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Deep Tissue Penetration of Bases and Steroids after Dermal Application in Rat

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Abstract—The local deep tissue penetration of bases such as diazepam, antipyrine, iodoantipyrine, haloperidol and steroids such as hydrocortisone, fluocinolone acetonide, testosterone and progesterone after dermal application as aqueous solutions was studied in a rat model. The extent of local, as distinct from systemic delivery, for each solute was assessed by comparing the tissue concentrations obtained below a treated site with those in contralateral tissues. Local direct penetration was evident for all solutes below the applied site, although depth of penetration varied between individual solutes. A physiological pharmacokinetic model was employed to estimate local tissue concentrations of various compounds after dermal application.

The concept of using the transdermal route to target deeper underlying tissues has received only limited attention. It is generally believed that following its passage through stratum corneum and epidermis, a topically penetrating molecule is efficiently removed by the dermal microcirculation. However, reports have appeared giving evidence of direct penetration of certain solutes below the topical site of application (Guy & Maibach 1983). Local subcutaneous drug delivery has been demonstrated for a diverse array of chemical substances such as dimethyl sulphoxide (Gorog & Kovacs 1968), salicylate derivative (Rabinowitz et al 1982; Baldwin et al 1984), lignocaine (Russo et al 1980; Singh & Roberts 1994a), steroids (Marty et al 1980, 1989), certain non-steroidal anti-inflammatory drugs (Ishihama et al 1979; Wada et al 1982; Poisson et al 1985; Giese 1990), and organophosphorous pesticides (Marty et al 1989).

There are conflicting reports in the literature as to the actual depth and quantity of these drugs delivered to the local subcutaneous structures after topical application. Localization of topically-applied triethanolamine salicylate in deeper underlying tissues has been demonstrated (Rabinowitz et al 1982; Baldwin et al 1984). On the other hand, Hlynka et al (1969) have reported the penetration of salicylic acid to a depth of only 250 μm below the applied site. Riess et al (1986) have reported significant concentrations of diclofenac in synovial fluid and tissue after topical administration on the hands of arthritic patients. In contrast, Radermacher et al (1991) have reported that distribution of topically-applied diclofenac to synovial fluid occurred mainly via the systemic blood supply and direct penetration if at all was minimal. The percutaneous application of an antifungal agent, flutrimazole in mini-pigs resulted in the compound accumulation only in the superficial layers of skin (100–200 μm) with very low deep-tissue penetration potential (Conte et al 1992).

We have recently studied the underlying tissue penetration of a variety of dermally applied non-steroidal anti-inflammatory agents in a rat model, and showed that they penetrated directly below the applied site only to a depth of about 3–4 mm (Singh & Roberts 1993c, 1994b). In contrast dermally applied lignocaine penetrated into the underlying muscle to a depth of about 1 cm (Singh & Roberts 1994a). The direct penetration of these solutes after topical application is most marked in the first 2 h of application (McNeill et al 1992; Singh & Roberts 1993c, 1994a).

In this study, we quantify the depth and extent of local tissue penetration of topically applied basic and steroidal solutes. The physiological pharmacokinetic model earlier described to define local distribution of topically applied solutes (Singh & Roberts 1993b,c, 1994a) was extended to present work to predict the underlying tissue concentrations of bases and steroids.

Theory

A physiologically based pharmacokinetic model has earlier been used to account for the dermal pharmacokinetics of topically applied solutes (Singh & Roberts 1993b,c, 1994a). Briefly, a solution of drug is applied directly to dermis (skin stripped of its epidermis) and the concentrations of solute in dermis and underlying tissues monitored with time. In this approach the solute is transported to the ith tissue compartment from the tissue compartment overlying it $(i + 1)$ and into the tissue compartment underlying it $(i - 1)$ (Fig. 1). The distribution into the plasma compartment is defined by the blood supply to individual tissues. The differential mass balance equations for the applied solution and underlying dermis and tissue compartments were derived (Singh & Roberts 1993b,c, 1994a) and can be written as:

For the solution:

$$V_s \frac{dC_s}{dt} = CL_{s\leftrightarrow d} C_o \exp(-k_{s\leftrightarrow d} t) \quad (1)$$

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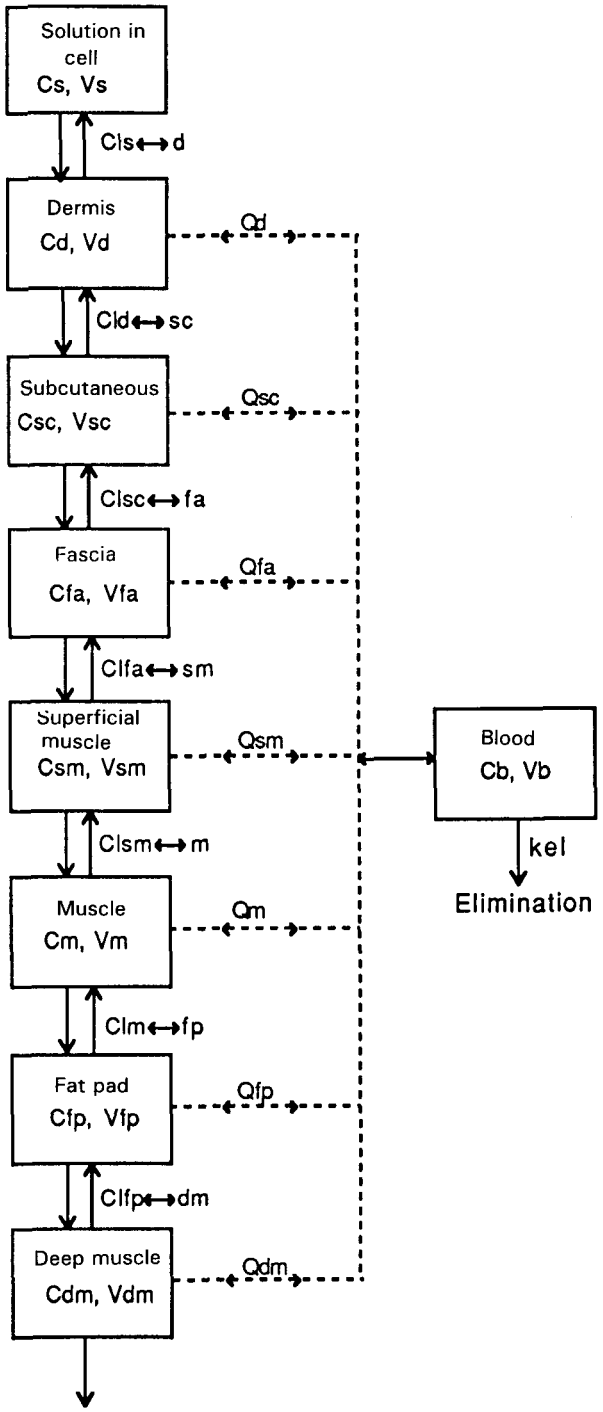

FIG. 1. A physiological pharmacokinetic model for local tissue penetration of compounds after dermal application.

For the dermis:

$$V_{ud}\frac{dC_{ud}}{dt} = V_d\frac{dC_d}{dt} = CL_{s\leftrightarrow d}C_o\exp(-k_{s\leftrightarrow d}t) + Q_d f_{ub}C_b - Q_d f_{ud}C_d + CL_{d\leftrightarrow sc}(f_{usc}C_{sc} - f_{ud}C_d) \quad (2)$$

where C_o and C_s are the concentrations in cell solution at zero time and at time t, V_s is the volume of the applied solution, V_{ud} and V_d are the apparent volumes of distribution of unbound solute in dermis and of total solute in dermis ($= V_{ud}f_{ud}$), respectively, $k_{s\leftrightarrow d}$ is the transfer rate constant between cell solution and dermis, f_{ud}, f_{ub} and f_{usc} are the fractions unbound in dermis, blood and subcutaneous tissue, C_b and C_d are the concentration of solute in blood and dermis, Q_d is the blood flow to dermis, $CL_{s\leftrightarrow d}$ is the clearance between cell solution and dermis, and $CL_{d\leftrightarrow sc}$ is the clearance between dermis and subcutaneous tissue.

For the underlying (ith) tissue:

$$Vu_{T.i}\frac{dC_{uT.i}}{dt} = V_{T.i}\frac{dC_{T.i}}{dt} = \{CL_{T.i+1\leftrightarrow i}(f_{uT.i+1}C_{T.i+1} - f_{uT.i}C_{T.i}) + Q_{T.i}(C_bRM_{T.i} - f_{uT.i}C_{T.i}) + CL_{T.i\leftrightarrow i-1}(f_{uT.i-1}C_{T.i-1} - f_{uT.i}C_{T.i})\} \quad (3)$$

where $Vu_{T.i}$ and $V_{T.i}$ are apparent volumes of distribution of unbound and total solute ($= V_{uT}f_{uT}$) in tissue, $CL_{T.i+1\leftrightarrow i}$ is the clearance between i + 1 tissue and ith issue, $CL_{T.i\leftrightarrow i-1}$ is the clearance between ith issue and i − 1 tissue, $C_{T.i}$, $C_{T.i+1}$, $C_{T.i-1}$ are the concentrations in the ith tissue, i + 1 tissue, and i − 1 tissue, respectively, $f_{uT.i}$, $f_{uT.i+1}$, $f_{uT.i-1}$ are the fractions unbound in the ith tissue, i + 1 tissue and i − 1 tissue, respectively, $Q_{T.i}$ is the blood flow to the ith tissue, and $RM_{T.i}$ is the ith tissue-plasma partition coefficient.

Materials and Methods

Chemicals and instruments

[^{14}C]Diazepam (sp. act. 53·8 mCi mmol^{-1}) was purchased from Amersham International, UK. [^{14}C]Iodoantipyrine (sp. act. 38·5 mCi mmol^{-1}), [^{14}C]antipyrine (sp. act. 54·5 mCi mmol^{-1}), [^{3}H]hydrocortisone (sp. act. 88 Ci mmol^{-1}), [^{3}H]progesterone (sp. act. 74·5 Ci mmol^{-1}) and [^{3}H]testosterone (sp. act. 80 Ci mmol^{-1}) were obtained from New England Nuclear, USA. [^{3}H]Haloperidol (sp. act. 30·7 Ci mmol^{-1}) and unlabelled compound were purchased from Janssen Pharmaceuticals, Sweden. [^{14}C]Fluocinolone acetonide (sp. act. 33·1 mCi mmol^{-1}) and unlabelled compound were gifts from Syntex Co., USA. All other chemicals were purchased from Sigma Chemical Company, USA. Zimmer's electrodermatome (Model 901, USA) was used for removing rat epidermis. Tissue solubilizer, NCS, and liquid scintillation fluids OCS (Organic Counting Scintillant) and BCS (Biodegradable Counting Scintillant) for tissue and aqueous samples, respectively, were purchased from Amersham International, UK. All other reagents used were of analytical grade. A liquid scintillation counter (Model MINIAX, Tri-carb 4000 Series, United Technologies Packard) was used to determine radioactivity in the samples.

Animals

Male Wistar rats, 300–350 g, were housed under standard laboratory conditions 20·0 ± 0·5°C, relative humidity 55–75% and supplied with normal pellet diet with free access to water. All experiments had previously been approved by the Animal Experimentation Committees of the University of Queensland and the Princess Alexandra Hospital.

Experimental design

Rats were lightly anaesthetized by pentobarbitone (35 mg kg^{-1}) and their body temperature maintained at 37°C by placing them on a heating pad. The hair from the 4 cm^2 dorsum area was removed by electric clippers and the epidermis removed by means of an electrodermatome set at a thickness of 80 μm (Singh & Roberts 1993a,b). A glass cell (i.d. 1·8 cm) was then adhered to the exposed dermis and warmed to 37°C by means of an external heating device. A solution of solute previously warmed to 37°C was introduced into the dermal glass cell and the solution stirred by a glass stirrer driven by an external motor (Siddiqui et al 1985; Singh & Roberts 1993a,b). The open end of the dermal absorption cell was sealed with parafilm to minimize losses due to evaporation.

Samples were removed from the dermal cell at various times and analysed for solute concentration. The glass cell containing drug solution was removed from the rat dermis at 2 h and a blood sample taken from the tail vein. The animals were then killed with an overdose of anaesthetic ether and the tissues below the treated site i.e. dermis, subcutaneous tissue, fascia, muscle lining or superficial muscle, muscle, fat pad and deep muscle, were dissected and placed in preweighed scintillation vials (Singh & Roberts 1993a,b). Similar tissues from the contralateral side were also removed. Tissue and plasma samples were stored at −20°C before analysis.

Another group of rats was initially anaesthetized by intraperitoneal injection of pentobarbitone (35 mg kg^{-1}) and, after removing epidermis as described above, killed by an overdose of anaesthetic ether. Dermal perfusion and tissue uptake studies were then conducted.

Sample treatment

Aqueous samples removed from the glass cells in in-vivo dermal studies were directly mixed with 5 mL liquid scintillation fluid BCS and counted on a liquid scintillation counter. The tissue samples were solubilized with 50 μL water and 1 mL tissue solubilizer NCS at 50°C for 6–8 h. After cooling the digested samples to room temperature (21°C), 0·03% of glacial acetic acid was added to each tissue sample followed by 10 mL organic scintillant OCS. The plasma samples were solubilized with tissue solubilizer (5 parts for 1 part plasma) at room temperature and treated with glacial acetic acid before adding OCS. Each sample was then counted in the liquid scintillation counter for 10 min (Singh & Roberts 1993a,b).

Data analysis

Zero time samples from the cell in dermal absorption studies were used to represent the initial solution concentration and ^{14}C or ^{3}H activity in the tissues and plasma converted to fraction of initial solution concentration (concentration fraction) (Singh & Roberts 1993a,b). Clearance into the dermis was estimated from the plot of percent solute remaining in the dermal perfusion cell with time using equation 4:

$$CL = kV \quad (4)$$

where k is the disappearance rate constant and V is the volume of solution applied to the dermis (Siddiqui et al 1985, 1989; Singh & Roberts 1993a,b). Adsorption of lipophilic solutes such as diazepam, haloperidol, testosterone and progesterone to glass has earlier been investigated and observed to be negligible (Dowsett et al 1984; Banerjee & Levitz 1985; Yliruusi et al 1986; Olcer & Hakyemez 1988; Martens et al 1990).

The tissue to plasma partition coefficients ($RM_{T,i}$) were calculated using pseudo steady-state plasma (C_p) and contralateral tissue concentrations ($C_{T,i}$) of bases and steroids 2 h after dermal perfusion according to equation 5:

$$RM_{T,i} = C_{T,i}/C_p \quad (5)$$

The MINIM computer program (Purves 1992) was used for nonlinear regression and numerical integration of equations 1, 2 and 3 using experimentally determined blood flows (Singh & Roberts 1993b) and estimated tissue-tissue clearances for lignocaine (Singh & Roberts 1994a) as constants. The predicted concentrations were compared with those obtained experimentally after application of bases and steroids to the rat exposed dermis.

Results

Disappearance of compounds into the dermis

Fig. 2 shows the fraction of solute remaining in solution applied to dermis. A first-order rate of disappearance is evident for all solutes. The values for the dermal clearance of solutes in rat dermis in-vivo deduced from the regression of data shown in Fig. 2 are given in Table 1. In general, the clearance of solutes in anaesthetized rats was higher than in rats killed before the dermal perfusion study was conducted. The mean clearance of 0·75 mL h^{-1} for anaesthetized rats corresponds to a dermal permeability coefficient of 0·29 cm h^{-1}, since the clearance and permeability-surface area product are identical terms.

Tissue distribution of compounds

Fig. 3 shows tissue concentration (expressed as fraction of initial concentration)-depth profiles for the solutes after 2 h application to rat dermis. The local tissue concentrations of solutes were higher in rats killed before the tissue uptake study was conducted. The underlying tissue concentrations of diazepam, haloperidol, testosterone and progesterone were higher than plasma concentrations up to a depth of about 1 cm, while the corresponding values for antipyrine, iodoantipyrine and hydrocortisone were higher than plasma concentrations up to a depth of about 6 mm. The underlying tissue concentrations of haloperidol, progesterone, hydrocortisone, fluocinolone acetonide and testosterone were also much higher than the concentrations in similar tissues on the contralateral side. The tissue concentrations of diazepam, antipyrine, iodoantipyrine and fluocinolone acetonide were also higher than concentrations in similar tissues from the contralateral side up to a depth of about 6 mm; at greater depths the levels were still higher though comparable with contralateral tissue concentrations.

A physiologically based pharmacokinetic model has earlier been suggested to describe local tissue distribution of polar nonelectrolytes (Singh & Roberts 1993b), salicylic acid and other non-steroidal anti-inflammatory drugs (Singh & Roberts 1993c, 1994b) and lignocaine (Singh &

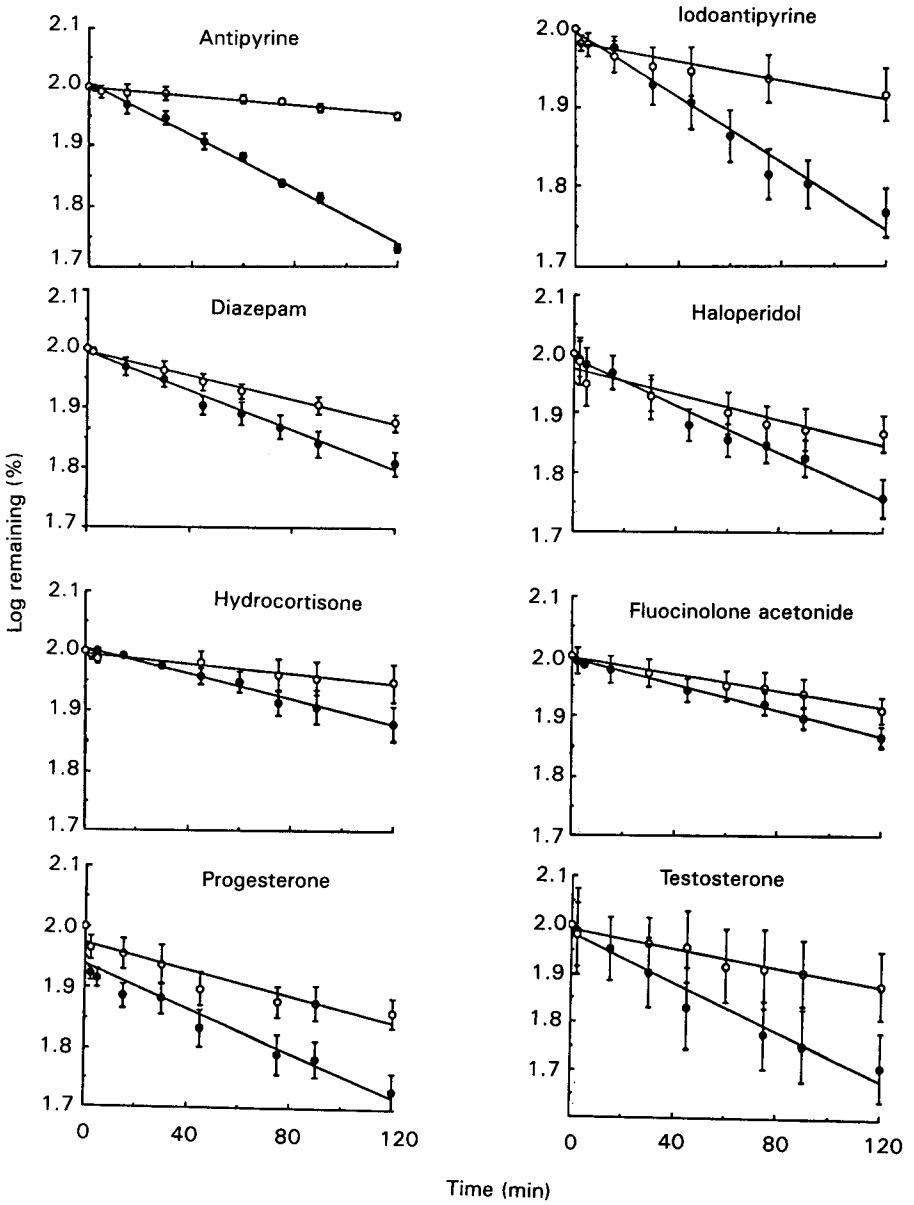

FIG. 2. Fraction of solute remaining to be absorbed after application of solutes to the exposed dermis of ● anaesthetized rats and ○ rats killed before the absorption study. Values are shown as mean ± s.d. (n = 3–4).

Roberts 1994a), after topical application. The tissue concentrations of bases and steroids in underlying tissues were predicted from the model shown in Fig. 1 and according to the equations 1, 2, and 3. The predicted levels were then compared with experimentally-observed tissue concentrations after application of various solutes to the rat exposed

Table 1. Clearance of solutes applied to rat exposed dermis in-vivo. (Mean ± s.d., n = 3–4.)

Compound	Dermal clearance		Dermal blood flow
	Anaesthetized animals ($mL\,h^{-1}$)	Killed animals ($mL\,h^{-1}$)	Blood supply ($mL\,h^{-1}$)
Diazepam	0·72 ± 0·11	0·42 ± 0·06	0·30
Antipyrine	0·93 ± 0·03	0·16 ± 0·03	0·77
Iodoantipyrine	0·87 ± 0·12	0·25 ± 0·06	0·62
Haloperidol	0·83 ± 0·05	0·44 ± 0·05	0·39
Hydrocortisone	0·47 ± 0·09	0·18 ± 0·03	0·29
Fluocinolone acetonide	0·43 ± 0·02	0·29 ± 0·04	0·14
Progesterone	0·74 ± 0·13	0·45 ± 0·06	0·30
Testosterone	1.02 ± 0·12	0·39 ± 0·01	0·63

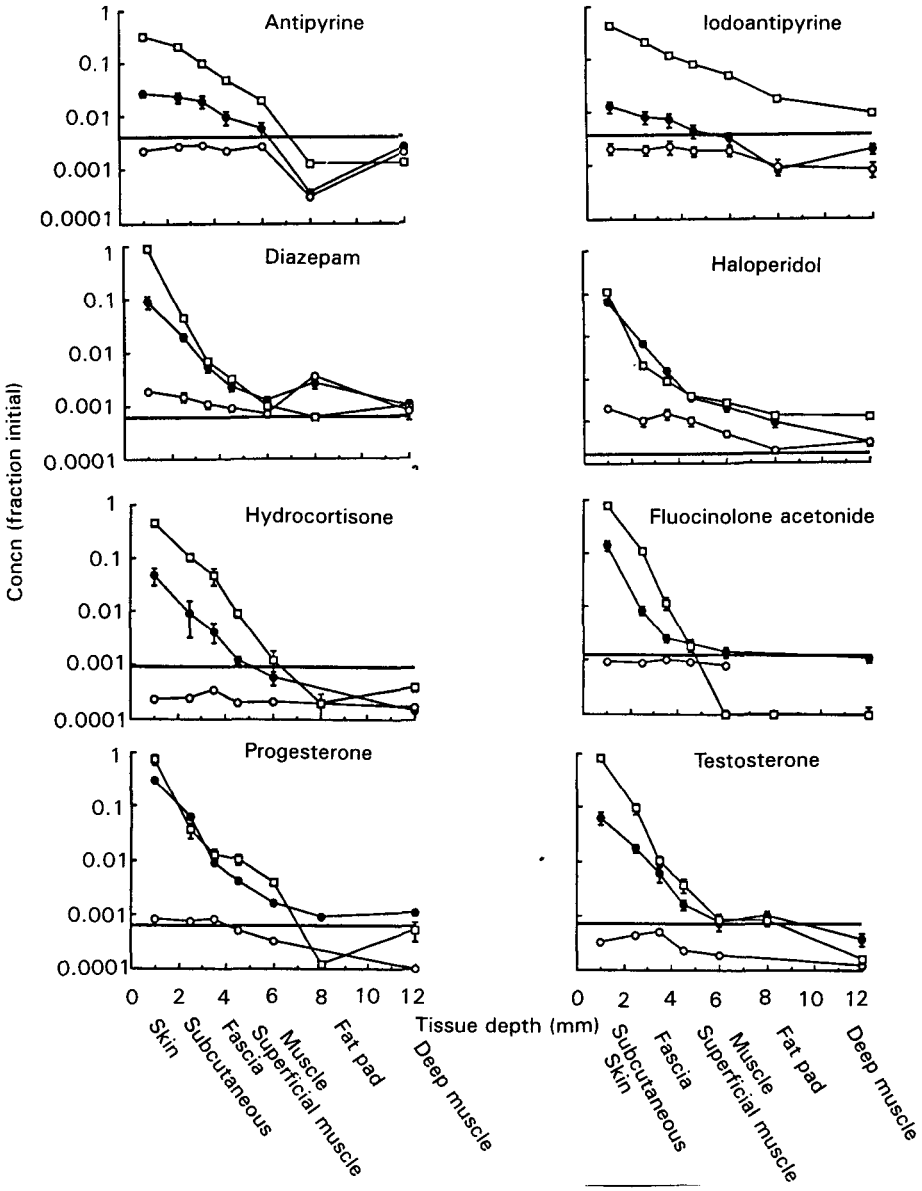

FIG. 3. Concentration (fraction of initial donor concentration) vs tissue depth profile of various solutes applied to rat dermis in-vivo. ● Underlying tissues after application to dermis of anaesthetized rat, ○ contralateral tissues after application to dermis of anaesthetized rat, □ underlying tissues after application to dermis of rat previously killed. Bold line represents plasma levels. Values are shown as mean ± s.d. (n = 3–4).

dermis (Fig. 4). The apparent tissue-plasma partition coefficients were estimated from the plasma and contralateral tissue concentrations 2 h after dermal perfusion, assuming a pseudo steady-state equilibrium. The establishment of equilibrium between contralateral tissue and plasma concentrations at 2 h has earlier been shown for salicylic acid (Singh & Roberts 1993c), piroxicam (McNeill et al 1992) and lignocaine (Singh & Roberts 1994a). The experimentally-determined concentrations compare reasonably well with the concentrations predicted from the model (Fig. 4).

Discussion

It has been previously shown that rat and human dermis have similar permeability characteristics (Singh & Roberts 1993a). The relative importance of tissue diffusion and perfusion processes in the dermis are most easily studied by placing the solute directly on the dermis in the absence of stratum corneum. The application of the drug solution directly on the dermis mimics perfect delivery through epidermis and overcomes the inherent differences in the permeability of the stratum corneum to solutes related to vehicle-skin, vehicle-drug and drug-skin interactions (Roberts 1991). The effect of the stratum corneum barrier on underlying tissue concentrations for different situations can be estimated by a convolution of the epidermal permeability kinetics with the underlying tissue kinetics (Singh & Roberts 1993c, 1994a,b). Whilst solutes are applied to the stratum corneum for their local or systemic effects, the iontophoresis of salicylic acid and lignocaine across intact

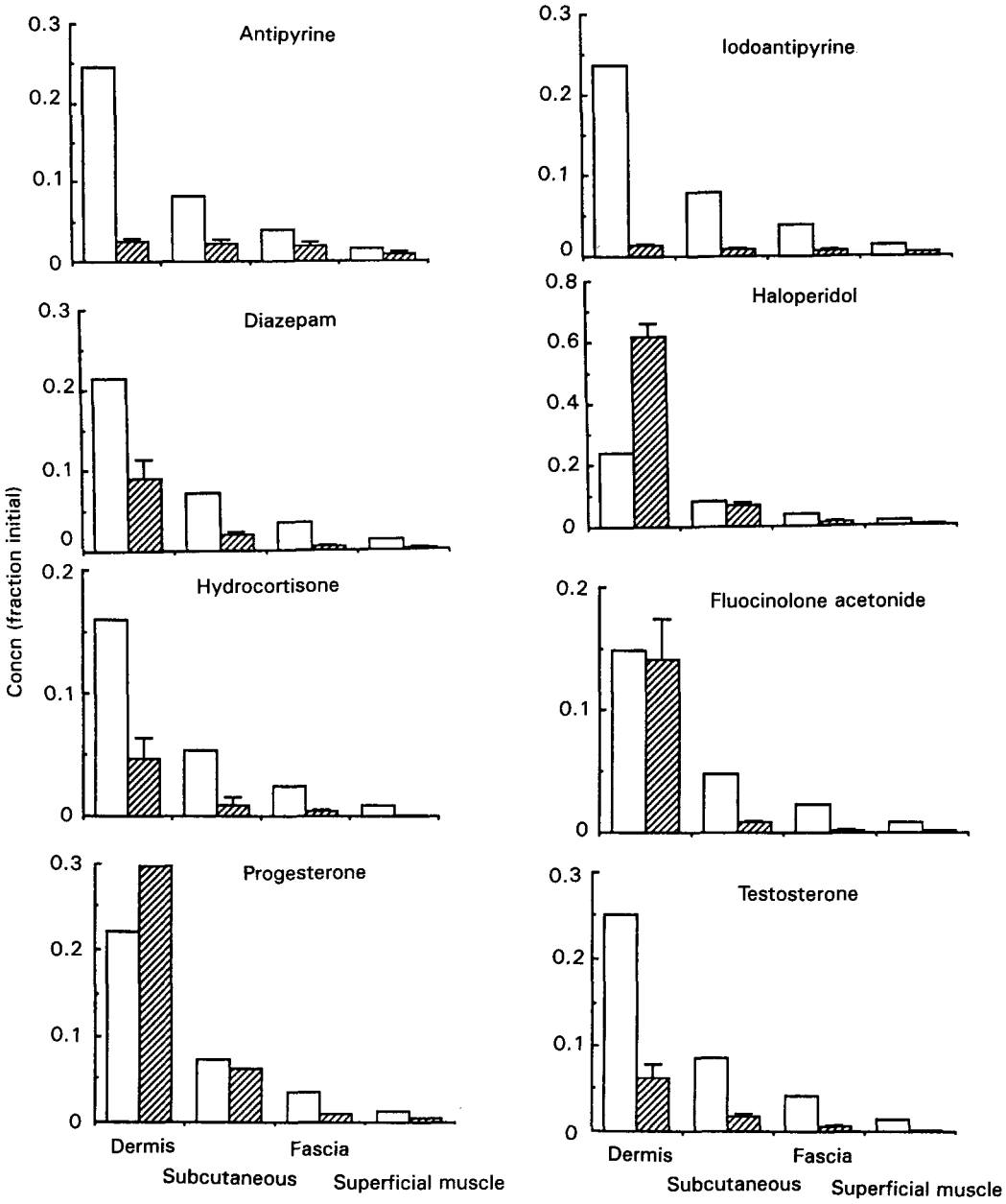

FIG. 4. Concentration (fraction of initial donor concentration) of solutes in underlying tissues. □ Predicted from the model shown in Fig. 1, ▨ experimentally observed values. Values are shown as mean ± s.d. (n = 3–4).

epidermis produced dermal concentrations (Singh & Roberts 1993a) comparable with the concentrations of these compounds applied on the rat exposed dermis (Singh & Roberts 1993a,c, 1994a). In these circumstances, the dermal absorption model, with convolution of epidermal transport as necessary, allows the tissue kinetics underlying epidermal passive, epidermal iontophoretic and passive dermal application to be studied.

The clearance of a compound applied to the dermis of a rat already killed before the study was carried out occurs mainly by diffusion, whereas the clearance in an anaesthetized rat is facilitated by both diffusion to deeper tissues and removal by the blood supply. The difference in dermal clearances between these animals should, therefore, represent the clearance due to viable blood supply (Siddiqui et al 1985, 1989; Roberts 1991; Singh & Roberts 1993b). The relative importance of blood supply in clearing topically applied solutes is apparent in Table 1, where the clearance in anaesthetized rats was higher than for killed rats. In addition, the clearance due to blood flow was observed to be dependent on solute size ($r^2 = 0{\cdot}70$) but not on lipophilicity. A similar size dependency has also been observed for other solutes (Singh & Roberts 1993b, 1994b), and also for drug absorption for muscle and subcutaneous tissue (Schou 1961; Nara et al 1992).

In the absence of blood flow any absorbed solute localizes in the tissues below the applied site by simple diffusion within and between the tissues. The high levels of any compounds in tissues below the applied site in the killed animal relative to the anaesthetized animal (Fig. 3) again

shows the importance of a viable blood supply in clearing topically applied compounds.

The concentrations of compounds in tissues below a dermally applied site in anaesthetized rats have been observed to peak twice; first at 2–4 h and then at around 10 h (McNeill et al 1992; Singh & Roberts 1993c, 1994a). The presence of the first peak has earlier been attributed to the direct penetration while systemic blood supply was mainly responsible for the second peak (McNeill et al 1992; Singh & Roberts 1993c, 1994a). Following a constant input source applied to epidermis, peak salicylate and lignocaine concentrations are achieved at 2 h and remain fairly constant thereafter (Singh & Roberts 1993c, 1994a). A 2-h time course was therefore selected to study the local tissue distribution-depth profiles for various solutes.

The results in Fig. 3 suggest that direct penetration is evident for all solutes, although the depth and extent of penetration varies for different solutes. The overall depth of direct penetration of about 1 cm is consistent with the results obtained previously with a model lipophilic solute, lignocaine, when the direct penetration of lignocaine was found to dominate in the first 2 h (in 16-h study) and up to a depth of about 1 cm (Singh & Roberts 1994a). About 80% of the observed lignocaine in underlying muscle ($\sim$ 8 mm) in the first 2 h was attributed to direct penetration (Singh & Roberts 1994a) after topical application. Thus, dermally applied compounds can by-pass the dermal microcirculation to reach underlying deeper tissues. The higher concentrations of the compounds in deeper tissues below the treated site as compared with similar tissues from an untreated site suggest some direct penetration in addition to predominant systemic distribution of drug.

We have previously reported that direct deep-tissue penetration of salicylic acid and other non-steroidal anti-inflammatory drugs is limited to a depth of about 3–4 mm below the applied site (Singh & Roberts 1993c, 1994b) while topically applied lignocaine can penetrate directly to a depth of about 1 cm (Singh & Roberts 1994a). This study observes that certain bases and steroids can penetrate directly into underlying tissues up to an average depth of about 8 mm. One way of interpreting the observed differences in tissue penetration of solutes lies in their lipophilicity. Salicylic acid and other non-steroidal anti-inflammatory drugs are predominantly ionized at the dermal pH of 7·4 and are therefore effectively removed by the blood supply. The bases and steroids used in the present work are predominantly un-ionized at the dermal pH. The more lipophilic solutes such as haloperidol, progesterone, testosterone and diazepam penetrated to a greater depth compared with the less lipophilic antipyrine, iodoantipyrine and hydrocortisone. Consistent with the above observations, other lipophilic solutes such as thyroxine, triiodothyronine and oestradiol have been shown to penetrate directly into local subcutaneous structures after topical application, while dexamethasone (a polar steroid) and di-isopropyl phosphate were effectively removed by the dermal blood supply (Marty et al 1989). Hydrocortisone, a polar steroid, shows direct penetration to a lesser extent than the more lipophilic progesterone. Triethanolamine salicylate, a lipophilic salt of salicylic acid, effectively penetrates underlying tissues after topical application (Rabinowitz et al 1982; Baldwin et al 1984) compared with ionized salicylic acid. The subcutaneous tissue/muscle accumulation of other lipophilic solutes such as di-isopropyl fluorophosphate, malathion, parathion, oestradiol and progesterone has also been shown in mouse and rat (Marty et al 1989). It was suggested that the blood supply to dermis is not capable of resorbing certain chemicals, and the substrate accumulates with time and is able to diffuse to deeper tissues (Marty et al 1989).

Rabinowitz et al (1982) have shown high levels of salicylate in local tissues after topical application of its triethanolamine salt to knees of dogs. Baldwin et al (1984) applied trolamine salicylate to biceps femoris in pigs and observed levels in underlying muscle ($\sim$ 4 cm) which were several times higher than those observed in contralateral muscle and in the blood. Riess et al (1986) have reported significant concentrations of diclofenac in synovial fluid and tissue after topical adminstration on the hands of arthritic patients. In contrast, Radermacher et al (1991) observed that distribution of topically applied diclofenac to synovial fluid occurred mainly via the systemic blood supply and direct penetration was minimal. A similar conclusion was drawn by Dawson et al (1988) for biphenylacetic acid. It was suggested that relatively higher diclofenac concentrations in synovial fluid observed by Riess et al (1986) were measured in smaller joints such as finger and wrist joints, probably reflecting a shorter diffusion distance (Radermacher et al 1991). The percutaneous application of an antifungal agent, flutrimazole in mini-pigs resulted in compound accumulation only in the superficial layers of skin (100–200 μm) with very low deep-tissue penetration potential (Conte et al 1992). The above observations suggest that the depth of penetration is an important parameter which should be stated in local tissue penetration studies. The depth of penetration may also be altered by biological variables such as presence or absence of adipose tissue or fat beneath the area of application (Rosenberg et al 1986). The observed differences in various studies may also be due to variables such as differences in formulation, method of application—solution, ointment or cream with or without rubbing—duration, application site and species studied.

The physiological pharmacokinetic model earlier used to describe the time course of solutes in underlying tissues after topical application (Singh & Roberts 1993b,c, 1994a,b) could be used to predict the tissue concentrations of various solutes in the present study (Fig. 4). In the model described, the two unknown variables are clearance between the tissues and unbound fraction of solute in the tissue. The blood flows to tissues were determined experimentally. In obtaining present predictions, we have assumed the unbound fraction to be constant and of similar magnitude in all tissues. A value of 0·5 estimated from a lignocaine study (Singh & Roberts 1994a) was used in the present study for all solutes. The predictions from the model were, however, found to improve for certain solutes such as antipyrine, iodoantipyrine, hydrocortisone, diazepam and testosterone by varying the unbound fraction of solute in the tissue. This limitation arises due to the lack of data on tissue binding (recognizing that tissue binding is different from plasma protein binding) of different solutes used in the present study. It is also recognized that another limitation in this analysis is the use of estimated tissue-tissue clearances of

lignocaine since the apparent tissue-tissue clearances may vary for different compounds. However, the goodness of fit and the known similarity of tissue diffusion coefficients for different solutes of a similar size (Paaske & Sejrsen 1989) appear to justify this assumption. The poor prediction in some cases may also be due to inherent differences in the tissue affinities for different solutes, nonlinearities in tissue binding (although trace concentrations of all solutes were used to avoid the nonlinearities in plasma and tissue binding), variations in local blood flow, plasma protein binding, dermis-water partitioning and tissue-partitioning with time and possible drug effects on membrane/blood flow.

Overall, this work suggests that basic solutes can substantially by-pass the dermal microcirculation and penetrate directly into underlying tissues after dermal application. The dermal blood supply is not a perfect sink for all topically applied compounds. A comparison of the results from this study and earlier work on the ionized non-steroidal anti-inflammatory drugs (Singh & Roberts 1994b) suggest that the un-ionized lipophilic forms of solutes are more likely to achieve greater local tissue penetration. Deeper tissue penetration may be desirable for the drugs hydrocortisone and fluocinolone acetonide for certain anti-inflammatory effects. Efforts are currently underway to develop transdermal patches for the systemic delivery of testosterone. In this instance, deeper penetration may be undesirable in that local undesirable effects on specific tissues below the applied site could arise.

The underlying tissue penetration of solutes after topical application involves a complex interplay of physiological variables such as tissue diffusion, tissue blood supply and its distribution, local and systemic metabolism, reversible and irreversible tissue binding, tissue-plasma partitioning, and physicochemical variables such as lipophilicity, molecular size, steric factors, state of ionization, protein binding, and aqueous and tissue solubility.

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References

Baldwin, J. R., Carrano, R. A., Imondi, A. R. (1984) Penetration of trolamine salicylate into the skeletal muscle of pig. J. Pharm. Sci. 73: 1002–1004

Banerjee, S., Levitz, M. (1985) On the stability of salivary progesterone under various conditions. Steroids 46: 967–874

Conte, L., Ramis, J., Mis, R., Forn, J., Vilaro, S., Reina, M., Vilageliu, J., Basi, N. (1992) Percutaneous absorption and skin distribution of [^{14}C] flutrimazole in mini-pigs. Arzneim. Forsch. 42: 847–853

Dawson, M., McGee, C. M., Vine, J. H., Nash, P., Watson, T. R., Brooks, P. M. (1988) The disposition of biphenylacetic acid following topical application. Eur. J. Clin. Pharmacol. 33: 639–642

Dowsett, M., Mansfield, M. D., Griggs, D. J., Jeffcoate, S. L. (1984) Validation and use of centrifugal ultrafiltration-dialysis in the measurement of percent free oestradiol in serum. J. Steroid. Biochem. 21: 343–345

Giese, U. (1990) Absorption and distribution of ibuprofen from a cream formulation after dermal administration to guinea pigs. Arzneim. Forsch. 40: 84–88

Gorog, P., Kovacs, I. B. (1968) Effect of dimethylsulfoxide (DMSO) on various experimental inflammations. Curr. Ther. Res. 10: 486–492

Guy, R. H., Maibach, H. I. (1983) Drug delivery to local subcutaneous structures following topical administration. J. Pharm. Sci. 72: 1375–1380

Hlynka, J. N., Anderson, A. J., Reidel, B. E. (1969) Investigation of intracutaneous drug absorption I. Development of a quantitative method of measurement. Can. J. Pharm. Sci. 4: 84–91

Ishihama, H., Kimata, H., Mizushima, Y. (1979) Percutaneous penetration of indomethacin. Experientia 35: 798–799

Martens, H. J., De Goede, P. N., Van Loenen, A. C. (1990) Sorption of various drugs in polyvinyl chloride, glass, and polyethylene-lined infusion containers. Am. J. Hosp. Pharm. 47: 369–373

Marty, J.-P., James, M., Hajo, N., Wepierre, J. (1980) Percutaneous absorption of oestradiol and progesterone: pharmacokinetic studies. In: Mauvais-Jarvais, P. (ed.) Percutaneous Penetration of Steroids. Academic Press, New York, pp 205–218

Marty, J.-P., Guy, R. H., Maibach, H. I. (1989) Percutaneous penetration as a method of delivery to muscle and other tissues. In: Bronaugh, R. L., Maibach, H. I. (eds) Percutaneous Absorption: Mechanisms, Methodology, Drug Delivery. Marcel Dekker, New York, pp 511–529

McNeill, S. C., Potts, R. O., Francoeur, M. L. (1992) Local enhanced topical delivery (LETD) of drugs: does it truly exist? Pharm. Res. 9: 1422–1427

Nara, E., Masegi, M., Hatono, T., Hashida, M. (1992) Pharmacokinetic analysis of drug absorption from muscle based on a physiological diffusion model: effect of molecular size on absorption. Pharm. Res. 9: 161–168

Olcer, M., Hakyemez, G. (1988) Investigations of some physicochemical properties of haloperidol which may affect its activity. J. Clin. Pharmacol. Therap. 13: 341–349

Poisson, M., Ralambosa, C., Blehaut, H., Astoin, J. (1985) Comparison of the concentration-effect relationship of a local anti-inflammatory agent and oral acetylsalicylic acid: the value of local application. Arzneim. Forsch. 35: 1550–1552

Paaske, W. P., Sejrsen, S. (1989) Permeability of continuous capillaries. Dan. Med. Bull. 36: 570–590

Purves, R. D. (1992) A Macintosh Application for Non-Linear Parameter Estimation. Program and Manual, Department of Pharmacology, University of Otago, Dunedin, New Zealand

Rabinowitz, J. L., Feldman, E. S., Weinberger, A., Schumacher, H. R. (1982) Comparative tissue absorption of oral ^{14}C-salicylate in humans and canine knee joints. J. Clin. Pharmacol. 22: 42–48

Radermacher, J., Jentsch, D., Scholl, M. A., Lustinetz, T., Frolich, J. C. (1991) Diclofenac concentrations in synovial fluid and plasma after cutaneous application in inflammatory and degenerative joint disease. Br. J. Clin. Pharmacol. 31: 537–541

Riess, V. W., Schmid, K., Botta, L., Kobayashi, K., Moppert, J., Schneider, W., Sioufi, A., Strusberg, A., Tomasi, M. (1986) Die perkutane resorption von diclofenac. Arzneim. Forsch. 36: 1092–1096

Roberts, M. S. (1991) Structure-permeability considerations in percutaneous absorption. In: Scott, R. C., Guy, R. H., Hadgraft, J., Bodde, H. E. (eds) Prediction of Percutaneous Penetration. IBC Technical Services Ltd, London, pp 210–228

Rosenberg, P. H., Kytta, J., Alila, A. (1986) Absorption of bupivacaine, etidocaine, lignocaine and ropivacaine into *n*-heptane, rat sciatic nerve and human extradural and subcutaneous fat. Br. J. Anaesth. 58: 310–314

Russo, J., Lipman, A. G., Cormstock, T. J., Page, B. C., Stephen, R. L. (1980) Lidocaine anesthesia: comparison of iontophoresis, injection and swabbing. Am. J. Hosp. Pharm. 37: 843–847

Schou, J. (1961) Absorption of drugs from subcutaneous connective tissue. Pharmacol. Rev. 13: 441–446

Siddiqui, O., Roberts, M. S., Polack, A. E. (1985) Topical absorption of methotrexate: role of dermal transport. Int. J. Pharm. 27: 193–203

Siddiqui, O., Roberts, M. S., Polack, A. E. (1989) Percutaneous

absorption of steroids: relative contributions of epidermal penetration and dermal clearance. J. Pharmacokin. Biopharm. 17: 405–424

Singh, P., Roberts, M. S. (1993a) Iontophoretic transdermal delivery of salicylic acid and lignocaine to local subcutaneous structures. J. Pharm. Sci. 82: 127–131

Singh, P., Roberts, M. S. (1993b) Blood flow measurements in skin and underlying tissues by microsphere method-application to dermal pharmacokinetics of polar non-electrolytes. J. Pharm. Sci. 82: 873–879

Singh, P., Roberts, M. S. (1993c) Dermal and underlying tissue pharmacokinetics of salicylic acid after topical application. J. Pharmacokin. Biopharm. 21: 337–373

Singh, P., Roberts, M. S. (1994a) Dermal and underlying tissue pharmacokinetics of lidocaine after topical application. J. Pharm. Sci. In press

Singh, P., Roberts, M. S. (1994b) Skin permeability and local tissues concentrations of non-steroidal anti-inflammatory drugs (NSAIDs) after topical application. J. Pharmacol. Exp. Ther. 268: 144–151

Wada, Y., Etoh, Y., Ohira, A., Kimata, H., Koide, T., Ishihama, H., Mizushima, Y. (1982) Percutaneous absorption and anti-inflammatory activity of indomethacin in ointment. J. Pharm. Pharmacol. 34: 467–468

Yliruusi, J. K., Uotila, J. K., Kristoffersson, E. R. (1986) Effect of flow rate and type of i.v. container on adsorption of diazepam to i.v. administration systems. Am. J. Hosp. Pharm. 43: 2795–2799