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Effect of penetration enhancers on the permeation of mannitol, hydrocortisone and progesterone through human skin

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Mannitol, hydrocortisone and progesterone were selected as model penetrants to assess the mode of action of eight potential penetration enhancers in human skin. Their partition coefficients, octanol : water and stratum corneum : water were measured and correlated with their postulated routes of penetration through human skin. The results suggest that mannitol penetrated via a polar route, hydrocortisone by a mainly lipid route and progesterone via a lipid pathway but its penetration rate was probably affected by aqueous layers. From permeation studies through cadaver skin in which an in-vivo mimic method was used, it was concluded that the penetration enhancers fell into three main categories: (1) solvents which enhanced permeation of polar and non-polar compounds e.g. 2-pyrrolidone, *N*-methylpyrrolidone, *N*-methylformamide and propylene glycol plus Azone; (2) enhancers which preferentially affected the polar route e.g. propylene glycol plus decylmethylsulphoxide, and (3) accelerants which mainly modified the non-polar route e.g. propylene glycol plus oleic acid, propylene glycol alone and, to a limited extent, water.

In recent years, there has been much interest in developing transdermal devices to deliver drugs such as hyoscine, nitroglycerin, oestradiol and clonidine to the systemic circulation. In attempting to achieve full flux control for such drugs when delivered from transdermal devices or other formulations, major problems arise because of the relative impermeability of human skin and its biological variability, person to person, site to site, and even centimetre by centimetre (Barry 1983). One solution would be to include in a formulation penetration enhancers which reversibly remove the barrier resistance of the stratum corneum and so permit the unhindered access of drug to the viable tissues and systemic circulation. To examine fundamental mechanisms, it is logical to investigate the action of a range of enhancers on a spectrum of model penetrants. Thus, this paper reports work on enhancers which may modify either the postulated lipid or polar route through human skin, or both, as assessed by how these accelerants alter the permeation of model penetrants having different polarities. It is widely accepted that such separate routes exist, but difficult to prove this conclusively (see Barry 1983; Flynn 1985).

The enhancers included materials expected to affect the polar route, e.g. 2-pyrrolidone, *N*-methylpyrrolidone, Azone and decylmethylsulphoxide (Cooper 1982; Southwell & Barry 1983; Stoughton & McClure 1983) and the non-polar route e.g. oleic acid (Cooper 1984). The penetrants chosen were hydrocortisone, progesterone and mannitol, as radioactive compounds, to cover a wide range of polarity. Mannitol, a hexahydric alcohol, was included as a highly polar model drug as many enhancers preferentially affect the polar penetration route (Southwell & Barry 1983). Much work has already been conducted using hydrocortisone and progesterone (e.g. Malkinson 1958; Scheuplein et al 1969; Ponec & Polano 1979) whereas studies using mannitol are scarce (Southwell et al 1981; Scott 1982; Akhter & Barry 1983). Some preliminary results were presented by Bennett & Barry (1984). The role of enhancers was recently reviewed (Woodford & Barry 1986).

Our permeation design was an 'in-vivo mimic' one which uses diffusion cells with a flow-through receptor solution corresponding to the blood supply and an unmixed donor phase representing a topical formulation. The advantages of this arrangement have been discussed by Akhter & Barry (1985). We used the so-called infinite dose technique with depleting solvent so that the drug source was maintained. Addition of an enhancer to a drug film deposited from acetone may alter the membrane/vehicle partition coefficient (Km), the apparent diffusion coefficient (D), or the membrane thickness (h). If the permeability coefficient (Kp) increases,

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then the flux will also increase (Kp = KmD/h). The technique can also be employed if the permeation is dissolution rate-limited as the maximum flux recorded after each addition of solvent can be related to the flux obtained at maximal thermodynamic activity for that system.

MATERIALS AND METHODS

Chemicals

Materials were: Azone (1-dodecylazacycloheptan-2-one, donated by Nelson Research), DCMS (decylmethylsulphoxide, donated by Proctor and Gamble), DMF (dimethylformamide, BDH, Analar), DMI (dimethylisosorbide, Atlas), NMF (*N*-methylformamide, donated by Aston University), NMP (*N*-methyl-2-pyrrolidone, BDH, GPR), OA (oleic acid, Sigma), PG (propylene glycol, BDH), 2P (2-pyrrolidone, BDH), acetone (BDH), octanol (BDH, specially pure), mannitol (BDH, Analar), hydrocortisone (Sigma), progesterone (Sigma), trypsin from bovine pancreas type III (Sigma), Soluene-350 tissue solubiliser (United Technologies Packard) and FisoFluor-1 (Fisons Scientific Apparatus). Radiochemicals were: [4-^{14}C]hydrocortisone (specific activity 55·5 mCi mmol^{-1}), [1-^{14}C]mannitol (60·1 mCi mmol^{-1}), [4-^{14}C]progesterone (56 mCi mmol^{-1}); radiochemical purities 99% (Amersham International).

Preparation of human skin membranes

Full-thickness Caucasian abdominal skin was prepared according to Coldman et al (1969). Excess fat was trimmed from specimens frozen at −24 °C and sections approximately 430 μm thick were obtained using a Davies Dermatome (Akhter & Barry 1985).

Stratum corneum samples were prepared by the heat-separation technique of Kligman & Christophers (1963). Epidermis was removed by incubating the membrane overnight at 37 °C, epidermal side down, on filter paper saturated with a solution of trypsin (0·0001%) containing 0·5% sodium bicarbonate. Mushy epidermis was removed before the horny layer was dried to constant weight in a desiccator.

Partition coefficient determinations

Partition coefficients of penetrants in octanol : water and stratum corneum : water systems were determined to define their polarities.

For octanol : water, 2 mL of octanol saturated with water and 2 mL of aqueous solution of penetrant saturated with octanol were shaken at 30 ± 0·2 °C for 24 h. Systems were centrifuged at 30 °C and radioactivity determined in 1 mL of each layer by adding to 10 mL FisoFluor-1 and counting in a Packard Liquid Scintillation Counter, Model Tri-Carb 460C; the experiment was repeated 4 times.

For stratum corneum determinations, weighed discs of dried stratum corneum (2 cm^2) were equilibrated for 14 days with 1 mL radiolabelled test solution. Samples were blotted on filter paper, and dissolved in 1 mL Soluene-350 in glass scintillation vials. 10 mL FisoFluor-1 containing 0·1 mL glacial acetic acid was added and samples were stored at room temperature overnight to allow chemiluminescence to subside. 10 mL of the same scintillant was added to duplicate 0·1 mL vehicle at equilibrium and the radioactivity was analysed as before. Eight determinations were made for each compound using stratum corneum from 5 different sources, male and female, and the Km values were calculated with reference to the dry weight of stratum corneum.

Diffusion experiments

An automated diffusion apparatus with stainless steel diffusion cells, diameter 4 mm, with donor at 22 °C and 60% r.h. and receptor at 30 °C, was used (Akhter et al 1984). The procedure was as follows.

(1) Full-thickness skin sections were partially hydrated by floating on water for 4 h before being mounted into the cells. The membranes were equilibrated for 3 days with water flowing through the receptor compartment and filling the donor section.

(2) Films of penetrant were deposited from 30 μL acetone containing 500 μg (40 μCi) [^{14}C]mannitol, hydrocortisone or progesterone. This procedure provided the first acetone control—AC_1.

(3) Receptor samples (4·0 mL) were collected every 4 h for addition to 10 mL Soluene-350 and subsequent scintillation counting.

(4) After 60 h, either a control (20 μL acetone–the second acetone control–AC_2) or a treatment with a potential penetration enhancer solution (Tr) was applied. Volumes were: 20 μL water, 10 μL 2P, NMP or NMF, 5 μL PG containing 2% Azone, PG with 5% oleic acid, or PG with 15% DCMS. Different volumes were chosen from preliminary experiments so as to ensure that the solvent remained on the skin for a time sufficient to dissolve the penetrant and produce a saturated solution. Additionally, the solvent volume was sufficient to saturate the first layer of the stratum corneum with penetrant but insufficient to leave excess liquid on the surface for an extended time. Hence greater volumes of acetone and water were required compared with PG solutions as both disappeared rapidly from the skin

surface. The amounts of Azone, OA and DCMS added to PG were selected to saturate the vehicle and thus provide maximum chemical potential.

(5) The penetration was monitored for a further 84 or 108 h.

(6) Acetone (20 μL) was added to provide the third acetone control–AC_3–and permeation was determined for a further 60 h to assess if the skin barrier had been modified by previous treatments.

(7) A further 20 μL acetone was delivered to a few cells, the fourth acetone control–AC_4, to assess if the increase in flux observed with AC_3 decreased with time.

RESULTS AND DISCUSSION

Partition coefficient data for the penetrants

Table 1 summarizes the partition coefficient data for mannitol, hydrocortisone and progesterone and compares them with literature values, where available. The latter data were reported at 25 °C whereas our work was at 30 °C. However, on theoretical grounds one would predict little difference between the values obtained at these temperatures, and Anderson et al (1976) showed that temperature change has little effect on the stratum corneum : water partition coefficients of phenolic compounds.

The experimental values related closely to literature data for hydrocortisone and progesterone thus confirming the validity of our method. Comparable results were not available for mannitol, the nearest being a butanol : water partition coefficient of −1·85 (Collander 1950). As butanol is more polar than octanol, a butanol : water partition coefficient for the highly polar mannitol would be expected to be closer to unity than that obtained from an octanol : water system. Our experimental value agreed with this hypothesis and illustrated that the mannitol data were of the correct order.

The three penetrants covered a wide polarity range. Mannitol, a highly polar molecule, should permeate preferentially through the polar pathways in the stratum corneum. Because of its very low partition coefficient and high hydrogen-bonding capability, suitable penetration enhancers should dramatically increase its permeation thus making mannitol a good test penetrant for the polar route. Progesterone had a high octanol to water partition coefficient and we would expect this lipophilic compound to penetrate the skin by a lipid route. Hydrocortisone, although usually referred to as a polar steroid, is a relatively non-polar material and is likely therefore to penetrate the skin mainly by a lipid route with a small fraction partitioning into the polar pathway.

Certain workers showed that for many body tissues, including the skin, 3 was the optimal log octanol to water Km for absorption (Fujita et al 1964; Hansch & Dunn 1972). At partition coefficient values above this, permeation generally becomes diffusion layer-controlled (Roberts et al 1978; Flynn 1985). Progesterone had a high octanol : water Km which suggests that clearance from the stratum corneum into the remaining epidermis and dermis could affect permeation so that the process may be modified by diffusion layers. In this paper, the term 'diffusion layers' includes remaining epidermis, dermis and aqueous stationary layers. Thus, enhancer effects may be masked because the controlling step does not reside entirely within the stratum corneum. In addition, the lipid nature of progesterone should enable it to move relatively easily through the stratum corneum and therefore there is not the same opportunity to show enhancement as for mannitol. Therefore, hydrocortisone might be expected to show increased rates compared with progesterone when penetration enhancers interact with the skin.

All stratum corneum : water results were closer to unity than the comparable values for the octanol : water system, emphasizing that the bulk properties of stratum corneum are more polar than octanol.

Table 1. Log partition coefficients for mannitol, hydrocortisone and progesterone determined at 30 °C; published values refer to 25 °C.

	Octanol : water		Stratum corneum : water	
Compound	Experimental[a]	Published	Experimental[a]	Published
Mannitol	−2·47 ± 0·08	—[b]	0·40 ± 0·20	—[b]
Hydrocortisone	1·38 ± 0·06	1·55[c]	0·90 ± 0·28	0·85[d]
Progesterone	3·78 ± 0·11	3·87[c]	2·28 ± 0·15	2·02[d]

[a] Data quoted to 3 significant figures ± s.d.
[b] No literature values available.
[c] Source Tomida et al (1978).
[d] Source Scheuplein et al (1969).

Diffusion experiments

The cumulative penetration of the test compound, with or without the addition of penetration enhancers, was measured over sequential 60 h periods (see Materials and Methods). Fig. 1 illustrates the

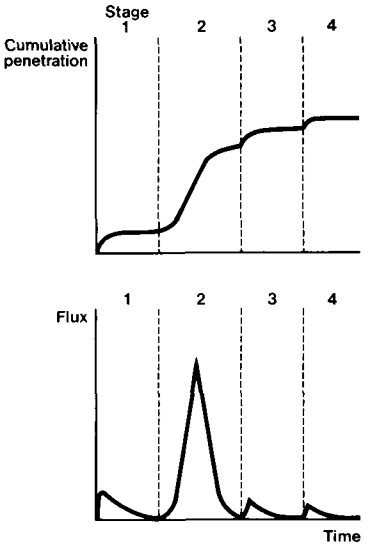

FIG. 1. Schematic diagrams of the cumulative permeation and the derived flux curves versus time illustrating the effects of the various stages of treatment.

general procedure with plots of cumulative penetration versus time and the derived flux or rate data as obtained by computer differentiation (slopes were obtained from adjacent experimental points). For clarity, it is useful to make some general comments on the stages in the permeation experiment as illustrated by this Figure.

After the initial application of drug in acetone at Stage 1, the acetone remained visible on the skin for approximately 5 min. As it evaporated, excess penetrant deposited as a film. Thus it was likely that during the initial minutes the flux into the skin was determined by the partition coefficient (stratum corneum : acetone). However, as the solvent evaporated, the chemical potential of the penetrant increased to a maximum at saturation when the driving force for partitioning into the skin was maximal. This optimum partitioning functioned until the acetone fully evaporated leaving solid material on the skin. The penetrant which had partitioned into the skin diffused through the membrane and into the receptor solution to provide a peak flux. With time the flux decreased, indicating that the initial high permeation rate was not sustained. The most probable explanation for this fall was that dissolution of the penetrant from the deposited film had become the rate-limiting step in percutaneous absorption; evidence for this explanation was that very little test compound penetrated the skin from a simple acetone deposition and microscopic examination of the skin surface revealed crystalline material (Akhter & Barry 1985).

Stage 2 was either a second acetone control application, AC_2, or a treatment with penetration enhancer, Tr (Fig. 1 illustrates the latter). With acetone the peak flux and the amount of test compound penetrating during a set period of 60 h was less than in Stage 1. This behaviour arose because the acetone had to dissolve the previously deposited film of penetrant before the compound could partition into the skin. Therefore, the actual time during which the maximal driving force into the skin operated was shorter. With some enhancers there was a long delay before the flux increased. This was probably because the partition coefficient (stratum corneum : treatment solvent) was so low that when the proposed enhancer dissolved the test compound, the latter remained preferentially in the enhancing vehicle rather than partitioning into the skin. Therefore, to obtain a reasonably consistent comparison of the amount of penetrant which had permeated the skin within a 60 h interval, the first 60 h after application was used if the time of maximum flux, T_{max}, was 30 h or less. If T_{max} was greater than 30 h, the chosen 60 h period was centred on the T_{max} point.

Stage 3 represents further addition of acetone-yielding AC_3. The purpose of this application was to determine if the prior treatment had damaged the skin. An increased permeation compared with that during Stage 1 would indicate that either the treatment had damaged the skin or it had established a penetrant reservoir within the stratum corneum.

To try to differentiate between these two possibilities, a further application of acetone was made in Stage 4—AC_4. With time the increase in permeation rate arising from a reservoir created by the treatment in Stage 2 should decrease whereas that due to irreversible damage of the stratum corneum will remain constant. Although each acetone control may form a reservoir, after Stage 1 their magnitudes should decrease because subsequent acetone controls allow less penetrant into the skin. Therefore, AC_4 should provide some indication as to which of the two alternatives operated.

The results of the permeation experiments are illustrated in Fig. 2 (mannitol), Fig. 3 (hydrocortisone) and Fig. 4 (progesterone). For each penetrant

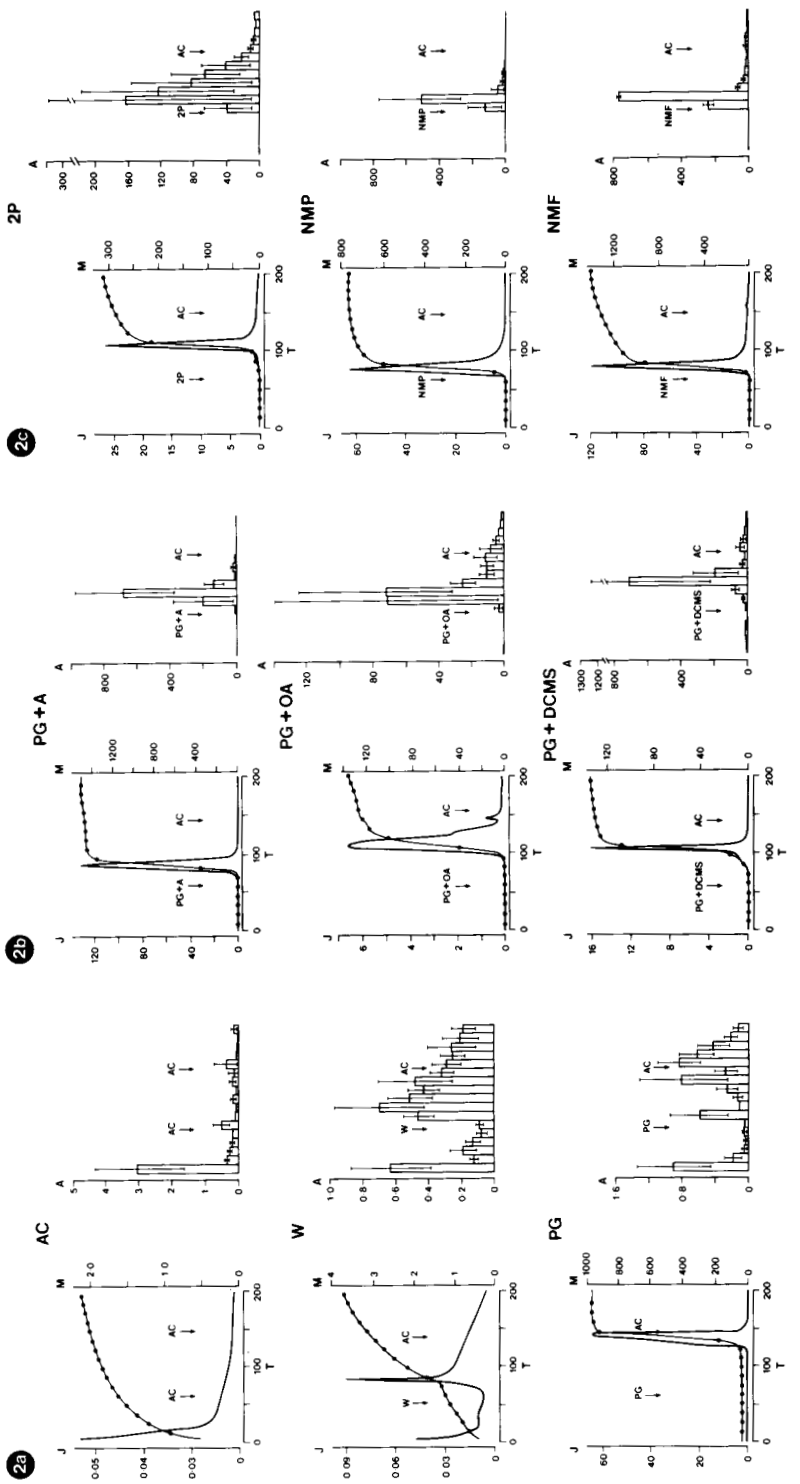

FIG. 2. Penetration of mannitol from acetone-deposited drug films and under the influence of potential penetration enhancers. Data illustrate an example of the flux (J, μg cm^{-2} h^{-1}) and cumulative penetration profiles (M, μg cm^{-2}) versus time (T,h) and histograms of amount penetrated in each 12 h period (A, μg cm^{-2}/12 h)—mean ± standard error (3 replicates). AC, acetone; W, water; PG, propylene glycol; A, Azone; OA, oleic acid; DCMS, decylmethylsulphoxide; 2P, 2-pyrrolidone; NMP, N-methylpyrrolidone; NMF, N-methylformamide.

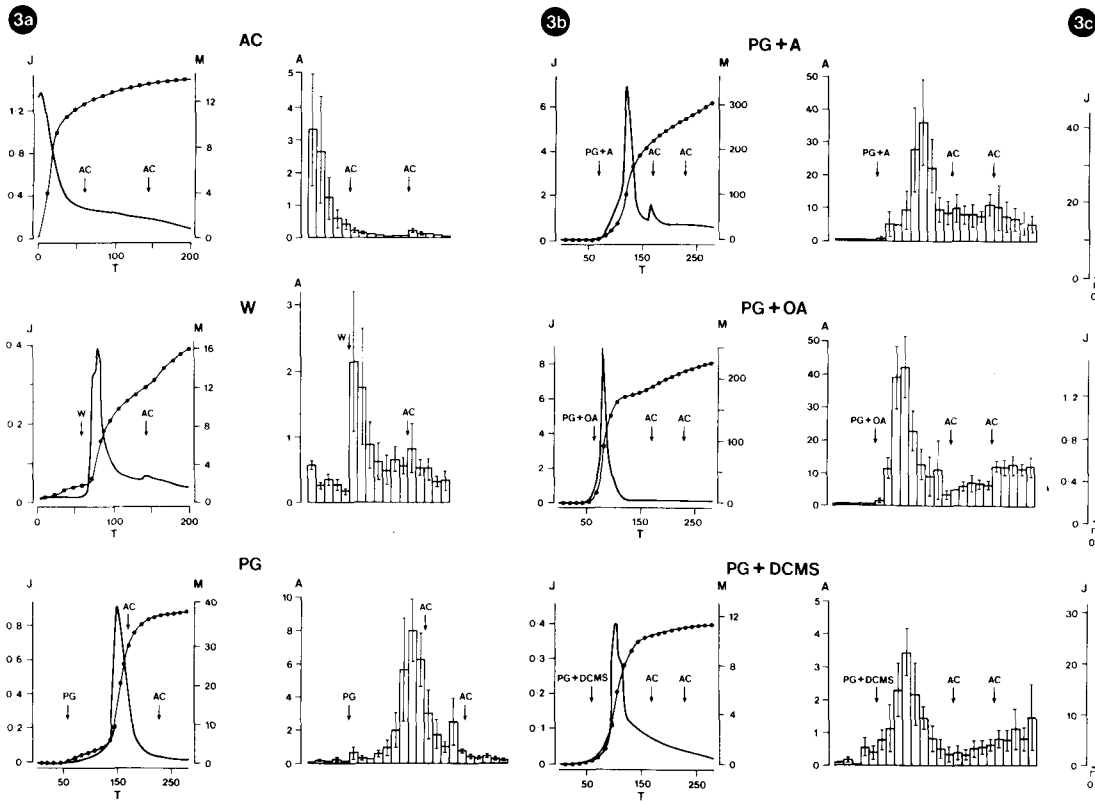

FIG. 3. Penetration of hydrocortisone from acetone-deposited drug films and under the influence of illustrate an example of the flux (J, μg cm^{-2} h^{-1}) and cumulative penetration profiles (M, μg cm^{-2}) amount penetrated in each 12 h period (A, μg cm^{-2}/12 h)—mean ± standard error (3 replicates). AC, aceto Azone; OA, oleic acid; DCMS, decylmethylsulphoxide; 2P, 2-pyrrolidone; NMP, N-methylpyrrolidone; NMF, N-

and treatment regime, the appropriate figure shows an example of a cumulative penetration profile and the derived flux curve, together with a histogram of amount penetrated in each 12 h period (mean and standard error for 3 replicates). These histograms illustrate the general variability of human skin, particularly as emphasized by penetration enhancer treatment (Southwell et al 1984).

To aid interpretation, selected values have been taken from these figures and listed in tables dealing with amounts penetrated and penetration rates (Tables 2 and 3).

Table 2 lists the amount of material which diffused through the skin membrane in a 60 h period during Stages 1, 2 and 3. To show which potential enhancers perform as such, it would be expected that the ratio of Total Tr Stage 2/Total AC_2 Stage 2 should be calculated, however, this would have introduced unacceptable errors due to the natural variations in the permeabilities of the human skin samples used. Fortunately, although the amount penetrating in Stage 1 for each set of cells varied with skin specimens used, this variation was carried through into Stage 2. Thus, any difference due to inherent permeability variations disappeared by calculating the ratio of Total Stage 2/Total Stage 1. As AC_1 was always greater than AC_2 (probably because of the time taken to redissolve the drug in Stage 2) the ratio of Total Stage 2/Total Stage 1 showed clearly which treatment materials enhanced the penetration of the test compounds i.e. if anything, this ratio would underestimate penetration enhancement which is

Table 2. Penetration of skin membranes from acetone-deposited films and under a range of potential penetration enhancers. Data reported as total penetrated in 60 h in each stage (μg cm^{-2}), delay (h) and duration (h).

Cell sets	Stage 1 AC_1 Total[b]	STAGE 2 AC_2 and Tr					Stage 3 AC_3 Total[b]
		Treatment	Delay[c]	Duration[d]	Total[e]	ER(A)[a]	
Mannitol							
1	1·98 ± 0·33	AC_2	—[f]	—[f]	0·94 ± 0·35	0·47	0·72 ± 0·38
2	1·13 ± 0·39	Water	32 ± 28	51 ± 9	2·17 ± 0·39	1·92	1·20 ± 0·52
3	1·23 ± 0·70	PG	—[f]	—[f]	1·54 ± 0·66	1·25	2·17 ± 1·36
4	2·32 ± 1·21	PG + Azone	21 ± 3	19 ± 1	1040 ± 360	446	12·0 ± 4·2
5	2·40 ± 0·69	PG + OA	24 ± 6	31 ± 5	195 ± 112	81·3	18·8 ± 6·3
6	3·83 ± 1·51	PG + DCMS	35 ± 4	15 ± 3	1010 ± 689	264	81·0 ± 10·9
7	1·66 ± 0·57	2P	28 ± 15	15 ± 1	744 ± 487	448	32·8 ± 7·7
8	2·83 ± 1·17	NMP	7 ± 3	29 ± 14	725 ± 385	256	16·7 ± 5·8
9	5·92 ± 1·82	NMF	4 ± 0	20 ± 0	1130 ± 89	191	40·0 ± 23·8
Hydrocortisone							
1	8·12 ± 4·42	AC_2	—[f]	—[f]	0·52 ± 0·11	0·06	0·43 ± 0·21
2	1·63 ± 0·06	Water	1 ± 1	56 ± 4	5·89 ± 2·74	3·61	2·51 ± 0·78
3	0·80 ± 0·32	PG	52 ± 17	48 ± 14	25·0 ± 1·7	31·3	8·75 ± 2·28
4	1·94 ± 0·30	PG + Azone	37 ± 8	70 ± 14	103 ± 38	52·9	44·3 ± 8·0
5	2·02 ± 0·71	PG + OA	17 ± 4	61 ± 8	122 ± 22	60·5	30·6 ± 6·9
6	1·24 ± 0·52	PG + DCMS	19 ± 3	47 ± 4	10·7 ± 2·5	8·65	2·36 ± 0·81
7	5·01 ± 3·47	2P	40 ± 4	46[g]	476[g]	95·0	—[h]
8	2·50 ± 0·81	NMP	16 ± 7	41 ± 13	21·7 ± 4·8	8·68	6·31 ± 1·36
9	2·05 ± 0·31	NMF	5 ± 1	37 ± 11	168 ± 55	82·0	7·92 ± 1·71
Progesterone							
1	3·81 ± 1·74	AC_2	—[f]	—[f]	0·75 ± 0·60	0·20	0·64 ± 0·52
2	3·78 ± 1·02	Water	0	29 ± 4	9·50 ± 0·48	2·51	1·56 ± 0·41
3	6·92 ± 1·41	PG	—[f]	—[f]	9·11 ± 1·71	1·31	3·65 ± 1·45
4	5·15 ± 1·54	PG + Azone	12 ± 10	93 ± 13	19·0 ± 6·2	3·69	5·77 ± 0·83
5	4·29 ± 0·96	PG + OA	12 ± 2	71 ± 13	8·40 ± 1·53	1·96	3·66 ± 0·90
6	8·64 ± 0·92	PG + DCMS	0	35 ± 7	31·4 ± 22·7	3·64	2·12 ± 1·71
7	4·01 ± 2·81	2P	40 ± 2	18 ± 2	90·5 ± 31·6	22·6	12·0 ± 4·31
8	1·12 ± 0·57	NMP	23 ± 11	41 ± 13	19·7 ± 5·9	17·5	4·47 ± 1·64
9	3·64 ± 1·55	NMF	3 ± 1	13 ± 1	97·6 ± 31·6	26·8	5·19 ± 1·98

[a] Enhancement Ratio (Amount) ER(A) = Total Stage 2/Total Stage 1.
[b] Total penetrated in first 60 h after application. Data quoted to 3 significant figures ± s.e.m.
[c] Time before peak became greater than 10% J_{max}. Data quoted in hours ± s.e.m.
[d] Time for which peak height is greater than 10% J_{max}. Data quoted in hours ± s.e.m.
[e] Total penetrated in 60 h, T_{max} ± 30 h or first 60 h of treatment. Data quoted to 3 significant figures ± s.e.m.
[f] At no time was flux less than 10% J_{max} therefore no values were obtained.
[g] Determined by extrapolation of curve, s.e.m. are therefore not quoted.
[h] Values not quoted due to effect of 2P remaining in skin.
Note: Abbreviations as in text.

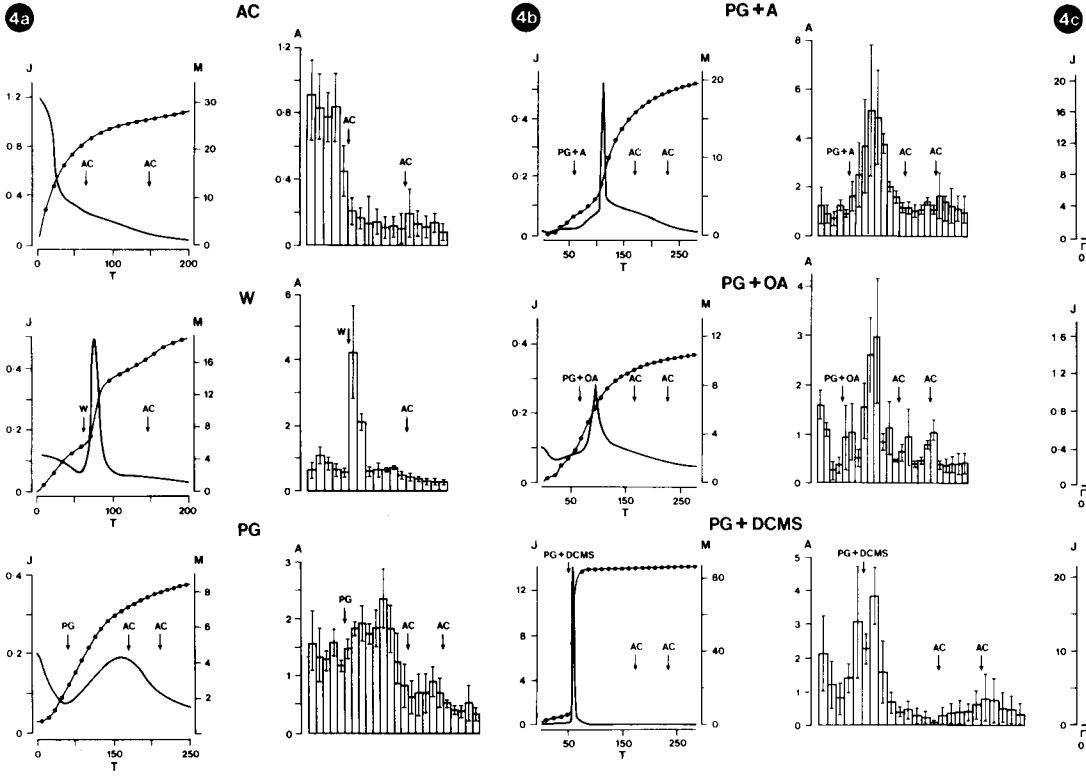

FIG. 4. Penetration of progesterone from acetone-deposited drug films and under the influence of poter trate an example of the flux (J, μg cm^{-2} h^{-1}) and cumulative penetration profiles in each 12 h period error (3 replicates). AC, acetone; W, water; PG, propylene glycol; A, Azone; OA, oleic acid; DCMS, decylme N-methylpyrrolidone; NMF, N-methylformamide.

preferable to overestimating the phenomenon. This value provides the Enhancement Ratio (Amount)–ER(A). With many of the potential enhancers there was a delay before the flux increased. Also the time during which the flux rose to a maximum after each application was variable. We defined the duration of effect as the time for which the flux (J) was greater than 10% J_{max}. The delay period was measured as the time before the flux reached 10% J_{max}. These values provided valuable information about the type of response for the different treatments.

Table 3 lists the maximum flux, J_{max} for Stage 2. To eliminate variation from the skin cells the J_{max} Tr (Stage 2) should be compared with J_{max} Stage 1 to assess whether the treatment enhanced permeation. However, in most situations J_{max} (Stage 1) occurred before the first sampling point and could not be calculated; we replaced it with J_{max} AC_2 (Stage 2). This provided the ratio J_{max} Tr Stage 2/J_{max} AC_2 Stage 2 recorded in Table 3, referred to as Enhancement Ratio (Rate)–ER(R). T_{max} was recorded as the time of onset of J_{max} as this indicated the delay time before each treatment exerted its maximal effect.

Thus, we analysed our data to provide details of both the relative amount of drug which penetrated the skin and also the maximum flux. We now discuss the most significant results obtained.

Mannitol permeation

Mannitol is a highly polar material (Table 1) and presumably penetrates the skin by the polar route. In view of its structure—with 6 OH groups—and very low partition coefficient, we would expect it to permeate skin slowly and to become extensively hydrogen-bonded to the tissue.

The results during deposition from acetone (Stage 1) confirm that mannitol permeation is slow, the average amount penetrating during the first 60 h being <3 μg (Table 2). However, in Stage 2 the dramatic effect of some of the potential penetration enhancers was evident. For example, although PG

Table 3. Variation in maximum flux (J_{max}, μg cm^{-2} h^{-1}) and time of J_{max} (T_{max}, h) during penetration of skin membranes from acetone-deposited films and under a range of potential penetration enhancers.

Stage 2 AC_2	Stage 2 Tr			
J_{max}[b]	Treatment	J_{max}[c]	T_{max}[d]	ER(R)[a]
Mannitol				
0·101 ± 0·048	Water	0·114 ± 0·050	22 ± 7	1·13
0·101 ± 0·048	PG	0·0784 ± 0·005	76 ± 14	0·772
0·101 ± 0·048	PG + Azone	98·5 ± 44·7	26 ± 3	970
0·101 ± 0·048	PG + OA	8·49 ± 6·03	45 ± 15	83·7
0·101 ± 0·048	PG + DCMS	64·7 ± 49·4	42 ± 6	638
0·101 ± 0·048	2P	65·3 ± 42·1	34 ± 21	643
0·101 ± 0·048	NMP	64·9 ± 43·4	17 ± 2	640
0·101 ± 0·048	NMF	121 ± 38·0	14 ± 0	1190
Hydrocortisone				
0·0237 ± 0·0063	Water	0·271 ± 0·125	17 ± 5	11·4
0·0237 ± 0·0063	PG	1·22 ± 0·17	89 ± 5	51·4
0·0237 ± 0·0063	PG + Azone	4·71 ± 1·41	65 ± 7	199
0·0237 ± 0·0063	PG + OA	5·51 ± 1·75	34 ± 2	233
0·0237 ± 0·0063	PG + DCMS	0·371 ± 0·083	39 ± 4	15·7
0·0237 ± 0·0063	2P	22·0 ± 11·1	57 ± 5	928
0·0237 ± 0·0063	NMP	21·2 ± 20·6	23 ± 11	896
0·0237 ± 0·0063	NMF	14·4 ± 8·3	14 ± 1	606
Progesterone				
0·0467 ± 0·0089	Water	0·477 ± 0·091	9 ± 3	9·57
0·0467 ± 0·0089	PG	0·240 ± 0·043	61 ± 3	5·14
0·0467 ± 0·0089	PG + Azone	0·703 ± 0·119	57 ± 8	15·1
0·0467 ± 0·0089	PG + OA	0·365 ± 0·089	38 ± 0	7·81
0·0467 ± 0·0089	PG + DCMS	0·046 ± 0·120	18 ± 2	9·55
0·0467 ± 0·0089	2P	11·4 ± 4·6	46 ± 0	244
0·0467 ± 0·0089	NMP	0·664 ± 0·281	32 ± 6	14·2
0·0467 ± 0·0089	NMF	12·4 ± 4·4	9 ± 1	265

[a] Enhancement Ratio (Rate), $ER(R) = \frac{J_{max}\ Tr\ Stage\ 2}{J_{max}\ AC_2\ Stage\ 2}$

[b] Mean of 3 cells where AC applied as control treatment ± s.e.m.

[c] Data quoted to 3 significant figures ± s.e.m.

[d] Data quoted to nearest number of hours ± s.e.m.

Note: Abbreviations as in text.

alone produced little increase in penetration, when used with either Azone or DCMS it caused the amount penetrated to be increased several hundred-fold as shown by the ER(A) values. These penetration enhancers produced a sharp response peak (Fig. 2). The long lag time recorded for all the PG solutions probably arose from the slow increase in the thermodynamic activity of mannitol at the skin surface as PG tended to remain on the stratum corneum for a protracted time i.e. the mannitol concentration in the vehicle increased only slowly. The effect of PG + Azone and PG + DCMS is further illustrated in Table 3, which compared the maximum rates of penetration. The ER(R) provided values of 970 for PG + Azone and 638 for PG + DCMS. When it is considered that the value obtained with PG alone was less than 1, it is clear that these materials produced very significant effects.

2P, NMP and NMF also dramatically enhanced mannitol penetration. T_{max} occurred much later with 2P than with the other two enhancers; the delay time would be longer with 2P than with NMP because the methyl group on NMP aids partitioning into the skin.

The effect seen with PG + OA was much smaller than that from the other enhancers, supporting the view that OA may preferentially affect compounds which penetrate via a non-polar route (Cooper 1984). However, there was a prominent enhancing effect compared with PG alone.

Water did not markedly increase mannitol penetration (Southwell 1981). As Behl et al (1980) showed that the extensiveness of the 'aqueous pore pathway' was relatively unaffected by hydration, our observation suggests that water permeates by an equivalent pathway to that taken by other small polar molecules such as methanol and ethanol.

Our evidence for mannitol leads us to conclude that this highly polar molecule is a useful model material for illustrating the effect of penetration enhancers which influence the polar route for permeation.

Hydrocortisone permeation

Although hydrocortisone is often referred to as a 'polar' steroid, it is relatively non-polar (Table 1) and we would expect it to use a predominantly non-polar route to permeate human skin. Therefore, vehicles that increase hydrocortisone penetration of the stratum corneum are likely to increase the permeation of other compounds which use the non-polar diffusion route.

As the partition coefficient of hydrocortisone is favourable for skin penetration, it might be expected that the effect of enhancers would be less dramatic than for mannitol. This is illustrated in the Tables and Figures. However, both water and PG increased hydrocortisone permeation although neither increased mannitol penetration. These increases correlate with the well-documented fact that water or hydration enhance the permeation of hydrocortisone (Scholtz 1961; Sulzberger & Witten 1961; Hall-Smith 1962; Vickers 1963; Feldmann & Maibach 1965). In our study, the increased permeation due to water was relatively small while PG increased the amount of hydrocortisone penetrating in 60 h by a factor of approximately 30 (Table 2) and also increased J_{max} by a factor of 50 (Table 3). Thus PG acted as a penetration enhancer for hydrocortisone. At first sight, it would appear that Azone and OA when in combination with PG also had dramatic effects. However, a large proportion of this effect was due to the activity of PG alone and this will be discussed later. The combination of PG + DCMS provided positive enhancement ratios but the values were less than those obtained for PG alone.

2P and NMF also greatly increased the absorption of hydrocortisone, showing that they act as enhancers for both the polar and non-polar route. With NMP however the rate of penetration was greatly increased but not the amount penetrated, because the rate peak was very sharp (Fig. 3).

Progesterone permeation

Progesterone is a non-polar steroid with a high partition coefficient (Table 1) and its penetration is likely to be at least partially under diffusion layer control (Barry 1983). Any enhancement effect will be limited as a system under total diffusion layer control (rate-limiting step in permeation not within the stratum corneum) would show no response to an accelerant which only modified the resistance of the horny layer. Also progesterone has a partition coefficient that promotes entry into the stratum corneum through which the drug diffuses relatively easily. This feature alone makes progesterone less useful than polar compounds for evaluating the effects of penetration enhancers. In general, there appears to be few compounds of satisfactory physicochemical nature for illustrating penetration enhancement as applied to the lipid route.

The results (Tables 2 and 3) showed much lower and more variable effects for progesterone penetration than for the other two compounds. 2P and NMF provided the greatest enhancement as judged by ER(A) and ER(R) values. Water and PG caused little enhancement, confirming that progesterone

permeation was at least partially under diffusion layer control. It proved difficult to draw definite conclusions as to whether or not the other potential enhancers aided the penetration of progesterone; NMP showed the greatest ER(R) effect.

Mixed penetration enhancers

The literature is controversial about whether PG is a penetration enhancer (Ritschel 1969). We found that it did not function as an accelerant for mannitol, or progesterone (probably due to diffusion layer effects) but it did enhance hydrocortisone permeation.

An alternative way of analysing our data so as to illustrate the additional effect arising from adding other enhancers to PG is to calculate the ratios 'Total amount penetrated from PG plus other enhancer/ Total amount penetrated for PG', making the value for PG alone equal to one. For mannitol we then found PG + Azone = 680, PG + OA = 130, and PG + DCMS = 660. The addition of OA to PG increased permeation but to a lesser extent than either Azone or DCMS, supporting the view that OA may preferentially affect non-polar compounds (Cooper 1984).

With hydrocortisone the ratio values were PG = 1, PG + Azone = 4·1, PG + OA = 4·9 and PG + DCMS = 0·4. PG alone enhanced the permeation of hydrocortisone and contributed much of the effect of the mixed enhancers. Thus, we were left with residual values of only 4 and 5 for PG + Azone and PG + OA, respectively, after the action due to PG had been removed. The slightly greater effect of PG + OA than that of PG + Azone may relate to OA being an accelerant for non-polar compounds (Cooper 1984).

PG + DCMS reduced the amount of hydrocortisone penetrating the skin (ratio = 0·4). One explanation for this is that DCMS, because of its surfactant structure, may form micelles in PG at the concentration used (15%) particularly when diluted with water passing from the receptor through the skin and into the donor. These micelles would contain much of the steroid and so inhibit drug partitioning into the skin. This explanation correlates with the results of Shinkai & Tanaka (1983) who showed that concentrations of lauric diethanolamide up to 7% increased the permeation of hydrocortisone while higher concentrations reduced penetration probably because of micellar entrapment of the steroid.

Table 2 also shows the delay and duration times for the various treatments. The addition of Azone, OA or DCMS to PG reduced the delay time before the increase in flux. (N.B. Because of the experimental arrangement, an actual delay time for PG with mannitol was not determined (Fig. 2) but it was certainly longer than for the mixed enhancers.) With mannitol the shortest delay time was observed with PG + Azone, a combination that did not reduce the delay time for hydrocortisone to the same extent. For hydrocortisone, the combinations PG + OA and PG + DCMS produced greater reductions in the delay time. If duration times are examined, it may be seen that, generally, treatments were effective for longer when hydrocortisone rather than mannitol was the test compound. However, the trend was similar except that PG + Azone had a shorter duration of action with mannitol than would have been expected from hydrocortisone results (see also Figs 2, 3). The explanation for the differences with PG + Azone could be that this mixture actually accelerated polar and non-polar compounds by different mechanisms.

It appeared that PG alone worked as an enhancer in situations where it built up in the stratum corneum and the solubility and thereby the partition coefficient of the penetrant increased in the tissue. This would occur when the penetrant is more soluble in PG than in water. There is then an added effect due to Azone, OA or DCMS depending on how these compounds affect the diffusion of the penetrant. Our data showed that PG had little effect on mannitol (a very water-soluble compound), a positive effect on hydrocortisone (a steroid more soluble in PG than in water) and little effect with progesterone (a steroid at least partially under diffusion layer control). The effects of Azone, OA and DCMS were then additive. It has recently been shown that Azone increases the penetration rate of PG (Evans personal communication; Hoelgaard & Møllgaard 1986) which could explain the increase in hydrocortisone permeation with PG + Azone compared with PG alone.

Further acetone application

Our general technique involved further treatment with acetone (Stage 3, illustrated in Tables 2 and 3) and a fourth application applied to cells previously treated with mixed enhancers (Stage 4, for brevity, data not shown). Our results may be summarized as (a) PG did not damage the skin but set up drug reservoirs, (b) the Azone from PG + Azone and the oleic acid from PG + OA may remain in the skin for a considerable time after application, and (c) acetone did not damage the stratum corneum.

CONCLUSIONS

(1) In attempting to probe the mechanism of enhancer action, it is necessary to use penetrants of different polarity and to test them with a variety of potential penetration enhancers.

(2) From our experimental data and theoretical considerations, we propose that mannitol permeates the skin by a polar pathway, hydrocortisone mainly by a lipid route, and progesterone by the same lipid route. Progesterone permeation was also affected by aqueous layers so that its clearance from the stratum corneum into the remaining epidermis, dermis and receptor militated against firm conclusions about enhancer activity.

(3) Mannitol showed dramatic penetration enhancement effects of up to several hundred fold, presumably because of its low partition coefficient and extensive hydrogen-bonding to the skin.

(4) 2-Pyrrolidone, *N*-methylpyrrolidone, *N*-methylformamide, propylene glycol plus Azone and propylene glycol plus decylmethylsulphoxide greatly increased mannitol permeation—this trend pointed to a polar route effect.

(5) Propylene glycol plus oleic acid slightly increased mannitol penetration which also supports a polar route effect.

(6) 2-Pyrrolidone, *N*-methylpyrrolidone, *N*-methylformamide, propylene glycol plus Azone, propylene glycol plus oleic acid and propylene glycol alone greatly increased hydrocortisone permeation, this was interpretable as a lipid route effect.

(7) Water and propylene glycol plus decylmethylsulphoxide slightly increased hydrocortisone penetration, suggesting a lipid route effect.

(8) Progesterone showed increased penetration with 2-pyrrolidone, *N*-methylpyrrolidone and *N*-methylformamide. However, because its permeation was affected by aqueous layers, it has limitations as a model drug for illustrating enhancer effects.

(9) Propylene glycol plus Azone affected mannitol and hydrocortisone permeation by different mechanisms.

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