J. Pharm. Pharmacol. 1985, 37: 27–37
Received 28 March, 1984

Absorption through human skin of ibuprofen and flurbiprofen; effect of dose variation, deposited drug films, occlusion and the penetration enhancer *N*-methyl-2-pyrrolidone

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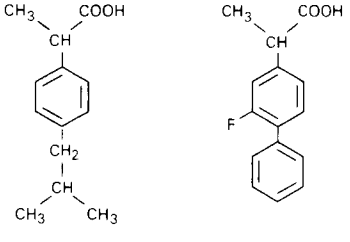
The penetration of ibuprofen and flurbiprofen, non-steroidal anti-inflammatory agents, was investigated from drug films deposited by acetone evaporation on cadaver skin in an open cell 'in-vivo mimic' design. Increased dosage did not produce a proportional increase in the permeation and maximizing the skin-drug contact did not increase penetration: both factors indicate that absorption from deposited drug films was dissolution rate-limited. Occlusion of the skin did not increase the dissolution rate of the deposited drug film, but did elevate the penetration of drug already present within the skin at the time of occlusion. The diffusion coefficients for both drugs were calculated by two methods, yielding $1{\cdot}8 \pm 1{\cdot}3 \times 10^{-11}$ cm^2 s^{-1} and $1{\cdot}0 \pm 0{\cdot}56 \times 10^{-11}$ cm^2 s^{-1} for ibuprofen and $1{\cdot}9 \pm 0{\cdot}59 \times 10^{-11}$ cm^2 s^{-1} and $0{\cdot}77 \pm 0{\cdot}23 \times 10^{-11}$ cm^2 s^{-1} for flurbiprofen. Increasing the acetone-skin contact time from 2 min to 2 h did not significantly alter the permeability of the skin. Absorption of flurbiprofen was similar from 10 and 100% saturated aqueous solutions, suggesting that the skin has a limited capacity for flurbiprofen transport beyond which further increase in drug penetration may be difficult. *N*-Methyl-2-pyrrolidone enhanced the penetration flux of ibuprofen sixteenfold and flurbiprofen, over threefold. The 'in-vivo mimic' design for permeation experiments has thus proved to be useful for evaluating the kinetics of topical therapy and the mechanism of action of potential penetration enhancers.

For the treatment of sunburn, rheumatoid arthritis and allied conditions, topical therapy with non-steroidal anti-inflammatory agents warrants investigation as an alternative to oral treatment.

The most widely reported in-vitro method for investigating skin absorption of drugs uses a membrane mounted between two fluid filled chambers. Drug permeation is monitored from the stratum corneum (SC) side (donor) through to the dermal side (receptor). However, this arrangement fully solvates or hydrates the skin, and thus does not mimic the normal in-vivo condition of the SC; such hydration may be expected to increase significantly the skin permeation of most compounds.

In our 'in-vivo mimic' design we exposed the SC surface to controlled environmental conditions and maintained the receptor at body temperature. The drug could then be applied to the skin as a solid drug film (via acetone evaporation), as discussed in this paper, or more generally as a thin liquid, cream or ointment film which is allowed to deplete in concentration. This system allows water and temeperature gradients to develop across the skin, similar to those found in-vivo. It enables the investigator not only to control the extent of skin hydration by occluding the donor chamber but also to observe the effects of dose size variation, changes in drug concentration and addition of vehicles (e.g. penetration enhancers) on the deposited film. The absorption of compounds can therefore be studied in the form and dose most applicable to their in-vivo use. (For a review of these concepts, see Barry 1983.)

To establish and check the validity of the technique we used acetylsalicylic acid. We then chose ibuprofen and flurbiprofen as model drugs because they are widely used, orally effective, non-steroidal anti-inflammatory agents and they represent weak acidic drug molecules—a class of compound little investigated in skin permeation



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studies. From the data presented here we assessed their absorption from deposited drug films and investigated the potential for using the technique as a model for the determination of penetration enhancer activity (Akhter et al 1982a, b). Thus the main questions the study attempts to answer are: what are the kinetics of skin penetration of phenylalkanoic acids applied to the SC in a volatile vehicle?; what role does drug dissolution play?; how does skin hydration effect the permeation of these molecules?; and is *N*-methyl-2-pyrrolidone a penetration enhancer for these carboxylic acids?

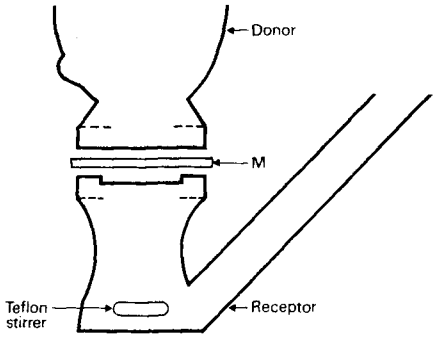

FIG. 1. Diffusion cell; M represents skin membrane.

MATERIALS AND METHODS

I *Preparation of skin membranes*

Strips of caucasian abdominal skin obtained at autopsy (male and female, age 68 ± 14 years) were used either immediately or stored at −24 °C. Specimens were thawed, washed with warm water or 0·1% Triton-X 100, clamped between two metal plates and frozen so as to provide an even surface. The plates were removed and full-thickness skin (~430 μm deep) was obtained by cutting the skin, when the dermis and epidermis were just mobile to the touch, with a Davies Dermatome (Duplex Electro Dermatome).

II *Chemicals*

Acetyl [carboxyl-^{14}C]salicylic acid, specific activity 111 μCi mg^{-1} was obtained from the Radiochemical Centre, Amersham. Ibuprofen, 2-(4-isobutylphenyl) propionic acid, specific activity 23 μCi mg^{-1}; flurbiprofen, 2-(2-fluoro-4-biphenylyl) propionic acid, specific activity 18 μCi mg^{-1} were both supplied courtesy of The Boots Company, Nottingham. The following non-labelled compounds were used: Soluene-350 (Packard Instruments Co. Inc.), Fisofluor 1-liquid scintillator (Fisons Scientific Apparatus Ltd), Triton-X 100 (BDH Chemicals Ltd), and *N*-methyl-2-pyrrolidone (BDH Chemicals Ltd).

III *Application method and penetrant assay*

Dermatomed skin (M) was mounted in glass diffusion cells to provide a membrane area of 2·27 cm^2 (Fig. 1). The donor side (SC) was exposed to 22 ± 1 °C and 60 ± 5% relative humidity and the receptor contained phosphate buffer pH 7·4 at 37 ± 0·5 °C (ibuprofen, pK_a 4·45 ± 0·05, and flurbiprofen, pK_a 4·13 ± 0·22, would be essentially fully ionized at this pH). After the skin had equilibrated for approximately 20 h, drug films were deposited by acetone evaporation (50 μl) from radiolabelled solutions.

The cumulative penetration of the drug was monitored by sampling 1 ml of the receptors at hourly intervals for the first 5 h, every 2 h for the next 8–10 h and then 4–6 hourly. The sample was added to 10 ml of FisoFluor 1 and analysed by scintillation counting (Packard Liquid Scintillation Counter, Model Tri-Carb 460C). Each sample removed was replaced with an equal volume of buffer; the entire receptor volume was frequently removed and replaced with fresh buffer, thus maintaining sink receptor conditions. The rate (flux) data were then derived from the cumulative profiles by determining the gradient; for clarity only half to one third of the data points obtained are shown in the cumulative profiles (Figs 2, 3).

At the end of the investigation residual drug remaining on the surface of the skin was estimated by washing with acetone, pooling the aliquots and then evaporating the acetone. Drug remaining within the SC (including some epidermis) and in the dermis (including some epidermis) was estimated after separating the tissues. (SC after this length of time peels readily from the dermis, therefore some epidermis is found adhering both to the SC and the dermis.) The tissues were solubilized separately in 1 ml of Soluene-350 plus 30–40 μl water for 2 h at 50 °C; radioactivity was estimated after adding 10 ml of FisoFluor 1.

IV *Preliminary investigation*

Acetylsalicylic acid, 18 μg containing 2 μCi per diffusion cell (3 replicates), was applied in 50 μl acetone as above and penetration was monitored for 130 h.

V *Primary investigation*

Ibuprofen and flurbiprofen at three dose levels each (0·1, 0·7 and 5·0 mg) containing 4 μCi per diffusion cell (4 replicates per dose, per drug) were applied in 50 μl acetone. The penetration profile was monitored for 180 h. During the first 60 h penetration was

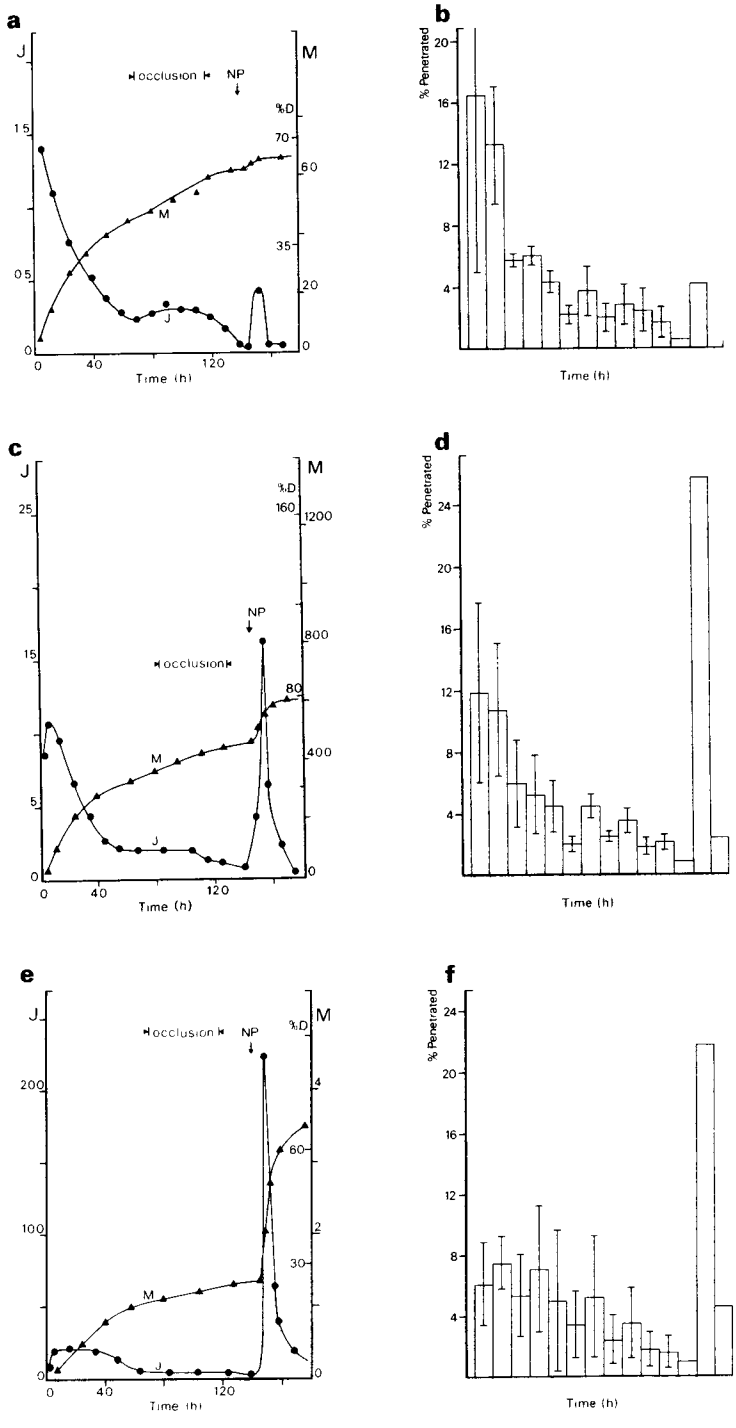

FIG. 2. Penetration of ibuprofen from 100 μg (a, b), 700 μg (c, d) and 5·0 mg (e, f) deposited drug films; effect of occlusion and *N*-methyl-2-pyrrolidone (NP). Data illustrate flux (J, μg 2·27 cm^{-2} h^{-1}) and cumulative penetration profiles (M, μg 2·27 cm^{-2}; % D, percent of cumulative) and histograms of % penetrated per 12 h period—average ±s.d. (3–4 replicates or average 2 replicates).

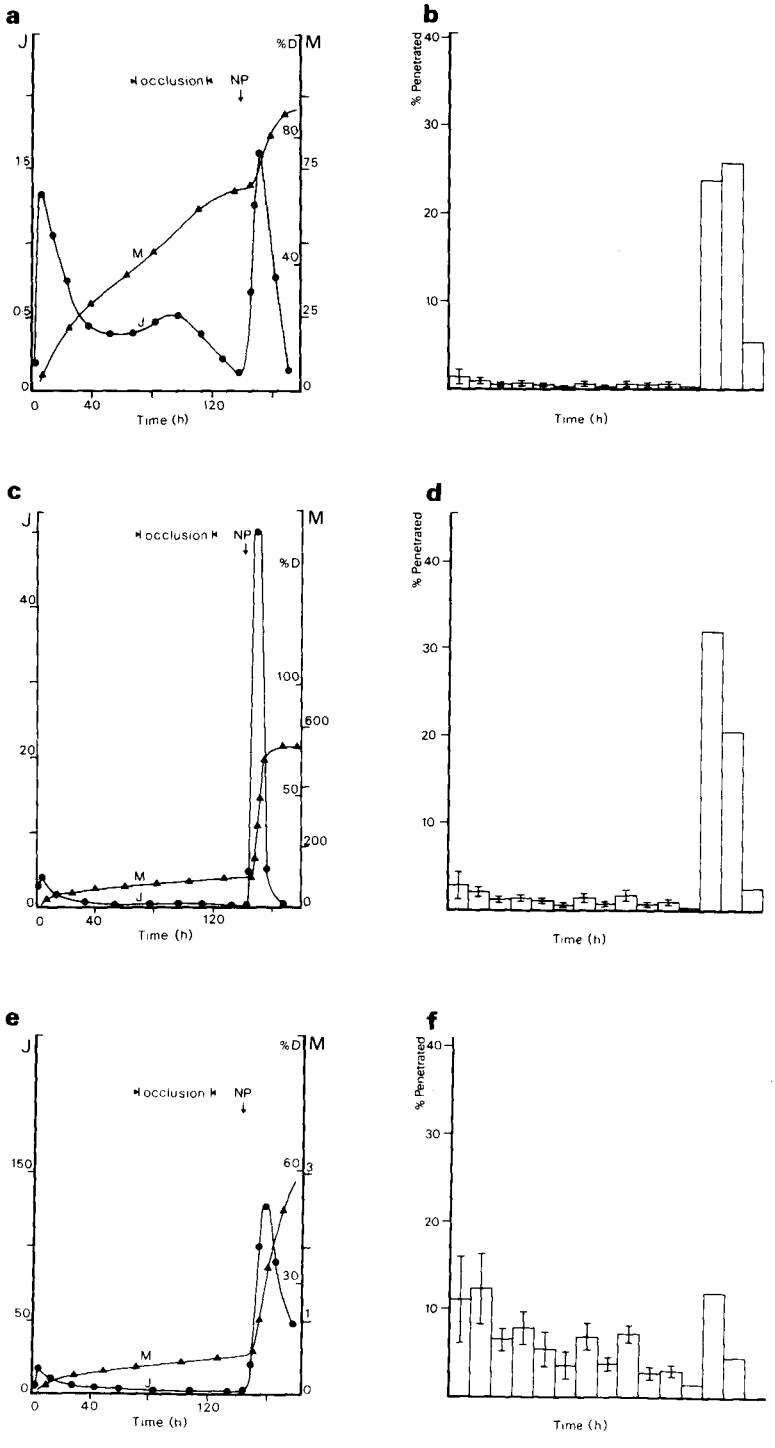

FIG. 3. Penetration of flurbiprofen from 100 μg (a, b), 700 μg (c, d) and 5·0 mg (e, f) deposited drug films; effect of occlusion and *N*-methyl-2-pyrrolidone (NP). Data illustrate flux (J, μg 2·27 cm^{-2} h^{-1}) and cumulative penetration profiles (M, μg 2·27 cm^{-2}; %D, percent of cumulative) and histograms of % penetrated per 12 h period (average of 3–4 replicates).

from acetone, and from a deposited drug film. The donor was then occluded with Parafilm for 40 h, the Parafilm was removed and the skin allowed to re-equilibrate to the controlled room conditions. Towards the end of the experiment 100 µl of *N*-methyl-2-pyrrolidone (NP) sufficient to cover the skin area was added. The maximum dose of either drug was completely soluble in this volume of NP. At the end of the investigation the content of drug in the skin and drug remaining on the surface of the skin was estimated as described in section III.

VI *Calculation of diffusion coefficient (D)*

Since no steady state diffusion is reached using the 'in-vivo mimic' approach, determination of D from lag time measurement is not feasible. Hence, D values were calculated from equation (1), assuming the thickness of the SC (l) to be 15 µm and the thickness of the initial layer deposited in the upper part of the SC to be negligible.

$$D = l^2/6t_{max} \quad (1)$$

The equation relies upon a knowledge of t_{max}, the time at which the penetration rate passes through a maximum. Equation (1) is derived from the general solution of the simple membrane problem (Scheuplein & Ross 1974; Crank 1975).

An alternative method of calculating D is represented by equation (2)

$$D = 0{\cdot}371\, l^2/t_{\frac{1}{2}} \quad (2)$$

which relies on estimating $t_{\frac{1}{2}}$—the time required for one-half of the diffusing fraction to penetrate the skin (Foreman et al 1983).

VII *Secondary investigations*

(a) *Drug film—skin contact.* Flurbiprofen, 10 mg containing 4 µCi per diffusion cell, was applied in acetone solution to four diffusion cells as above. After drug film deposition a 50 g brass weight was placed on the skin in two diffusion cells; the other two cells were left as normal.

Skin was mounted in another six diffusion cells and washed with 1 ml of Triton-X 100 (0·1%) for 1 min and rinsed with 6 × 1 ml aliquots of warm water. All cells were equilibrated to conditions described in section III. Flurbiprofen, 10 mg containing 4 µCi per diffusion cell, was then deposited from acetone solution as usual. A 50 g brass weight was placed on the skin in three diffusion cells; the other three cells were left as normal. The cumulative penetration was monitored for 60 h.

(b) *Flurbiprofen penetration from 10 and 100% saturated water solutions.* Six diffusion cells were prepared in the normal manner and 10 mg flurbiprofen (4 µCi) applied to each as a drug film. Permeation was monitored for approximately 80 h, then the remaining drug on the skin was removed by acetone washing. The diffusion cells were allowed to stand for 50 h and penetration of any remaining drug was monitored.

Solutions of flurbiprofen (1 ml) representing 10 and 100% aqueous saturation were added to each of three cells. The penetration profiles were further monitored for approximately 60 h, with frequent refreshment of the donor to prevent depletion of drug or vehicle.

(c) *Penetration from flurbiprofen discs.* Flurbiprofen discs, 210 mg containing 2 µCi per disc, were prepared using an infrared spectrophotometer disc holder (pressure 1 Ton cm^{-2}). The discs were placed on the skin surface (one specimen) in 4 diffusion cells and the cumulative penetration was monitored for 80 h.

For comparison with the discs, flurbiprofen, 10 mg containing 2 µCi per diffusion cell, was applied in acetone solution to two diffusion cells, thus depositing a drug film. The penetration was monitored for 80 h.

(d) *Acetone contact time.* Flurbiprofen, 10 mg containing 4 µCi per diffusion cell, was deposited from acetone solution (50 µl) in two diffusion cells and the penetration was monitored for 60 h. The same quantity of drug was also applied to another three diffusion cells in 500 µl of acetone and the donor chambers were immediately occluded to prevent acetone loss. Occlusion was removed 2 h later and the acetone allowed to evaporate. The cumulative penetration was monitored for 60 h.

(e) *Flurbiprofen penetration in previously prehydrated and normal skin.* Flurbiprofen, 10 mg containing 4 µCi per diffusion cell, was deposited from acetone solution in eleven diffusion cells. The penetration profile was monitored for 50 h. Another 15 diffusion cells were prepared and the donor chambers occluded for 30 h. Flurbiprofen, 10 mg containing 4 µCi per diffusion cell, was then deposited from acetone solution and the drug permeation monitored for 50 h.

RESULTS

I *Preliminary investigation*

Preliminary in-vitro investigations using acetylsalicylic acid produced a cumulative penetration of 21·2 ± 3·8% of applied dose after 120 h, and a maximum flux of 0·28 ± 0·06% dose h^{-1}. These results were compared with the in-vivo date of Feldmann & Maibach (1970) who reported a cumulative penetration of 21·8% of applied dose after 5 days, and a maximum flux of 0·43% dose h^{-1}. Aspirin and salicylic acid permeation is similar (Feldmann & Maibach 1970; Franz 1978) and hence no corrections were made for any aspirin hydrolysis.

II *Primary investigation*

Table 1 indicates that the diffusion of both ibuprofen and flurbiprofen applied in the volatile solvent, acetone, increases with dose but not in direct proportion. Figs 2 & 3 represent absorption of ibuprofen and flurbiprofen, respectively, from three doses, illustrating example data for flux and cumulative penetration profiles, together with histograms (average of 4 replicates) of % penetration in 12 h intervals. Both figures further illustrate an initial ibuprofen or flurbiprofen rate maximum (at about 10 h) due to partitioning from acetone solution and subsequent permeation; this maximum fell to lower penetration rates from the deposited drug film. Occlusion produced no apparent increase in the flux of either drug; however, the sudden increase in the penetration rate following NP addition was significant. The data in Table 2 show percentage of ibuprofen or flurbiprofen remaining on the surface of the skin, in the SC and in the dermis, at the end of the investigation.

III *Diffusion constants for ibuprofen and flurbiprofen*

The diffusion constant calculated by using equation (1) was 1·8 ± 1·3 × 10^{-11} cm^2 s^{-1} for ibuprofen and 1·9 ± 0·59 × 10^{-11} cm^2 s^{-1} for flurbiprofen. By using equation (2) the diffusion constant for ibuprofen was 1·0 ± 0·56 × 10^{-11} cm^2 s^{-1} and flurbiprofen it was 0·77 ± 0·23 × 10^{-11} cm^2 s^{-1}. These values represent an average of 12 replicates.

IV *Secondary investigations*

(a) *Drug film—skin contact.* There was no marked difference between permeation of flurbiprofen from normal skin and that from normal skin plus the weight (data expressed as maximal rate obtained (J_{max}; μg cm^{-2} h^{-1}) and cumulative penetration (M; μg cm^{-2}) at J_{max} and at 60 h were respectively sample A 6·6, 9·7, 97 (n = 2); A + weight 6·1, 9·2, 75 (n = 2); B (washed) 3·8 ± 1·2, 9·6 ± 4·9, 93 ± 26 (n = 3) B washed + weight 3·1 ± 0·62, 6·9 ± 2·7, 67 ± 17 (n = 3)). The data further suggest that there was no appreciable difference between Triton-X 100 washed skin and Triton X-100 washed skin plus the added weight.

(b) *Flurbiprofen penetration from 10 and 100% saturated water solutions.* Fig. 4 compares the penetration of flurbiprofen from a 10 mg deposited drug film with flurbiprofen penetration from a 10% saturated solution. The Figure indicates that when we removed the deposited drug film and replaced it with a 10% saturated solution, there followed a lag time after which the solute permeation reached and maintained a steady state flux. Maximal flurbiprofen absorption from the drug film (0–20 h) and from the 10% saturated solution was 5·2 ± 3·6 μg cm^{-2} h^{-1} (n = 3) and 20 ± 7·9 μg cm^{-2} h^{-1} (n = 3) repectively (representing a ratio of ~1 : 4).

A comparison of flurbiprofen absorption from 10 and 100% saturated solutions (20 ± 7·9 μg cm^{-2} h^{-1} and 22 ± 14 μg cm^{-2} h^{-1}, respectively) indicates that the penetration was essentially the same.

The figures quoted above were derived from three replicates, representing three separate skin specimens.

Table 1. Ibuprofen and flurbiprofen penetration from 0·1, 0·7 and 5·0 mg doses. Data expressed as μg cm^{-2} penetrated (M) and ratio of M (M*) relative to 0·1 mg dose at 11, 24 and 49 h; average of 3–4 replicates.

Drug	Applied dose (mg)	11 h		24 h		49 h	
		M	M*	M	M*	M	M*
Ibuprofen	0·1	8·7 ± 5·6	1·0	13 ± 7·0	1·0	19 ± 6·9	1·0
	0·7	40·0 ± 23·0	4·6	77 ± 30·0	5·9	110 ± 39·0	5·8
	5·0	140·0 ± 67·0	16·0	310 ± 10·0	24·0	590 ± 210·0	31·0
Flurbiprofen	0·1	5·1 ± 2·7	1·0	11 ± 4·7	1·0	17 ± 6·0	1·0
	0·7	9·3 ± 5·6	1·8	16 ± 6·3	1·5	25 ± 7·1	1·5
	5·0	27·0 ± 19·0	5·3	49 ± 29·0	4·5	74 ± 42·0	4·4

Table 2. Ibuprofen and flurbiprofen skin drug balance from 0·1, 0·7 and 5·0 mg deposited drug films. Data expressed as: percentage (%) drug remaining on the skin (Ac wash), in the stratum corneum (SC), in the dermis (D) and their ratios (%*) relative to the dermis. Data represent average of 3–4 replicates.

	Dose					
	0·1 mg		0·7 mg		5·0 mg	
	%	%*	%	%*	%	%*
Ibuprofen						
D†	0·20 ± 0·12	1·0	0·42 ± 0·32	1·0	0·38 ± 0·16	1·0
SC†	4·0 ± 4·6	20·0	3·6 ± 4·8	8·6	3·3 ± 0·22	8·7
Ac wash	2·4 ± 2·5	12·0	3·4 ± 4·9	8·1	3·4 ± 1·5	9·0
Flurbiprofen						
D†	0·66 ± 0·17	1·0	1·1 ± 0·35	1·0	0·92 ± 0·24	1·0
SC†	3·4 ± 2·7	5·2	14.0 ± 10·0	13·0	4·7 ± 1·6	5·1
Ac wash	6·3 ± 4·3	9·6	20·0 ± 5·0	18·0	11·0 ± 13·0	12·0

† Plus some epidermis; see text for further details.

(c) *Penetration from flurbiprofen discs.* The penetration of flurbiprofen from the disc was 0·004 $\%h^{-1}$, while penetration from the deposited drug film was 0·02 $\%h^{-1}$.

(d) *Acetone (Ac) contact time.* Fig. 5a, b shows the effect of increasing the acetone contact time from 2 min to 2 h on flurbiprofen permeation. Fig. 5b indicates that flurbiprofen starts to penetrate within a short time when the acetone contact time was 2 min. However, Fig. 5a shows that increasing the acetone contact time to 2 h prevented significant flurbiprofen permeation until the acetone was allowed to evaporate.

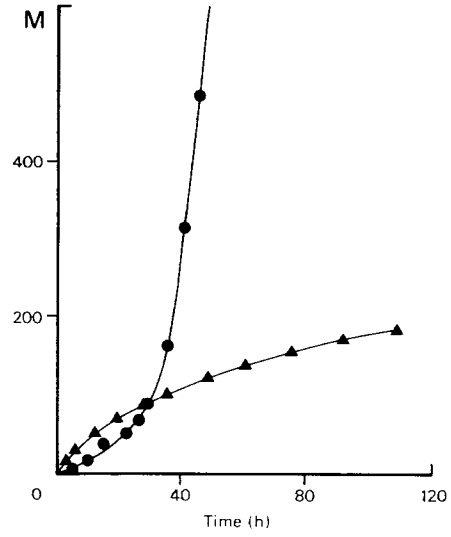

FIG. 4. Absorption of flurbiprofen from a 10 mg deposited drug film (▲) and from a 10% flurbiprofen solution in water (●). Data expressed as cumulative penetration (M, μg 2·27 cm^{-2}) against time (h).

Once the acetone had evaporated and its effect had been removed, the absorption of flurbiprofen was not substantially different for the two acetone contact times and the maximum fluxes were similar: Data expressed as maximal rate obtained (J_{max}; μg cm^{-2} h^{-1}); cumulative penetration (M; μg cm^{-2}) at 2, 6, 12, 24, 48 and 60 h; and the time at which J_{max} occurs (T_{max}, h) are respectively at 2 min Ac time 12, 27, 43, 93, 130, 170, 190, 1·9 (n = 2) and 2 h Ac time: 10 ± 1·7, 0·77 ± 0·21, 38 ± 4·2, 87 ± 14, 140 ± 18, 210 ± 16, 220 ± 16, 4·8 (n = 3).

(e) *Flurbiprofen penetration in previously prehydrated and normal skin.* Fig. 6 shows that the permeation of flurbiprofen through prehydrated occluded skin (i.e. occluded before drug deposition) and non-occluded skin was similar.

Fig. 7 represents the penetration of ibuprofen (a, b) and flurbiprofen (c, d) from 0·1 mg deposited drug films. The figure shows that occlusion elevated and maintained the flux of both drugs (Fig. 7a, c), provided the onset of occlusion was not delayed beyond the time required for the acetone peak to decay. However, the increase in penetration was less pronounced or not observed if the acetone peak had already mainly or fully decayed (Fig. 7d, b, respectively).

The overall effect on the three doses due to acetone, drug film dissolution, occlusion and NP treatment is summarized in Fig. 8.

DISCUSSION

From our preliminary investigations with acetylsalicylic acid, we can infer that our technique represents a valid 'in-vivo mimic' design and we can

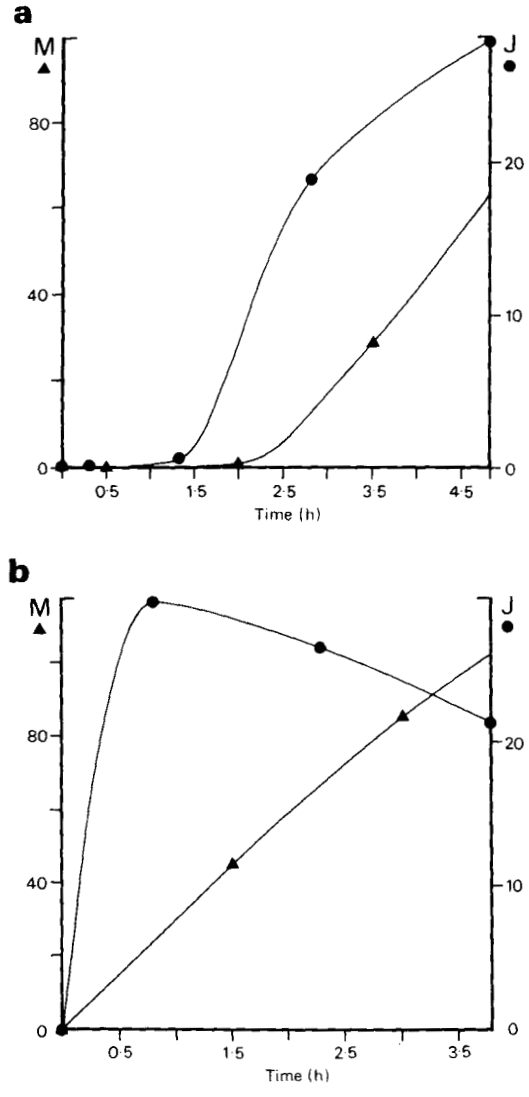

FIG. 5. Penetration of flurbiprofen, effect of acetone contact time. Data illustrate acetone contact time of 2 h (a) and 2 min (b); flux (J, μg 2·27 cm^{-2} h^{-1}; ●) and cumulative profiles (M, μg 2·27 cm^{-2}; ▲).

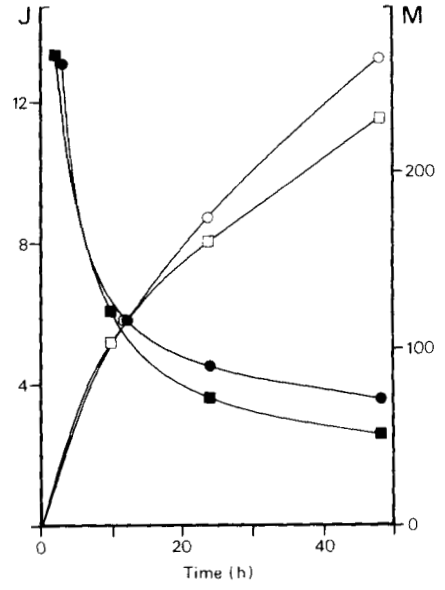

FIG. 6. Flurbiprofen permeation from 10 mg deposited drug films through prehydrated (○, ●) and normal (□, ■) skin. Data expressed as flux (J, μg 2·27 cm^{-2} h^{-1}, ●, ■) and cumulative profiles (M, μg 2·27 cm^{-2}; ○, □).

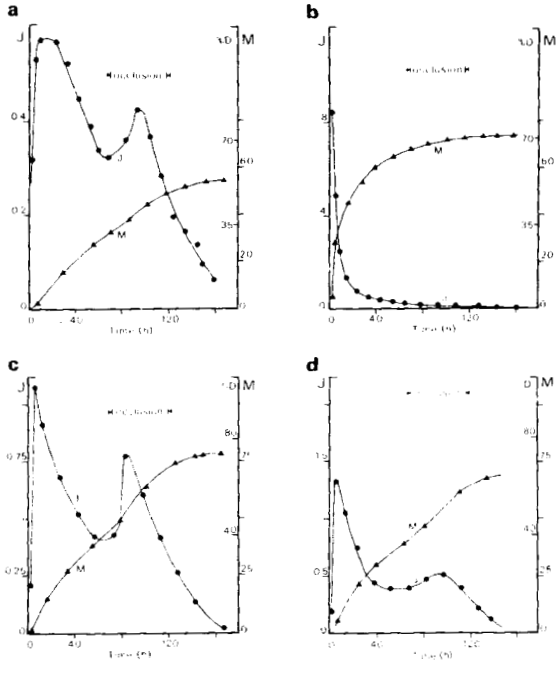

FIG. 7. Penetration of ibuprofen (a, b) and flurbiprofen (c, d) from 0·1 mg deposited drug films. Data illustrate flux (J, μg 2·27 cm^{-2} h^{-1}) and cumulative profiles (M, μg 2·27 cm^{-2} %D, percent of cumulative).

move on to consider the results of our experiments with the phenylalkanoic acids.

When the acetone solution was layered onto the skin most evaporated within 2 min and relatively little passed into the skin. Initially a small quantity of drug partitioned from the acetone solution into the skin as governed by the partition coefficient of the drug (SC : acetone). As the solvent evaporated the thermodynamic activity of the solute increased and eventually became greatest at saturation, when the driving force was maximal for the partitioning of

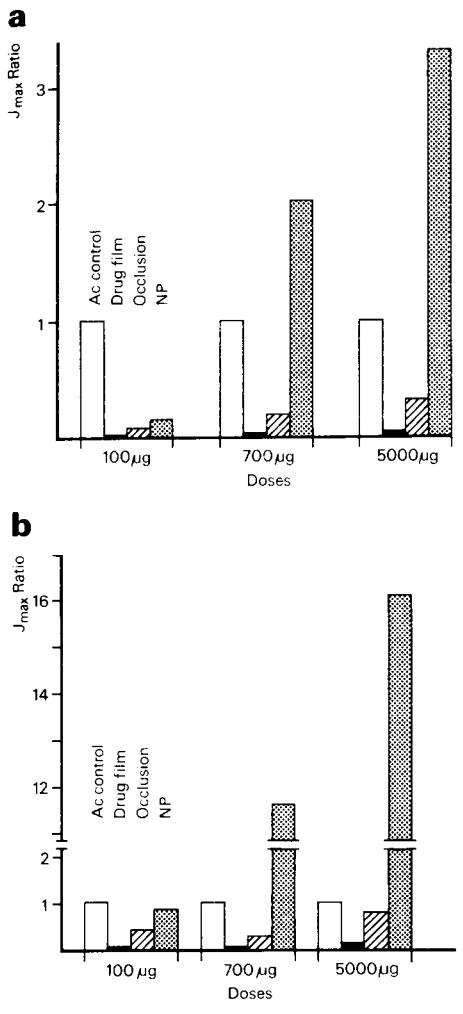

FIG. 8. Ibuprofen (a) and flurbiprofen (b) penetration from 0·1, 0·7 & and 5·0 mg doses. Overall effect on penetration due to acetone (Ac control), deposited drug film (Drug film), occlusion, and *N*-methyl-2-pyrrolidone (NP) treatment. Maximal rates obtained are expressed as ratios relative to Ac control (J_{max} ratio).

ibuprofen or flurbiprofen into the SC. Further evaportion of acetone precipitated excess drug as a deposited film on the skin. The drug which partitioned into the upper layers of the skin diffused to the receptor and produced the initial or 'acetone' maximum in the flux profile (Figs 2, 3). However, with time, the acetone peak flux fell to a mimimum, for both drugs, indicating that the initial high rate of permeation was not sustained. Presumably this was because of the slow dissolution of the deposited drug film, i.e. during this period drug penetration may have been dissolution rate-limited. In agreement with this concept Table 1 shows that the different doses did not produce a 50 fold increase in the amounts penetrated. From Figs 2, 3 we can show the pseudo-lag times to be relatively short (ibuprofen 1·8 ± 0·4 h, n = 11; flurbiprofen 1·9 ± 0·7 h, n = 12) indicating that the drugs penetrated the skin readily, but the penetration was not sustained. Both these observations support the concept of dissolution-limited diffusion from the deposited drug film, as at the time of the acetone maximum, the quantity of drug on the skin surface had not depleted significantly.

Although the diffusion constants, calculated by the two methods, were similar for both drugs, flurbiprofen permeated to a lesser extent than ibuprofen. This was confirmed by data in Table 2 which show that larger amounts of flurbiprofen remained on the skin at the end of the investigation. This may have been due to differences in partition coefficient effects and dissolution kinetics of the drugs on the skin surface. Calculation of D values by the two methods provided similar results of the right order of magnitude for such compounds, confirming the value of these methods for estimating the diffusion coefficient.

We performed secondary investigations to confirm whether or not the penetration of ibuprofen and flurbiprofen deposited from acetone was dissolution rate limited. Scheuplein (1976) suggested that polar compounds permeate from aqueous solution predominantly via shunt routes. The initial maximum in the flux profile (Figs 2, 3) may thus have been due to penetration via shunt routes through the skin as suggested by the short lag times. However, the rate of drug permeation was not maintained beyond the initial maximum. This behaviour may have arisen because the absorption was dissolution rate-limited or because of depletion of the drug directly above the shunt routes. Microscopical examination of the drug film showed large crystals of ibuprofen and flurbiprofen which were interwoven to produce the drug film. The presence of air spaces between the crystals indicated the possibility of poor skin-drug contact and, possibly, consequently low absorption. The presence of loose desquamating SC cells on the skin surface could also contribute to poor skin-drug film contact. To minimize such effects we washed the skin surface with 0·1% Triton-X 100 to remove loose desquamating SC cells (McGinley et al 1969) and then with distilled water to remove the surfactant, before adding the drug in acetone. We also placed weights on the drug films to ensure close contact of drug and skin. Maximizing skin-drug contact pro-

duced no appreciable difference in penetration when compared to normal penetration. Thus, poor skin-drug contact was not a limiting factor for the penetration of these drugs from deposited films.

When we removed the deposited drug film and replaced it with a 10% saturated solution of flurbiprofen, there followed a lag time after which the solute permeation reached and maintained a steady state flux as indicated in Fig. 4. The steady state flux was approximately four times larger than the flux from the deposited drug film and this further suggested that the absorption of these drugs from deposited films was dissolution dependent. Results also indicated that the penetration of flurbiprofen from 10 or 100% saturated solutions was similar and this provided an example of non-Fickian diffusion. This behaviour also suggested that the capacity of the skin had reached a limit and it could not accommodate the passage of drug beyond that produced by the 10% saturated solution. The limit may have been reached before the 10% level, but this feature was not investigated further.

Partitioning of ibuprofen or flurbiprofen from the acetone solution was responsible for the initial increase in the cumulative penetration profile (Figs 2, 3 up to 80 h). The very low permeation obtained after the acetone maximum may have been due to a depletion of the remaining drug deposited in the SC from the acetone solution and a slight steady dissolution of the deposited drug film, or both. To resolve this point we prepared flurbiprofen discs and placed them on the skin surface, in order to remove the initial partitioning from acetone. However, absorption from the drug film was approximately 5 times higher than from the disc and no initial penetration maximum (c.f. acetone maximum) was observed from the disc. This suggested that the low penetration beyond the acetone maximum was due both to deposited drug film dissolution and depletion of drug from the initial acetone partitioning.

The volume of acetone drug solution used was sufficient to completely cover the skin surface; the acetone rapidly evaporated, remaining on the skin for under 2 min before depositing the drug film. To assess the effect on the skin arising from acetone contact we increased the acetone contact time from approximately 2 min to 2 h, by covering the donor chamber and preventing acetone evaporation. Results (Fig. 5a, b) showed that little flurbiprofen permeated until the acetone had evaporated. This indicated that partitioning from acetone solution into the horny layer was not favourable until evaporation of the acetone caused an increase in the solute concentration. The eventual penetration flux maxima and cumulatives from the two acetone contact times were similar. Hence an increase in the acetone contact time did not elevate the penetration of flurbiprofen, i.e. the acetone did not damage the skin. This confirmed the steroid work of Scheuplein & Ross (1974) who also showed that acetone did not harm the SC membrane.

Flurbiprofen permeation was similar through prehydrated occluded and non-occluded skin (Fig. 6). Occlusion did not enhance the absorption of ibuprofen or flurbiprofen from deposited drug films (Figs 2, 3), i.e. it did not increase dissolution rate. However, it did elevate the flux of drug already present in the horny layer, as seen when the solute was present in the skin in significant amounts (Fig. 7a, c). This increase was not observed if onset of occlusion was delayed beyond the time required for the acetone peak to decay (Fig. 7b).

In our investigations the relative humidity (RH) was set at 60 ± 5%; however, under occlusion, the RH was greater and the pliability of human SC increased. Takahashi et al (1981) report that water absorbed into human SC is 'bound water' in a RH of 0–60% but 'free water' above 60% RH. They further suggested that free water breaks hydrogen bonds in keratin. Table 2 shows that ibuprofen and flurbiprofen were present in the SC at the end of the investigation—perhaps bound to the keratin matrix. Under occlusion the presence of free water may lead to the destruction of hydrogen bonds between the drug molecules and skin and result in a slight increase in the flux as observed in Fig. 7a, c. The total water content of the SC is increased by free water and this may also have contributed to the increase in penetration observed in Fig. 7a, c. Thus, Scheuplein (1967) suggested that the energy of activation of polar molecules is reduced when free water is present.

The overall effect on the three doses due to acetone, dry film dissolution, occlusion and NP treatment is summarized in Fig. 8. Addition of NP dissolved the drug film and removed the dissolution step. The ibuprofen or flurbiprofen then penetrated from this solution according to their concentrations and partition coefficients to create another significant concentration gradient in the skin. NP also permeated the skin readily (45 $\mu l\ cm^{-2}$ was absorbed into the upper layers of the SC within 2–3 h), and so increased progressively with time the chemical potential and thermodynamic activity of the drug remaining in the solution, as for acetone. This further promoted the passage of the drug into the skin. However, more significantly the absorbed NP

acted as a penetration enhancer and changed the diffusional resistance of the membrane (i.e. NP increased the diffusion coefficients of the drugs). The result of the sequence of events of drug dissolution, partitioning and NP penetration was to produce a large peak in the flux (Figs 2, 3). Thus the flux increased sixteen-fold for flurbiprofen and over three times for ibuprofen, relative to the acetone peak. Fig. 8 appears to suggest that NP decreased the flux of both drugs from the 100 μg dose; this false impression is because little drug remained in or on the surface of the skin when the NP was added. Figs 2, 3 show that at the onset of NP treatment over 60% of both drugs had already penetrated. Thus the accelerant effect of NP at this drug dosage was not observed.

The NP enhancer effect was transient, in that the peak due to the accelerant quickly fell as the drug film redeposited and the NP content of the membrane depleted; the flux began to return to the basal dissolution limited level, indicating the reversible accelerant effect of NP.

These investigations suggest that both ibuprofen and flurbiprofen do penetrate the skin and may be formulated to provide local physiological concentrations in the treatment of sunburns, arthritis and allied conditions, provided a systematic approach is employed. The study emphasizes the value of closely duplicating the in-vivo situation for percutaneous evaluation.

We conclude that the phenylalkanoic acids, ibuprofen and flurbiprofen, permeate the skin readily when applied in a small quantity of volatile vehicle. Evaporation of the vehicle precipitates the drug as a solid film and this is accompanied by a fall in the penetration rate due to the dissolution limited permeation of the drug film. The dissolution and subsequent penetration from the drug film is not increased by maximizing the skin-drug contact. Occlusion does not increase the dissolution rate of the drug film and therefore does not enhance the absorption. However, occlusion can increase the penetration of drug already present in the stratum corneum. *N*-Methyl-2-pyrrolidone acts as a penetration enhancer for these carboxylic acids and can significantly increase the quantity of drug permeating across the skin.

The skin has a limited capacity for phenylalkanoic acid transport beyond which further increase in drug absorption is difficult to achieve without using penetration enhancers.

These experiments emphasise the value of the 'open cell' technique as a method for topical drug studies, particularly for screening molecules which may have accelerant properties.

Acknowledgements

The authors thank the SERC and Boots Drug Co. for support in respect of a CASE studentship for SAA.

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