values after 4 days) agree quite well with a literature value. Møllgaard and Hoelgaard (1983b) applied a solvent-deposited dry ES film to human skin and reported a steady-state flux of 0.011 $\mu$g $cm^{-2}$ $h^{-1}$. No true steady-state was observed over our 4-day period, but an 'average' value of 0.025 $\mu$g $cm^{-2}$ $h^{-1}$ can be calculated. It should be noted that although ES is a sparingly water-soluble compound, 'sink' conditions were maintained throughout the diffusion run by using a receptor fluid flow rate of 2 ml $h^{-1}$. (In our previous steady-state work, we compared both water and ethanol/water (50 : 50, v/v) as receptor solutions – the results indicated that sink conditions were achieved using either receptor.) For more lipophilic molecules, a surfactant may be added to the receptor fluid (Bronaugh, 1985).

Figures 1, 2, 5 and 6 show that more 5FU penetrated the skin than ES – perhaps a surprising result, as the stratum corneum preferentially dissolves lipophilic molecules. A possible explanation may involve solvent contact time; this was 10–15 min for 5FU (acetone/ethanol, 100 $\mu$l) but less than 5 min for ES (acetone, 30 $\mu$l). Thus with 5FU more drug may initially have penetrated via the shunt route. Also, as 5FU is not very soluble in acetone/ethanol, the drug would have been at saturation for most of the 10–15 min solvent contact time, thus exhibiting maximum thermodynamic activity for longer. ES is very soluble in acetone, so it would have been at saturation for a short period only. Finally, the longer solvent contact time for 5FU may allow some acetone/ethanol to partition into the upper skin layers, which itself allows 5FU to partition in. Consequently, more 5FU may have partitioned into the tissue than ES, forming a larger 'top layer reservoir'.

Inspection of Figs. 5 and 6 reveals a similar trend in accelerant effectiveness for ES penetration compared to 5FU permeation. Again, the aqueous-based enhancers had little or no influence, whereas all PG-based accelerants increased steroid permeation. PG itself enhanced the 24-h penetration of ES by 9-fold, comparable to the 11-fold value obtained by Møllgaard and

Hoelgaard (1983b) using the same drug and accelerant concurrently. Azone, OA and DCMS in PG yielded ER's of 28, 36 and 7, respectively, after 24 h. Thus OA pretreatment was more effective in promoting ES penetration than was Azone in the first 24 h, although after 96 h there was no significant difference between the two sets of results. This same order of effectiveness of the PG-based enhancers was reported previously by Barry and Bennett (1987) who used hydrocortisone as a model drug. DCMS in PG was no more effective than PG alone. These results contrast somewhat with our previous steady-state work; with this, only pretreatment with 5% OA in PG significantly increased steroid absorption, producing an ER of 3.5. Once again, this demonstrates the importance of experimental design in examining enhancer effectiveness.

Typical cumulative and rate plots for ES permeating untreated and enhancer-pretreatment epidermis are depicted in Figs. 7 and 8. The 'shunt route' peak observed with 5FU was not so obvious with ES (untreated skin). Again, this may reflect the much shorter solvent contact time in the latter situation. The rate of ES permeation drops with time, again suggesting that the process becomes dissolution rate-limited with respect to the surface film as the top layer reservoir depletes. ES diffusion plots for skin pretreated with aqueous-based accelerants show peak rates just after drug application. Generally, the peak rates are higher than for the control sample but then fall quickly – it is possible that the wet skin surface allows some ES to remain in solution longer than for the control, enabling more drug to be transported down the appendages initially.

With the PG-based accelerant pretreatments (Fig. 7), penetration rate maxima occur at $7.4 \pm 1.7$, $7.1 \pm 3.1$, $3.9 \pm 1.1$ and $15.0 \pm 2.8$ h for 2% Azone in PG, 15% DCMS in PG, 5% OA in PG and PG, respectively. Barry and Bennett (1987) found a similar order in time delay following application of the same PG-based accelerants directly to a dried hydrocortisone film. Figures 7 and 8 profiles are similar to their 5FU counterparts, and a similar accelerated diffusion mechanism can be proposed. The presence of PG in the membrane aids the partitioning of drug into the

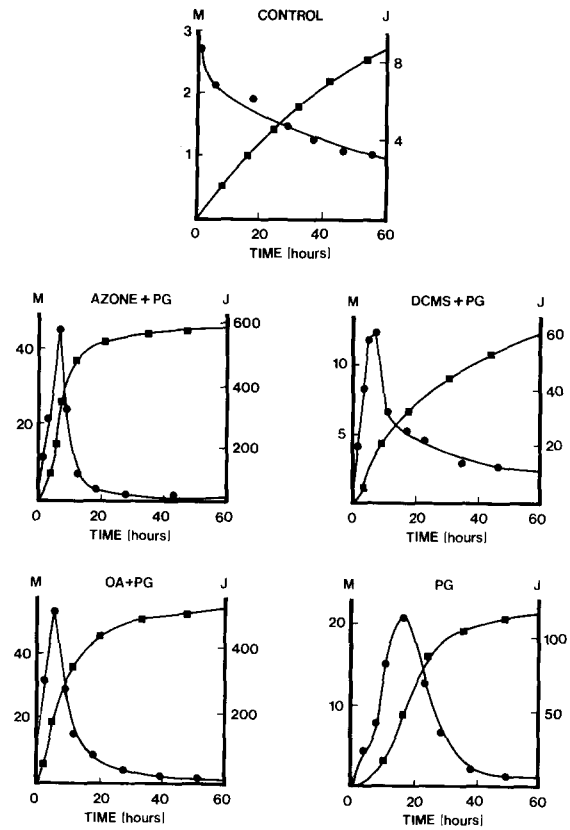


Fig. 7. Typical cumulative (squares; M, CPM $cm^{-2}$) and penetration rate (circles; J, CPM $cm^{-2}$ $h^{-1}$) profiles for oestradiol permeating untreated and PG-based accelerant-treated human epidermis from a 0.5 mg dried film.

skin; PG also solvates the tissue, reducing drug–tissue interactions, and hence increases drug mobility. Thus a larger top layer reservoir forms than in the control run which also depletes quickly. Azone and OA may not only increase drug mobility within the tissue, but allow in larger concentrations of PG; this, in turn, would allow in more Azone or OA. Thus, with these two accelerants, the ER is greater than for PG alone, and the peak flux occurs sooner. OA in PG was the most effective ES promoter (after 24 h), as it was in the steady-state work. DCMS has been reported to be a polar drug accelerant (Cooper, 1982) – indeed, 15% DCMS in PG did not enhance ES delivery more than did PG after 24 h.

The aqueous based accelerants had no significant effect on the ES penetration at the 24-h

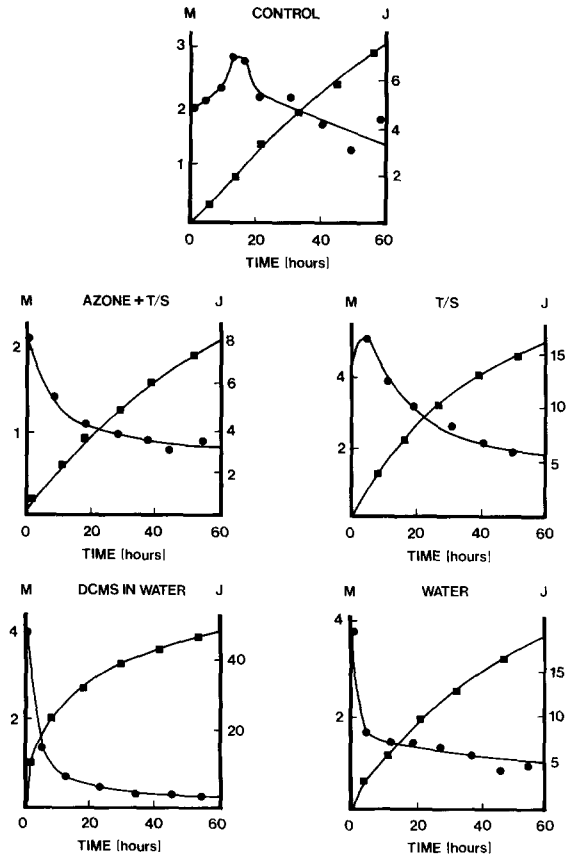


Fig. 8. Typical cumulative (squares; M, CPM $cm^{-2}$) and penetration rate (circles; J, CPM $cm^{-2}$ $h^{-1}$) profiles for oestradiol permeating untreated and aqueous-based accelerant-treated human epidermis from a 0.5 mg dried film.

point. This is perhaps not surprising as the presence of water will not greatly aid the ES skin-solvent partition coefficient. Also, water may be lost by evaporation comparatively quickly from the top tissue layers, and thus not act as a tissue solvator.

The overall picture from the finite dose experiments is that pretreatment with PG- and PG-based accelerants does aid drug penetration. The aqueous-based accelerants are at best (for 5FU) only moderately effective, and for ES, ineffective. The results of this and our two previous investigations have revealed some insights into the action of penetration enhancers and the route of drug permeation through the skin as summarised below.

*Accelerant action and the route of drug permeation*

Our previous DSC studies (Goodman and Barry, 1989) indicated that the most important action of accelerants on the stratum corneum barrier is to disrupt the lipid bilayer structure and to increase the fluidity of this region. Drug mobility in this less rigid environment will then be greater. However, many accelerants additionally interact with intracellular protein. They may solvate the horny cell contents themselves, displace bound water, expand the structure and compete with drugs for hydrogen-bonding sites. Some accelerants appear to interact with the lipid bilayers only, e.g. Azone. Both in the present work and in our previous steady-state studies, we have observed that the effectiveness of such promoters may be enhanced by formulating them with a cosolvent such as PG, which appears to solvate intracellular protein. Thus a particularly effective accelerant combination is one that will increase the permeability of both intercellular lipid bilayers and the proteinaceous horny cells. A third feature is that when a significant amount of solvent enters the stratum corneum, it may alter the partitioning behaviour of the drug or a co-administered lipid enhancer. The triad of features (Lipid interaction, Protein alteration and Partitioning phenomena) may be referred to as the LPP theory.

When investigating accelerant effectiveness in vitro, experimental design is an important factor. With the finite dose approach used here, PG pretreatment enhanced both polar and relatively non-polar drug delivery. In the previous steady-state work, the glycol was apparently ineffective; however, that study utilised fully hydrated skin. The effects on the tissue of water, itself a penetration enhancer, probably masked the enhancing ability of PG. Additionally, drugs of different polarities should be used as models – some accelerants may only promote polar drug diffusion, e.g. DCMS, or the converse.

Previously there has been much conjecture on the route of drug permeation through the stratum corneum. In the 'brick and mortar' view of the stratum corneum, with protein cells surrounded by intercellular lipid bilayers, both intercellular and transcellular diffusion is possible (the latter route must still cross intervening lipid regions). Elias et

al. (1981) favour the intercellular route as the predominant pathway as they claim that drug permeation rate is influenced by the lipid content of the tissue. Indeed, it is likely that this route will be most significant for highly lipophilic drugs. However, recent work (Oakley and Swarbrick, 1987) on the partitioning of ionizable drugs into the stratum corneum has suggested that ionized materials may be located in the aqueous regions. Additionally, a 'random' brick and mortar model for drug transport across the horny layer has recently been devised (Tojo, 1987). This model suggests that transcellular diffusion is the dominant pathway for water and salicylic acid. Our DSC (Goodman and Barry, 1989) and diffusion work investigating penetration enhancer actions also suggests that transcellular diffusion may also be important not only for polar materials, but for relatively non-polar ones (such as ES) as well. Accelerants are most effective when they interact with both intercellular and transcellular corneum components. This appears to be the situation for ES – thus this relatively non-polar steroid may well diffuse, at least in part, via the transcellular route. This is supported by the recent study of Raykar et al. (1988), examining the role of protein and lipid domains in the uptake of various steroids.

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