Research Article

Transdermal Drug Transport and Metabolism. I. Comparison of *In Vitro* and *In Vivo* Results

Donald B. Guzek,[1] Alane H. Kennedy,[1] Stephen C. McNeill,[1] E. Wakshull,[1] and Russell O. Potts[1,2]

Received May 18, 1988; accepted August 22, 1988

Using excised human skin and tissue grafted to athymic mice, the *in vitro* and *in vivo* delivery and metabolism of a salicylate diester were compared. Concentration profiles of this drug and its metabolites were obtained for the outer several hundred microns of the skin. These results show significant differences in the extent of enzymatic cleavage and distribution of metabolites between *in vitro* and *in vivo* studies. Furthermore, these data suggest that *in vitro* results may overestimate metabolism because of increased enzymatic activity and/or decreased capillary removal.

KEY WORDS: epidermal drug metabolism; human skin; athymic mice.

INTRODUCTION

Far from being a passive membrane or "black box" for the passage of drugs, the skin has significant metabolic activity. As pointed out in several recent reviews, metabolic activity spans a broad range of oxidative, reductive, hydrolytic, and conjugative reactions (1–3), making the skin a source of extrahepatic metabolism of many xenobiotics and topically applied drugs.

With the recent appreciation of the enzymatic activity of skin, several groups have begun to study dermal and epidermal metabolism using a variety of techniques. Kao and coworkers chose *in vitro* model systems and, in a series of experiments, have shown that metabolic activity is maintained for long periods of time only if samples are maintained under rather stringent tissue culture conditions (4–7). Other investigators have chosen to investigate dermal metabolism *in vivo* using laboratory animals (8–11). In an effort to provide data relevant to humans *in vivo* yet not put subjects at risk, Krueger and his colleagues have developed a perfused flap model involving human skin grafted to an athymic rat (12). It is only in the latter study that a systematic comparison of *in vivo* and *in vitro* flux results was presented. The objective of the study presented here is to compare further *in vivo* and *in vitro* measurements of drug delivery and metabolism, with particular emphasis on the concentration profiles within the skin under steady-state conditions.

The transport and metabolism of drugs in the skin places two kinetic events in competition. Results of recent experimental (13,14) and theoretical (15–19) investigations have suggested that diffusional and metabolic processes in the skin are intimately related, with one often having a profound effect on the other. For example, Hadgraft has shown that compounds which reside in the skin for extended lengths of time undergo more metabolic change (19).

In this study, a diester derivative of salicylic acid (A) which is enzymatically cleaved to a monoester (B) and, ultimately, salicylic acid (C) was chosen as a model compound for the study of transdermal delivery and metabolism. The delivery and metabolism of these compounds were examined *in vivo* using human skin grafted to athymic mice and *in vitro* using human skin obtained during surgery. Drug distribution and hydrolysis were determined in skin samples by extending sectioning techniques (20–22) to include high-performance liquid chromatographic (HPLC) analysis of radiolabeled drug and metabolites. The results show that the drug distribution and metabolism profiles obtained *in vitro* and *in vivo* are substantially different. These differences can be explained in terms of the relative contribution of both transport and hydrolysis to the ultimate distribution of drug and metabolites.

MATERIALS AND METHODS

Synthesis and Formulation of Salicylate Esters

The diester and monoester were synthesized from ^{14}C-salicylic acid (New England Nuclear) via conventional esterification reactions. The structure of the diester (A), monoester (B), and salicylic acid (C) are shown in Fig. 1. In the studies presented here, R_2 represents a methyl group, while R_1 represents an ethyl carbonate group. As shown in Fig. 1, the radiolabel was incorporated in the carboxyl group of salicylate and remained intact in all three molecules.

In all experiments, 25% (w/w) of the ^{14}C-labeled diester at a specific activity of 220 dpm/nmol was place in a water/oil (49/51, w/w) vehicle, in which the oil phase consisted of stearic acid (13%) glyceryl monostearate (8%), anhydrous lanolin (2%), polysorbate-85 (2%), and sorbitan tristearate (1%).

[1] Pfizer Central Research, Groton, Connecticut 06340.
[2] To whom correspondence should be addressed.

0724-8741/89/0100-0033$06.00/0

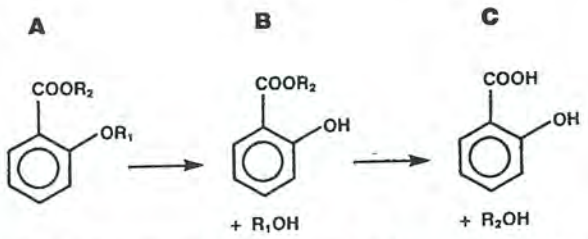

Fig. 1. A schematic representation of the diester (A), monoester (B), and salicylate (C) compounds studied. R_1 = ethyl carbonate; R_2 = methyl.

Approximately 50 mg of formulation was spread evenly over 1 cm^2 of skin with a glass rod to initiate each experiment.

Chromatographic Analysis

The monoester, diester, and salicylate were separated on HPLC using a 25-cm-length, 10-μm C18 Microbondbpak column (Waters) with a 1.5-cm-length, 7-μm precolumn (RP-18, Brownlee Newgaurd) at 21°C. The mobile phase consisted of acetonitrile, water, and tetrahydrofuran at a volume ratio of 50:50:1 and was pumped through the columns at 1 ml/min using a constaMetric III pump (LDC/Milton Roy). Samples were injected automatically (Water WISP 710B), and 0.5-ml fractions of the eluate collected in a fraction collector (ISCO Model 328). The collected eluate was mixed with scintillation fluor, and radioactivity measured as disintegrations per minute (dpm) using liquid scintillation counting. Under these chromatographic conditions salicylate and the di- and monoesters eluted at 2.5, 9.5, and 16.0 min, respectively, both individually and in a mixture.

In Vitro Flux

Samples of human facial skin obtained from surgery were cut to a uniform 300-μm thickness with a dermatome immediately prior to each study. The sample was mounted in an open-top diffusion cell with the dermis side in contact with 5 ml of phosphate-buffered saline (PBS), pH 7.2, which was stirred rapidly. The stratum corneum side was covered with a thin layer of the radiolabeled formulation, and the entire diffusion cell maintained in a water bath at 32°C. All human skin samples were used within 4 hr of surgical removal. At periodic intervals, 100 μl of the receiver buffer was removed and replaced with fresh buffer. The radioactivity of these samples was determined by liquid scintillation counting. At the end of each experiment (approximately 6 and 24 hr), the entire contents of the receiver side were analyzed by HPLC.

Skin Homogenization and Inhibition of Hydrolysis

The skins of two hairless mice (Charles River) were homogenized in Hank's balanced salt solution containing 5 m*M* Hepes, pH 7.2 (3.6 g of tissue in 10 ml), at 4°C with a tissue homogenizer (Kinematica, Lucern, Switzerland). The homogenate was centrifuged for 2 × 10 min at 3500 rpm in a Sorval RT6000B refrigerated tabletop centrifuge (Dupont), and the pellet discarded. The turbid supernatant was then used in the hydrolysis experiments.

The following proteinase inhibitors (from 100× stock solutions) were added to the supernatant (final volume, 1 ml in a 1.5-ml microfuge tube) and incubated at room temperature for 45 min at the concentrations indicated in Table I and the figure legends: Diisopropylfluorophoshate (DFP; in ethanol), phenylmethylsulfonylfluoride (PMSF; in ethanol), EGTA (in Hank's balanced salt solution), *N*-methyl maleimide [NMM; in 50% dimethyl sulfoxide (DMSO)], and iodoacetamide (IA; in Hank's balanced salt solution). Controls contained the appropriate vehicles. The inhibitors DFP, PMSF, and EGTA were obtained from Sigma (St. Louis, Mo.), while NMM and IA were synthesized at Pfizer. Twenty microliters of radiolabeled diester (0.7 mg/ml in H_2O, 100,000 dpm/20 μl) was then added to the supernatant and incubated at 37°C for 0–180 min. The incubation was terminated by removing 100 μl of the mixture and adding it to 200 μl of methanol at 4°C. The samples were then centrifuged at 12,000*g* for 4 min, and 200 μl of the supernatant was analyzed by HPLC and liquid scintillation counting as described above.

Athymic Mice Surgery

Human facial skin obtained as a by-product of face-lift surgery, was dermatomed to a 300-μm thickness and orthotopically grafted to athymic mice (Charles River) as previously described (23). Pre- and postauricular, nonterminal hair-bearing sites of approximately 1-cm diameter were grafted to each flank of a single animal. Animals used in the studies reported here all had viable grafts several months after surgery. All animals were housed in individual isolator units with autoclaved cage bedding, water, and food and were kept in a controlled-environment (light, temperature, and humidity) room.

Measurement of Drug Distribution

Samples of skin were treated with topically applied diester (approximately 50 mg/cm^2) for the desired period of time. At the end of the experiment, the athymic mice were sacrificed and the human skin was quickly excised. The excised sample was placed dermis side down on a microtome chuck maintained at −17°C. A metal ring of a thickness slightly greater than that of the skin sample was placed around the tissue, and the ring filled with embedding medium (O.C.T., Tissue-Tek). A flat, precooled surface was pressed against the stratum corneum, resulting in a flat outer surface which was frozen in 10–20 sec. Samples were maintained in liquid nitrogen until needed for further processing.

Frozen samples were mounted in a microtome (AO Histostat), and 40-μm sections, parallel to the outer surface, were obtained to a depth of about 300 μm. Each section was placed in a hand-held homogenizer with 100 μl of the mobile phase and ground with a small ground-glass insert. The mobile phase was removed, and the entire process repeated two more times. The pooled mobile phase was then centrifuged for 5 min at maximum speed (Eppendorf Model 5415). The supernatant was removed and filtered through a 0.5-μm filter into HPLC injection tubes. Two hundred microliters was then automatically injected (WISP Waters) onto the HPLC and analyzed as described above.

In *in vitro* drug distribution experiments, the skin sample was placed, dermis side down, in a petri dish on filter

paper saturated with PBS. Drug in the vehicle was spread on the stratum corneum surface of the sample. At the end of the experiment, excess drug was removed from the surface, and this tissue processed as described above for *in vivo* samples.

RESULTS

Figure 2 shows the total *in vitro* flux of the ^{14}C-labeled compound and its metabolites through human skin. These results show that after a short lag period of a few minutes, there is a linear increase in the total dpm with time for the remainder of the experiment. The slope of these data was obtained by linear regression analysis ($r = 0.987$). This value, when divided by the specific activity of the permeant (2.2×10^{11} dpm/mol) and the cross-sectional area of the skin (1.8 cm^2), yielded a steady-state flux of 12.5 nmol/cm^2/hr.

While flux measurements provide information on the total mass of material passing through the skin, the precise molecular species present remain unknown. Thus, samples obtained from the receiver (dermis) side of the diffusion cell were subjected to HPLC analysis. These results showed that all radioactivity in the receiver chamber at 6 and 24 hr eluted with a retention time identical to that of salicylic acid. In a control experiment, intact compound was incubated in the receiver buffer. Once again, all radioactivity was in the form of salicylic acid. Samples taken from the donor chamber, however, showed only intact diester. Thus, on passage through the skin or upon incubation with buffer in contact with the dermis, the diester was hydrolyzed to salicylic acid. In contrast, diester in contact with the stratum corneum side of the skin remained unhydrolyzed for the duration of the experiment.

In order to determine whether the hydrolysis was chemical or enzymatic, intact diester was incubated in buffer and human or mouse skin homogenates. Samples were then analyzed by HPLC techniques for the amount of the diester, monoester, and salicylate. The data, presented in Fig. 3, show the rapid hydrolysis of diester in human skin homogenate, reaching baseline levels within 60 min. In contrast, the monoester increased to maximum concentration after about 30 min and decreased steadily thereafter. Finally, the

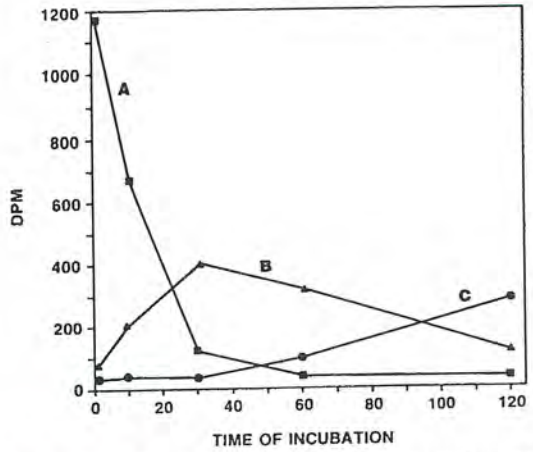

Fig. 3. The change in concentration of the diester (A), monoester (B), and salicylate (C) vs the time of incubation in skin homogenate.

levels of salicylic acid increased continuously throughout the course of the experiment. This time course strongly suggests a precursor–product relationship of diester to monoester to salicylate. In a control experiment, the diester was incubated in buffer alone. Over a range of pH from 3 to 10, no significant hydrolysis was noted during the time course of the experiment.

To verify further the enzymatic nature of the hydrolysis, mouse skin homogenate experiments were performed in the presence of various inhibitors of enzyme activity. As shown by the results in Fig. 4, complete hydrolysis was observed after about 30 min in mouse skin homogenate. The addition of phenylmethylsulfonylfluoride (PMSF) to the incubation medium resulted in a dose-dependent decrease in hydrolysis, while the addition of 1 m*M* (or even 0.2 m*M;* data not shown) diisopropylfluorophosphate (DFP) resulted in complete inhibition of hydrolysis over 180 min of incubation. Several other enzyme inhibitors were also tested, with the results summarized in Table I. These results show that EGTA, *N*-methyl maleimide (NMM), and iodoacetate (IA) had no effect on hydrolysis.

In order to determine the extent of hydrolysis and lo-

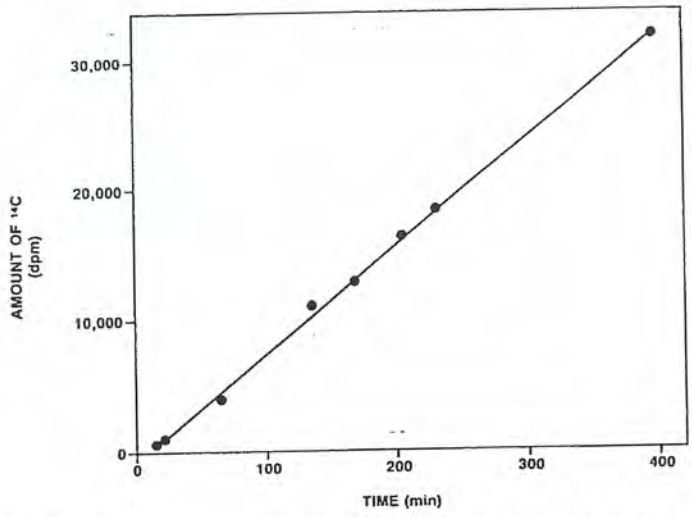

Fig. 2. The appearance of ^{14}C-labeled material in the receiver chamber of an *in vitro* diffusion experiment using human skin.

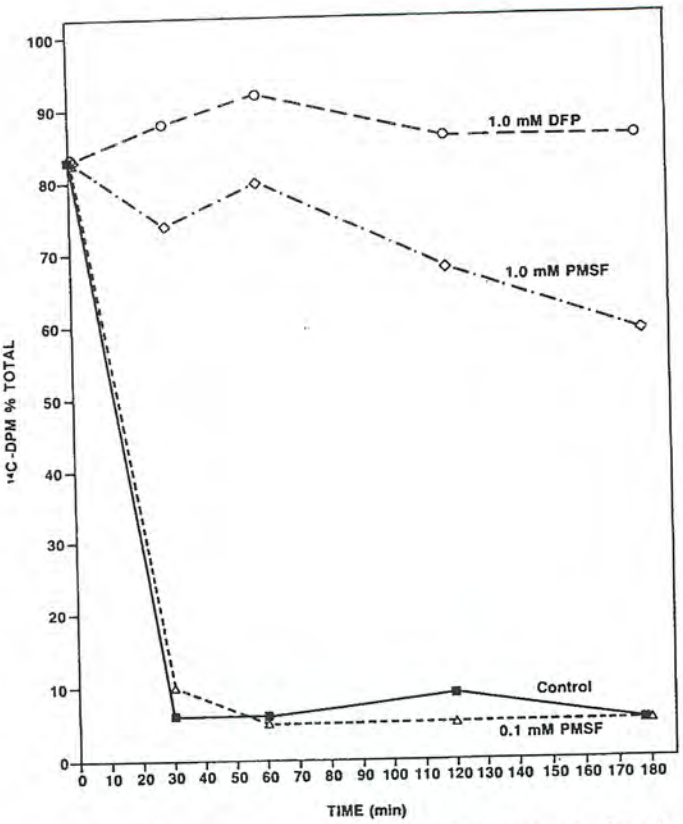

Fig. 4. The decrease in concentration of the diester vs time for hydrolysis in skin homogenate alone and in the presence of the esterase inhibitors DFP (diisopropylfluorophosphate) and PMSF (phenylmethylsulfonylfluoride).

calization of metabolites within the skin, samples were frozen and cut into serial 40-μm sections, parallel to the skin's surface, and the radioactivity was extracted and analyzed by HPLC. The results obtained *in vivo* and *in vitro* are shown in Figs. 5a and b, respectively. Each plot represents the average of duplicate samples in two experiments obtained 6 hr after application of a topical formulation containing the ^{14}C-labeled diester.

Table I. Effect of Various Protease Inhibitors on the Hydrolysis of the Diester by Mouse Skin Homogenates

Incubation conditions	Diester remaining after 120 min (%)
Buffer (pH 3–10)	>99
Skin homogenate	<5
+DFP (0.2–1 m*M*)	~80
+PMSF	
1 m*M*	~70
0.1 m*M*	~5[a]
+EGTA (5 m*M*)	5
+NMM (5 m*M*)	6
+IA (5 m*M*)	6

[a] At 0.1 m*M*, PMSF did not inhibit hydrolysis of the diester to the monoester but effectively inhibited conversion of the monoester to salicylate. Thus, the percentage of radioactivity as the monoester was 72% for 0.1 m*M* PMSF and 20% for 1 m*M* PMSF, whereas both concentrations resulted in only a 5% conversion to salicylate. This suggests that two enzymes with differing sensitivities to PMSF are responsible for each step of the enzymatic conversion. No attempts to characterize this phenomenon further have been made.

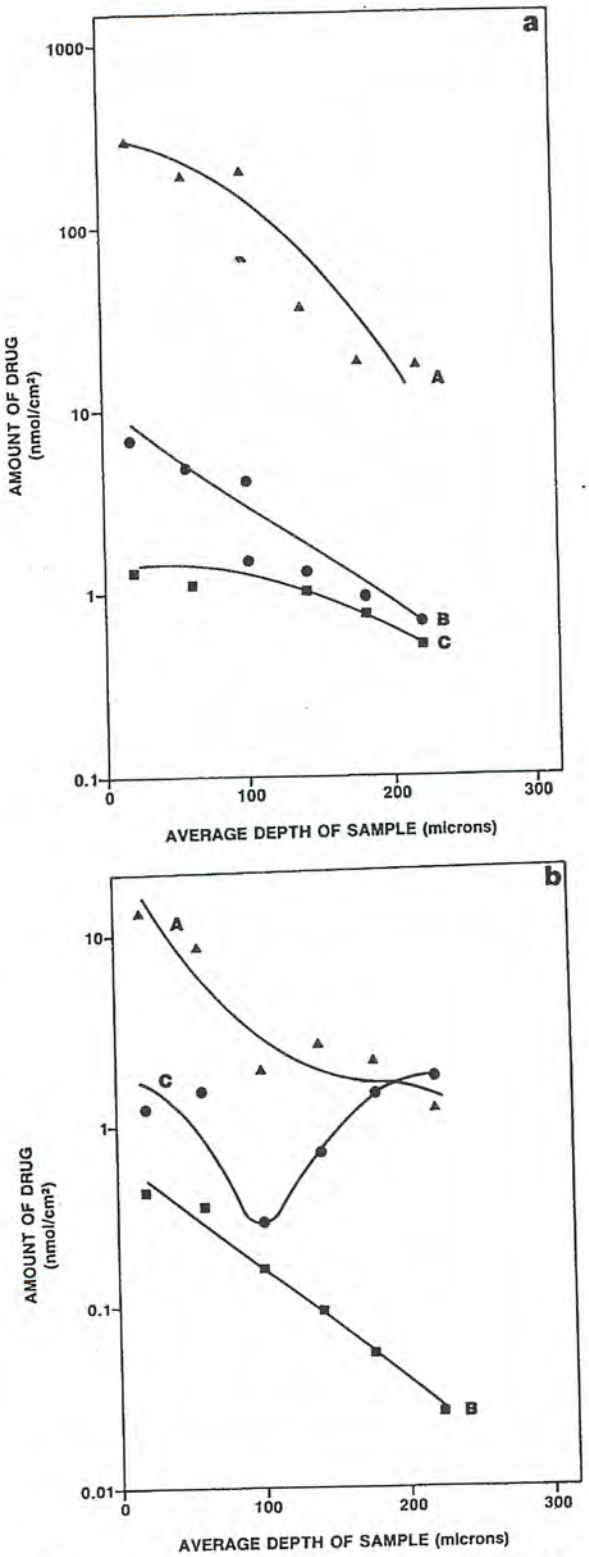

Fig. 5. The concentration of the diester (A), monoester (B), and salicylate (C) as a function of the depth within human skin either grafted to athymic mice (a) or *in vitro* (b). Results were obtained after 6 hr of topical drug application.

Figure 5a shows the concentration of the diester and its metabolites as a function of the depth within a sample ob-

tained with human skin grafted to athymic mice. At each layer examined, approximately 50-fold more diester than metabolites was found. Note also the exponential decrease in all three with increasing depth, showing a significant gradient of drug across this tissue.

Figure 5b shows the concentration profiles obtained with human skin *in vitro*. Samples were placed on filter paper moistened with phosphate-buffered saline during the 6 hr of drug application. At the end of this period the samples were processed as described above. The results show approximately 10-fold more diester than metabolites in the outer layers of the skin but comparable concentrations in deeper sections. In marked contrast to the *in vivo* results above, salicylate showed a small initial decrease in concentration to a depth of about 100 μm, followed by an increase in concentration with further increases in depth, consistent with results obtained by others (2) showing a buildup of drug in the lower layers of skin under *in vitro* conditions. In addition, the net concentration gradient of salicylate was near zero.

In order to investigate time-dependent changes in enzymatic hydrolysis, concentration profiles were also obtained at 24 hr. The *in vivo* and *in vitro* results are shown in Figs. 6a and b, respectively. The results in Fig. 6a also show the standard error associated with the triplicate determinations made under these conditions. For the sake of clarity, error bars are presented only for the data from the outermost section. These values, however, are typical for data obtained at all depths. The *in vivo* results (Fig. 6a) show a concentration gradient for the mono- and diesters across the skin but little change in salicylic acid, showing nearly complete removal of this more polar compound from the skin. In marked contrast to all other results, those obtained *in vitro* at 24 hr show little diester remaining (less than 1 nmol/cm^2 at all depths), while concentrations of salicylate in excess of 20 nmol/cm^2 are measured throughout the sample, suggesting nearly complete hydrolysis but inefficient removal under *in vitro* conditions. Finally, like the 6-hr *in vitro* results, these 24-hr *in vitro* data show an increase in the concentration of salicylate in deeper sections of the skin. The difference, however, between the maximum and the minimum values of salicylate concentration are not statistically significant.

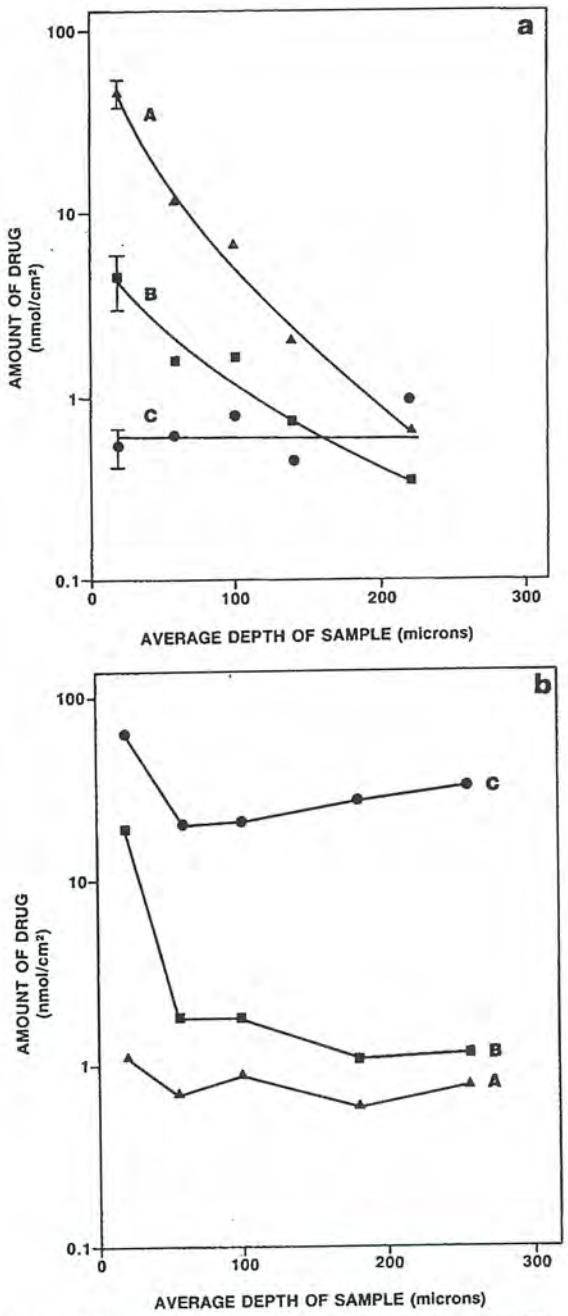

Fig. 6. The concentration of the diester (A), monoester (B), and salicylate (C) as a function of the depth within human skin either grafted to athymic mice (a) or *in vitro* (b). Results were obtained after 24 hr of topical drug application.

DISCUSSION

The permeation of ^{14}C label through human skin *in vitro* is characterized by a short lag time (estimated to be 2 min) followed by the rapid establishment of steady-state kinetics with a flux of 12.5 nmol/cm^2/hr (Fig. 2). Analysis of the contents of the receiver side of the diffusion cell at 6 and 24 hr by HPLC showed that all of the radioactivity injected was salicylic acid. Furthermore, the diester incubated in receiver buffer exposed to the dermis showed only ^{14}C-salicylate upon HPLC analysis. In contrast, no hydrolysis was observed after several days of contact of the diester with the stratum corneum side of the skin or in buffer alone. Given the stability of the diester in buffer and in the vehicle in contact with the stratum corneum, it is unlikely that either chemical hydrolysis or bacterial esterase activity is responsible for hydrolysis. Rather, it appears that the diester was enzymatically hydrolyzed to salicylic acid either on passage through the skin or while in the buffer in contact with the skin. Complete hydrolysis was also observed for the octanoate, tetradecanoate, and oleate (R_1) and methyl (R_2) diesters under identical experimental conditions (data not shown). Similar results were obtained for the hydrolysis of 5-fluorouracil and metronidazole esters (23) and hydrocortisone 21-heptanoate (25) upon passage through the skin. The results presented here are in contrast, however, to those obtained for the permeation and metabolism of estradiol mono- and diacetate (14) and hydrocortisone 21-acetate (25) through mouse skin. Those results showed significant concentrations of both esters in the receiver chamber and may reflect species differences between the studies and/or differ-

ential hydrolysis of salicylate, hydrocortisone, and estradiol esters.

In order to demonstrate further the enzymatic hydrolysis of the diester, this compound was incubated in buffer and skin homogenates. The results show that over a pH range of 3 to 10, no breakdown of the diester was detected after several hours of buffer alone. In contrast, as shown by the results in Fig. 3, incubation in a human skin homogenate resulted in a rapid disappearance of the diester. In addition, the results show a transient increase followed by a decrease in the monoester concentration. Throughout the time course of the experiment there was a continuous increase in the salicylate concentration. These results are consistent with the enzymatic hydrolysis of the diester to the monoester and, ultimately, to salicylic acid.

In order to shed further light on the enzymatic nature of the hydrolysis, various enzyme inhibitors were added to a mouse skin homogenate mixture. The results (Fig. 4 and Table I) show that while metalloproteinase (EGTA) and thiol proteinase inhibitors (NMM and IA) had no effect on hydrolysis at the concentrations tested, DFP and PMSF, which are serine proteinase and esterase inhibitors, dramatically inhibited hydrolysis. Another esterase inhibitor has been used to verify the enzymatic hydrolysis of hydrocortisone esters by mouse skin (25). Thus, the results presented here strongly indicate that the hydrolysis of the di- and monoester to salicylate is due to skin esterase activity.

While skin homogenates provide strong evidence for enzymatic activity in skin, all information about enzyme localization is lost. In order to localize the enzymatic activity within the skin and to gain some insight into the distribution of metabolites, the concentration profiles of the mono- and diester and salicylate were determined for the outermost several hundred microns of the tissue. *In vivo* results were obtained with human skin grafted to athymic mice. The grafted tissue, by its very survival, demonstrates a competent vasculature. In addition, numerous biological and histological tests have shown that grafted tissue retains characteristics of human skin (23). The concentration profiles obtained at 6 hr under these *in vivo* conditions (Fig. 5a) show an exponentially decreasing concentration of all three, consistent with measures of distribution of other topically applied drugs obtained *in vivo* with human subjects (21,22). In addition, the results show a diester concentration which is about 50-fold greater than that of the monoester and salicylate at each level examined. Thus, only a few percent of the drug localized in the outer 200 μm of the skin *in vivo* is hydrolyzed.

The results shown in Fig. 5b were obtained *in vitro* with human skin samples in the absence of sink conditions in the underlying dermal tissue. Two interesting observations can be made relative to the results obtained *in vivo*. First, the diester concentration *in vitro* is only about 10-fold greater than that of the metabolites in the outer layers and comparable to that of salicylate in the deepest layers. Thus, greater apparent hydrolysis occurs *in vitro* than *in vivo*. Second, the salicylate concentration actually increases in the deeper sections of the skin. This is most likely due to a lack of capillary perfusion and consequent drug clearance *in vitro*, as suggested by others (20–22). Thus, the results of *in vitro* experiments suggest that the diester incubates longer in the enzymatic milieu, resulting in greater hydrolysis, and that the hydrolytic products accumulate due to a lack of competent capillary removal. Such changes in drug removal may dramatically affect epidermal/dermal drug delivery and metabolism.

The *in vivo* and *in vitro* concentration profiles of the mono- and diester and salicylate were also obtained after 24 hr of application. As shown in Fig. 6a, there is a significant gradient of both esters across the skin *in vivo* but relatively little salicylate at any level. These results are consistent with efficient removal of the most polar compound, while the more lipophilic mono- and diester remained in the skin. Furthermore, the concentrations of all three compounds are less at 24 than at 6 hr, reflecting the time-dependent depletion of the finite dose of the diester applied to the skin. The relative concentration of the diester is about 10-fold greater than the metabolites near the surface but comparable at deeper levels. In contrast, after 6 hr *in vivo* the ratio of the diester to its metabolites (Fig. 5a) is only 0.01 at all levels. Thus, there is a greater extent of hydrolysis *in vivo* at 24 hr than at 6 hr. The greater relative hydrolysis *in vivo* at 24 hr may reflect a concentration dependence of the hydrolysis since less drug is in the skin due to the finite dosage protocol used.

Similar distribution results were obtained for the hydrolysis of hydrocortisone 17-butyrate 21-propionate in dog skin (11). Following topical application of ^{3}H-labeled drug, the stratum corneum was removed and then a punch biopsy taken of the remaining skin. Using a combined HPLC and radiometric assay similar to the techniques described here, these investigators showed that the diester was hydrolyzed in the skin to the monoester and parent compound. Furthermore, about 50% of the diester was hydrolyzed in the deeper tissue, while about 85% remained intact in the stratum corneum. Based on these results, they concluded that esterase activity was much greater in viable epidermal and dermal tissue than in the stratum corneum. While the stratum corneum was not specifically assayed in the studies presented here, it is entirely contained within the first 40-μm section. Our *in vivo* results (e.g., Fig. 6a) show that the extent of hydrolysis is minimal in this outer section and, thus, are consistent with little enzymatic activity in the stratum corneum.

The results obtained *in vitro* at 24 hr (Fig. 6b) are markedly different from the *in vivo* results. In particular, there is a negligible concentration of the diester at any level, with almost-complete conversion to salicylic acid. Also, as with the 6-hr *in vitro* data, the concentration profile of salicylic acid actually increases with depth after the first two sections. Furthermore, the salicylate concentration is much greater at 24 than at 6 hr. These results are entirely consistent with *in vitro* results obtained for a number of other topically applied drugs (22) and are most likely due to lack of drug removal in *in vitro* experiments. Complete diester hydrolysis can similarly be explained, since it is not removed and, thus, incubates in the enzymatic milieu of the skin.

Kao and co-workers have extensively examined the changes in metabolic activity of skin maintained *in vitro* (4–7). Their results showed that a significant number of cytoplasmic and mitochondrial enzymes are released from skin due to excision of the sample, often to a greater extent than seen in subsequent freeze–thaw cycles or chemical treat-

ment (5). Similarly, Hoelgaard and co-workers have shown significant release of esterase activity from human skin into the receiver chamber during *in vitro* diffusion experiments (24). Therefore, it is not unreasonable to assume that esterase activity is released in the preparation of *in vitro* samples. Alternatively, the increased esterase activity noted here may reflect biochemical changes (e.g., changes in pH and/or liberation of lysosomal esterases) in the tissue due to decreased skin viability *in vitro*. Regardless of the precise mechanism, the increased hydrolysis associated with *in vitro* samples of human skin can be attributed to both decreased removal of drug due to lack of capillary exchange and increased enzymatic activity.

In conclusion, we have compared the *in vivo* and *in vitro* concentration profiles of salicylic acid and salicylate esters in human skin. The results show that hydrolysis of these "prodrug" compounds is enzymatic. Furthermore, only the most polar metabolite was found in the receiver buffer of the *in vitro* flux experiments, suggesting that hydrolysis of lipophilic esters may serve as an efficient elimination mechanism. A similar hypothesis has been proposed for the elimination of benzo(*a*)pyrene from cutaneous tissue (8). In addition, the results show that *in vitro* measurements may overestimate metabolic activity due to the lack of perfusion and/or release of enzymes. In contrast, human skin grafted to athymic mice maintains competent vascular perfusion and is not subjected to surgical procedures which would release enzymatic activity during the experimental time frame. Thus, skin grafted to athymic mice and rats (12) would appear to be more reflective of the metabolic and transport properties of human skin *in vivo*. The possible extension of these findings to other drugs and other enzymatic acitivites is under way in our laboratory.

REFERENCES

1. D. R. Bickers. In V. A. Drill and P. Lazar (eds.), *Current Concepts in Cutaneous Toxicology*, Academic Press, New York, 1980, pp. 95–126.
2. D. A. W. Bucks. *Pharm. Res.* 1:148–153 (1984).
3. J. Hadgraft. In H. Bundgaard (ed.), *Design of Prodrugs*, Elsevier, Amsterdam, 1985, pp. 271–289.
4. J. Kao, J. Hall, and J. M. Holland. *Toxicol. Appl. Pharmacol.* 68:206–217 (1983).
5. J. M. Holland, J. Y. Kao, and M. J. Whitaker. *Toxicol. Appl. Pharmacol.* 72:272–280 (1984).
6. J. Kao, F. K. Patterson, and J. K. Hall. *Toxicol. Appl. Pharmacol.* 81:502–516 (1985).
7. J. Kao and J. Hall. *J. Pharm. Exp. Ther.* 241:482–487 (1987).
8. H. Mukhtar and D. R. Bickers. *Drug Metab. Dispos.* 11:562–567 (1983).
9. W. A. Khan, M. Das, S. Stick, S. Javed, D. R. Bickers, and H. Mukhtar. *Biochem. Biophys. Res. Comm.* 146:123–126 (1987).
10. Y. Ozawa, I. Koyama, S. Murayama, and T. Nadai. *Chem. Pharm. Bull.* 33:5113–5118 (1985).
11. W. Wiegrebe, A. Retzow, E. Plumier, N. Ersoy, A. Garbe, H.-P. Faro, and R. Kunert. *Arzneim.-Forsch.* 34:48–51 (1984).
12. G. G. Krueger, Z. J. Wojciechowski, S. A. Burton, A. Gilhan, S. E. Huether, L. G. Leonard, U. D. Rohr, T. J. Petelenz, W. I. Higuchi, and L. K. Pershing. *Fund. Appl. Toxicol.* 5:s112–s121 (1985).
13. M. Loden. *J. Invest, Dermatol.* 85:335–339 (1985).
14. K. H. Valia, T. Kakuji, and Y. W. Chien. *Drug Dev. Ind. Pharm.* 11:1133–1173 (1985).
15. K. Tojo, K. H. Valia, G. Chotani, and Y. W. Chien. *Drug Dev. Ind. Pharm.* 11:1175–1193 (1985).
16. J. L. Fox, C.-D. Yu, W. I. Higuchi, and N. F. H. Ho. *Int. J. Pharm.* 2:41–57 (1979).
17. R. H. Guy and J. Hadgraft. *Int. J. Pharm.* 11:187–197 (1982).
18. R. H. Guy and J. Hadgraft. In H. I. Maibach and N. Lowe (eds.), *Models in Dermatology, Vol. 2*, Karger, Basel, 1985, pp. 170–177.
19. J. Hadgraft. *Int. J. Pharm.* 4:229–239 (1980).
20. A. Zesch and H. Schaefer. *Arch. Derm. Forsch.* 246:335–354 (1973).
21. A. Zesch and H. Schaefer. *Arch. Derm. Forsch.* 252:245–256 (1975).
22. H. Schaefer, A. Zesch, and G. Stuttgen. *Skin Permeability*, Springer-Verlag, Berlin, 1982.
23. G. G. Krueger and R. A. Briggaman. In J. Fogh and B. P. Giovanella (eds.), *The Nude Mouse in Experimental and Clinical Research, Vol. 2.*, Academic Press, New York, 1982, pp. 301–322.
24. A. Hoelgaard, B. Mollgaard, J. M. Anderson, and S. W. Kim (eds.). In *Advances in Drug Delivery Systems*, Elsevier, Amsterdam, 1986, pp. 111–120.
25. W. Smith. Ph.D. thesis, University of Michigan, Ann Arbor 1982.