Drug Distribution in Human Skin Using Two Different *In Vitro* Test Systems: Comparison with *In Vivo* Data

Heike Wagner,[1] Karl-Heinz Kostka,[2] Claus-Michael Lehr,[1] and Ulrich F. Schaefer[1,3]

Received July 28, 2000; accepted September 15, 2000

Purpose. Two *in vitro* test systems used to study drug penetration into human skin—the Franz diffusion cell (FD-C) and the Saarbruecken penetration model (SB-M)—were evaluated, and the results were compared with data gained under analogous *in vivo* conditions.

Methods. Excised human skin was used in all *in vitro* experiments. Flufenamic acid dissolved in wool alcohols ointment, was chosen as a model drug, and the preparation was applied using 'infinite dose' conditions. To acquire quantitative information about the drug penetration, the skin was segmented into surface parallel sections at the end of each experiment, first by tape stripping the stratum corneum (SC), and second by cutting the deeper skin layers with a cryomicrotome. The flufenamic acid was extracted from each sample and assayed by high performance liquid chromatography (HPLC). For *in vivo* experiments, only the tape stripping technique was used.

Results. a) *Drug penetration into the SC:* In both *in vitro* test systems the total drug amounts detected in the SC were found to increase over the different incubation times. Similar conditions were obtained *in vivo,* but on a lower level. Using Michaelis–Menten kinetics, the m_{max} value was calculated for the skin of two donors. The relations of the m_{max} values for the FD-C and the SB-M closely correspond (1.26 [donor 1] and 1.29 [donor 2]). A direct linear correlation of the drug amount in the SC and the time data were found for *in vivo* with both *in vitro* test systems.

b) *Drug penetration into the deeper skin layers:* The detected drug amounts in the deeper skin layers continuously increased with the incubation time in the SB-M, while in the FD-C, only very small drug amounts were observed after incubation times of 30 and 60 minutes. It was also noticed, that the drug amounts rose steeply at time points 3 and 6 hours. Additional studies showed a remarkable penetration of water into the skin from the basolateral acceptor compartment in the FD-C. This could explain the different drug transport into the deeper skin layers between the two *in vitro* test systems.

Conclusions. Both *in vitro* models showed comparable results for the drug penetration into the SC and a robust correlation with *in vitro* data. Different results were obtained for the deeper skin layers. Whether a correlation between *in vitro* and *in vivo* data is also possible here has to be investigated by further experiments.

KEY WORDS: Franz diffusion cell; Saarbruecken penetration model; skin segmentation; *in vitro–in vivo* correlation; flufenamic acid; dermal penetration.

[1] Dept. of Biopharmaceutics and Pharm. Technology, Saarland University, D-66123 Saarbruecken, Germany.

[2] Dept. of Plastic and Hand Surgery, Caritaskrankenhaus, D-66822 Lebach, Germany.

[3] To whom correspondence should be addressed. (e-mail: ufs@rz.uni-sb.de)

INTRODUCTION

In the context of dermal and transdermal drug delivery, there has been an intensified interest in *in vitro* test systems over the past few years. Such test systems are not only needed for the development of new dosage forms or the evaluation of pharmaceutical and cosmeceutical products, but also to obtain information about the effects of drugs and/or excipients on the barrier function of the skin. In fact, this kind of information could be gained best from *in vivo* experiments on humans, but studies with volunteers do not easily allow the determination of drugs in the deeper skin layers, because this usually requires invasive techniques. Therefore, the use of excised human skin mounted on suitable *in vitro* test systems may be an alternative.

In the present study, two different *in vitro* test systems were evaluated. The first test system chosen is the Franz diffusion cell (FD-C), which was originally developed to study transdermal drug permeation (1–3). The second test system is the Saarbruecken penetration model (SB-M), which has been developed by Professor H. Loth and his coworkers in our laboratories earlier (4–6). Here, in contrast to the FD-C, no liquid acceptor medium is used to avoid nonphysiological hydration of the tissue. Which system best mimicks the *in vivo* conditions is of essential interest for the use of *in vitro* test systems as a surrogate for *in vivo* experiments. To monitor the drug penetration into human skin, flufenamic acid dissolved in wool alcohols ointment was applied to the skin for different incubation times. Flufenamic acid can be easily extracted from the skin samples and monitored by HPLC in a range of ng/ml. To achieve well-defined kinetic conditions, the 'infinite dose' dosage was used (7). Corresponding *in vivo* experiments were carried out on six volunteers using the volar forearms to determine the drug amount penetrated into the SC.

MATERIALS AND METHODS

Materials

The following chemicals and equipment were used: Flufenamic acid modification I (Kali-Chemie Pharma, Hannover, D); wool alcohols ointment, Multifilm kristall-klar and Fixomull® (Beiersdorf, Hamburg, D); Soerensen phosphate buffer pH 7.4, Ringer solution, McIlvaine citric acid–phosphate buffer pH 2.2 and NaOH (all components from Merck, Darmstadt, D); Plastibase® (Heyden GmbH, München, D); methanol (Baker, Deventer, NL); 5(6)-carboxyfluorescein and rhodamine B (Sigma Chemical Co.; St. Louis; USA); Finn Chambers (Hermal, Reinbek, D); light microscope (Olympus BH-2; Olympus, Hamburg, D); thickness meter, model 5041 type (VZR) with tactile probe (MT) 10B (accuracy ± 1 μm; Heidenhain Co., Traunreut, D); cryomicrotome HR Mark II, model 1978 (SLEE, Mainz, D); Franz diffusion cell type 4G-01-00-20 (Perme Gear, Riegelsville, PA, USA); isocratic HPLC consisting of a 655 A 40 autosampler, L4250 detector, L 6220 pump, 6000K data interface and 5 μm LiChrospher® 100/RP-18 column/12.5 cm × 4 mm (Merck-Hitachi, Darmstadt, D); laser scanning confocal imaging system MRC-1024 (Bio-Rad Laboratories, Muenchen, D); argon ions laser (American Laser Corp., Salt Lake City, USA); Zeiss axiovert 100 microscope (Carl Zeiss, Oberkochen, D).

Flufenamic acid (0.9% W/W) dissolved in wool alcohols ointment (German Pharmacopoeia 1999) was used as the drug preparation. Wool alcohols ointment generally consists of 6% wool alcohols, 0.5% cetostearyl alcohol, and 93.5% white petrolatum. Ingredients of the wool alcohols ointment may penetrate into the skin and influence the solubility and distribution of the drug in the different skin layers. Flufenamic acid has a melting point of 132.8°C (modification I) and a pk_a of 3.9. The solubility in water is limited, but can be increased with lipophilic solvents as well as with alkaline solutions. At 32°C, 0.9% flufenamic acid is completely dissolved in the ointment base (8). To avoid recrystallization, the preparation was stored at 32°C. Before application of the ointment the absence of crystals was verified by light microscopic inspection. The ointment base consisted of lipophilic components only; therefore flufenamic acid most likely is not dissociated within the preparation.

Determination of the Thickness of the Skin and the SC

To observe possible volume changes of the skin during the *in vitro* experiments, the thickness of the skin was measured before and after the experiments with a thickness meter at an accuracy of ±1 μm. The thickness of the SC in a cross section of the skin was determined by a light microscopic procedure and an ocular micrometer. For the analysis of the *in vivo* experiments the SC thickness was assumed to be 20 μm.

Skin Dissection

Excised human skin from Caucasian female patients who had undergone abdominal plastic surgery was used. Immediately after excision the subcutaneous fatty tissue was removed using a scalpel. The cut into 10 × 10 cm pieces, wrapped in aluminum foil and stored in polyethylene bags at –26°C until use. Previous studies in our laboratories have shown that the skin remained stable upon freezing as the penetration of drugs and the thickness of the SC was not affected after a freezing period of 3 and 6 months, respectively (9,10). These results are in accordance with other laboratories (11,12). For penetration experiments, skin disks were punched out, thawed, cleaned with cotton soaked with Ringer solution, and transferred onto the respective test system. In both cases, the same size of the skin area was available for drug incubation.

Dosage Regime and Incubation Times

To maintain 'infinite dose' conditions (7), an ointment layer of 2 mm thickness was applied to the skin. Such conditions foster a very reproducible way of application, that steady state concentration of flufenamic acid in the stratum corneum is expected, and that effects caused by rubbing were avoided. The drug preparation was applied to the skin for 4 different periods of time: 0.5, 1, 3 and, 6 hours (*in vitro*) or 0.25, 0.5, 1, and 3 hours (*in vivo*).

Franz Diffusion Cell (FD-C) Experiments

In the FD-C, the skin was positioned between the donor compartment containing the drug preparation and the receptor compartment, filled with Soerensen phosphate buffer pH 7.4 (1–3). The temperature was kept at 32 ± 1°C by a water jacket. The acceptor fluid was mixed with a magnetic stirring bar (500 rpm). To achieve higher reproducibility, the skin was prehydrated with the basolateral receptor medium for 30 minutes. The drug preparation was filled into a plastic cylinder (ointment layer = 2 mm), which was carefully positioned on the surface of the skin (Fig. 1a). One sample from the acceptor medium was taken before and after each experiment to determine transdermal permeation of flufenamic acid.

Saarbruecken Penetration Model (SB-M) Experiments

A more detailed description of this model can be found elsewhere (4–6). The skin was put onto a filter paper soaked with Ringer solution and placed into the cavity of a teflon bloc. Compared to the FD-C, nonphysiological hydration of the skin was avoided here due to the absence of the liquid acceptor medium. The drug preparation was filled into a cavity of a teflon punch of 2 mm depth. The punch was applied on the surface of the skin, and a weight of 0.5 kg was placed on the top of the punch for 2 minutes to improve the contact between the skin and the drug preparation. After 2 minutes, the teflon punch was fixed in its position, and the gap between the two teflon parts was sealed with Plastibase® to avoid water loss from the skin (Fig. 1b). The whole apparatus was transferred into a plastic box and was placed in a water bath at 32 ± 1°C for thermostatization (8).

Segmentation of the Skin and Calculations of the Drug Concentration—skin depth profiles

To compare the results of both models, the skin was always treated in exactly the same manner at the end of all experiments. First, the remaining ointment was removed by wiping the skin with cotton. Second, the skin was transferred into a special apparatus where it was mounted on cork discs using small pins (6). The stretching of the skin going along with that, helped to overcome problems of furrows (13) in the

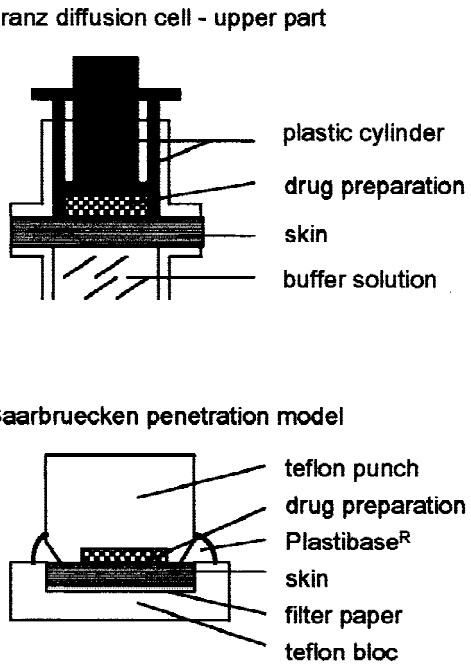

Fig. 1. *In vitro* test systems for the study of drug penetration.

subsequent tape-stripping procedure. For this procedure, the surface of the skin was covered with a teflon mask with a central hole of 15 mm in diameter. The skin was successively stripped with 20 pieces of adhesive tape (size = 15 × 20 mm) placed on the central hole (14). In a standardized procedure each tape was charged with a weight of 2 kg for 10 seconds, removed rapidly and combined in 6 pools of 1, 1, 3, 4, 5, and 6 strips for analytical purposes. Due to this standardized procedure, each of the removed cell layers had nearly the same thickness, which had been shown in previous studies in our laboratories (6,9). The number of tape strips was linearly correlated with the remaining thickness of the SC. Other laboratories had reported analogous data (15). Dividing the predetermined SC thickness by 20 would give the thickness of the SC layers per strip. The first tape strip was always discarded because of potential contamination by residual drug on the surface of the skin.

After the tape-stripping, the skin was rapidly frozen in a stream of expanding carbon dioxide, and a specimen with a diameter of 13 mm was taken out of the stripped area and transferred into a cryomicrotome. The skin was cut into surface parallel sections and collected according to the following scheme: #1 = incomplete cuts; #2–5 = 2 × 25 μm sections; #6–9 = 4 × 25 μm sections; #10–11 = 8 × 25 μm sections; #12 = rest of the residual tissue. The thickness of the incomplete cuts and the skin rests were calculated over their weights relating to a standard cut with known weight and thickness.

In vivo Experiments

Six human volunteers (three male, three female), aged 23–29 years, from whom informed consent was obtained, participated in the study. They were in good health and had no history of any dermatological disease. A template of Fixomull® with four holes was fixed on the volar left and right forearm of each volunteer. The distance between the outmost located holes and the elbow or the wrist, respectively, was 5 cm, the distance between two successive holes was 2 cm. Each hole released an area of 15 mm in diameter and represented one experimental area. The drug preparation was applied by Finn Chambers, 15 mm in diameter and 1 mm in depth.

The tape-stripping procedure was performed as described before, except for exerting the pressure just with the forefinger. To increase reproducibility the procedure was performed by the same person according to the AAPS/FDA Workshop Report (14) and the Guidance for Industry.

Assay Method

The flufenamic acid was extracted from the adhesive tape, the skin cuts, and the filter paper with 1.5 ml 0.05 M NaOH by shaking for 2 hours at room temperature and centrifugation of the skin cuts at 800 × g for 30 minutes.

HPLC Procedure

20 μl of the supernatants were injected directly onto the HPLC system. An 80/20 (V/V) mixture of methanol/Mcllvaine citric acid–phosphate buffer pH 2.2 was used as a mobile phase. At a flow rate of 1.2 ml/min, the retention time of flufenamic acid was 3.5 ± 0.2 minutes. The detector was set at 284 nm. Unknown flufenamic acid concentrations were calculated against known standards via the method of area under the absorption-time curves. The method provided good linearity ($r = 0.999$) over a concentration range of 50 to 2000 ng/ml and a recovery of 97.8 ± 3.8%.

Measurements of the Hydration of the Skin/Confocal Laser Scanning Microscopy

The transport of water from the basal side into the excised skin specimen was studied as follows: The thickness of the skin before and after the experiments was measured using a thickness meter. The transport of the hydrophilic marker compound 5(6)-carboxyfluorescein, dissolved in the receptor medium (0.01 mmol/l (FD-C) and 0.1 mmol/1 (SB-M), respectively) was investigated from the basolateral side of the skin to the apical side. The experiments were carried out as usual. To counterstain the SC a solution of 0.01 mmol/1 (FD-C) or 0.1 mmol/l (SB-M) rhodamine B in Soerensen phosphate buffer pH 7.4 was applied for the last 30 minutes. Cross sections were investigated with a laser scanning confocal imaging system (magnification 2.5, laser power 3%, scan modus slow, gain 1000, measurement Kalman and method red/green fluorescence [filter system A1 and A2—488 and 514 nm]).

RESULTS

To reduce the influence of inter-individual differences between various skin donors, all *in vitro* experiments were carried out with the skin from the same donor (donor 1). To confirm these results skin of an additional donor (donor 2) was used.

Drug Penetration Time Course

Drug Amounts in the SC

Fig. 2a demonstrates the total amounts of flufenamic acid which were found in the SC. Only slight differences could be seen using the two *in vitro* models. For both test systems, an increase of the detected drug amount was observed over the experimental time period. In the FD-C, the drug amounts rose until 3 hours incubation time. A plateau at a level of about 3500 ng/cm^2 flufenamic acid was reached thereafter. The SB-M showed a continuous increase, indicating a constant rate of penetration throughout the experiment. A plateau did not seem to have been achieved during the tested incubation time.

In the *in vivo* experiments the flufenamic acid amounts found in the SC were clearly less than those found *in vitro.* Although the changes in the detected drug amounts were small, no plateau seemed to have been reached in the 3 hours incubation time.

Drug Amounts in the Deeper Skin Layers

Figure 2b shows the results for the deeper skin layers. In the SB-M, there was a continuous increase of penetrated flufenamic acid until 3 hours incubation time, and then remained at the same level after 6 hours. No drug could be found in the filter paper beneath the skin. In the FD-C, the detected flufenamic acid amounts after 0.5 and 1 hour incubation time were very low, but a steep increase was observed after 3 and 6 hours (to amounts even higher than in the SB-M). Although relatively high flufenamic acid amounts were detected in the

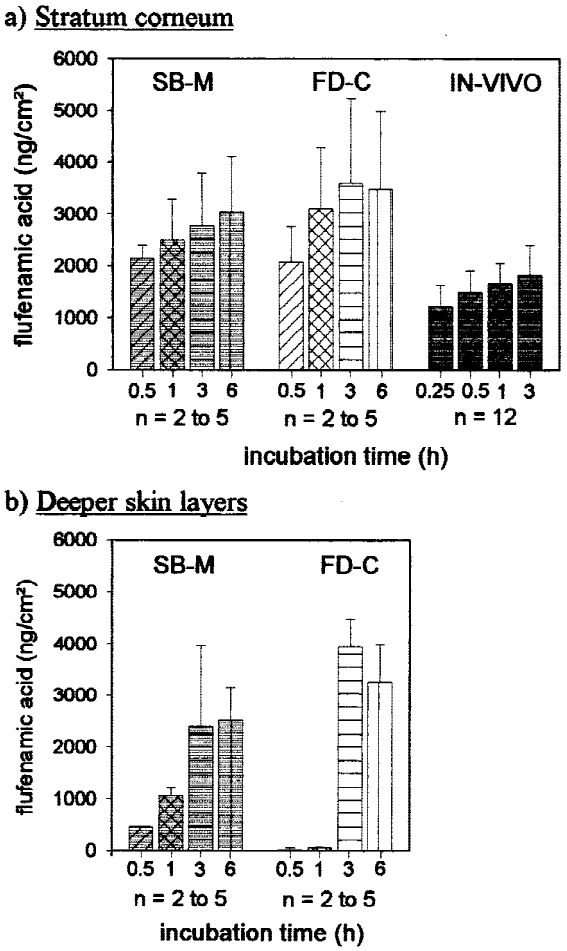

Fig. 2. Drug penetration time course. Mean of the flufenamic acid amounts [(ng/cm^2) + s.d.; n = 2 to 5 (*in vitro*) and n = 12 (*in vivo*)].

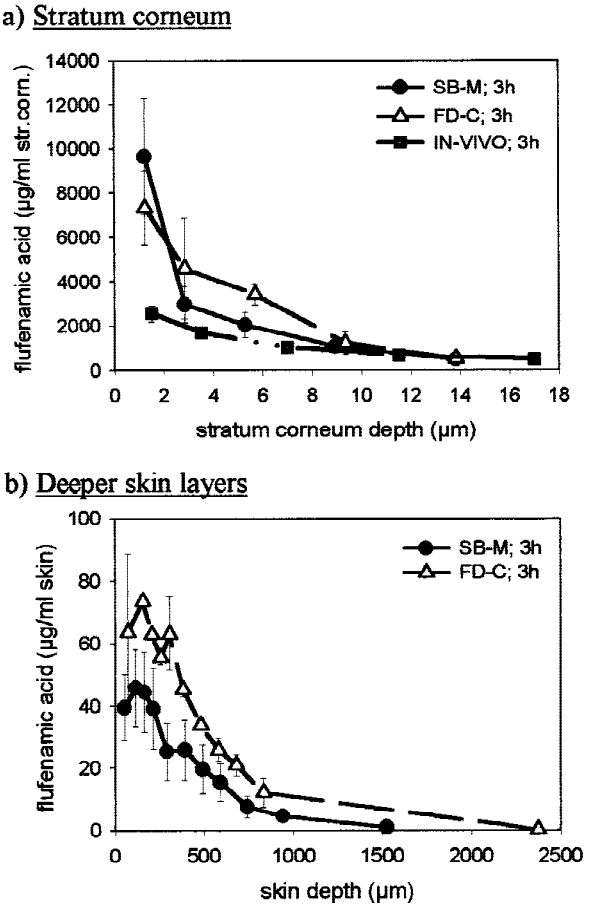

Fig. 3. Drug concentration–depth–profiles. Mean of the flufenamic acid values [(µg/ml skin) ± s.e.; n = 2 to 5 (*in vitro*) and n = 12 (*in vivo*)]; incubation time: 3 hours.

deeper skin layers after the longer incubation times, no flufenamic acid was found in the buffer beneath the skin which corresponded with data from other laboratories (16,17) detecting 'lag times' of about 4 hours using rat abdominal skin or SC sheets isolated from hamster cheek pouch in permeation experiments (18,19). Obviously, the tissue was acting as a sink itself for the drug for up to 6 hours in both test systems. *In vivo* data could not be obtained because of the invasive techniques needed.

Drug Concentration Depth Profiles

Fig. 3 shows an example (3 hours incubation time) for the drug concentration–depth–profiles which resulted from the detected flufenamic acid amounts of the various samples.

SC Depth–Profiles

In both *in vitro* designs, nonlinear drug concentration–SC depth–profiles were observed: A steeper profile in the SC layers next to the surface and a flatter profile in the deeper SC layers. While the flatter part of the profiles was more or less the same for both *in vitro* test systems, the initial gradient appeared to be steeper in the SB-M than in the FD-C. This characteristic curve shape was found at all time points which were investigated (data not shown).

In the *in vivo* experiments, the drug concentration–SC depth–curves showed nonlinear profiles as well, but with a gradient less steep the first tissue layers.

Deeper Skin Layers Depth–Profiles

The profiles of the FD-C and the SB-M (Fig. 3b) demonstrate a continuous increase of the penetrated flufenamic acid amount into the deeper skin layers at the time point 3 hours. In contrast to the SB-M, practically no profiles could be determined for the FD-C after 0.5 and 1 hour, due to the very low drug amounts after 0.5 and 1 hour in all skin segments. After 6 hours, the drug concentration–skin depth–profiles of the FD-C reached higher flufenamic acid levels than the SB-M (data not shown).

Skin Hydration *In Vitro*

Measurements of the skin's thickness served as an indicator for the skin's hydration. The FD-C showed an increase in the thickness of the skin after longer incubation times, which evened out at a level about 118.1 ± 0.2% after 6 hours. In contrast, nearly no increase in the thickness of the skin could be detected with the SB-M (103.7 ± 3.4% after 6 hours).

The increased penetration of water into the skin from the basolateral receptor compartment of the FD-C was also demonstrated by the penetration of the hydrophilic marker compound 5(6)-carboxyfluorescein after an incubation time of 6

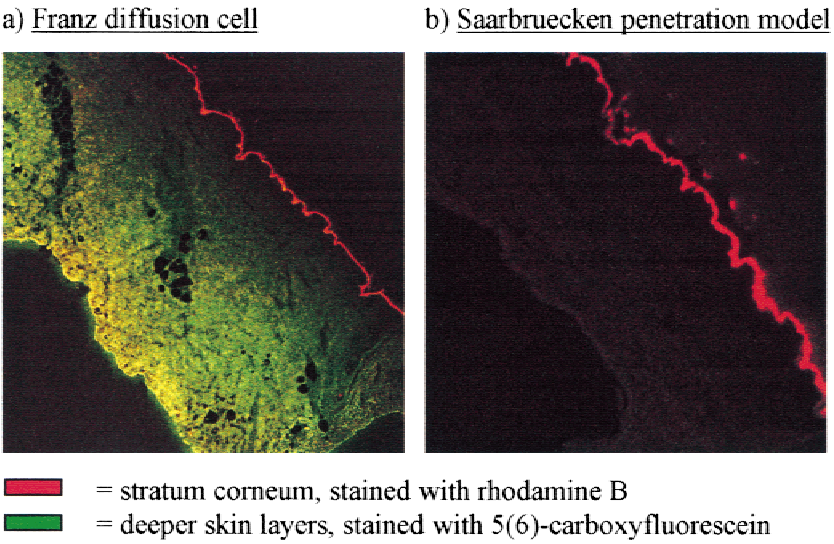

Fig. 4. Basolateral penetration of 5(6)-carboxyfluorescein into the skin. Confocal laser scanning microscopy; incubation time: 6 hours. a) FD-C: 0.01 mmol/l 5(6)-carboxyfluorescein. b) SB-M: 0.10 mmol/l 5(6)-carboxyfluorescein.

hours (Fig. 4). In contrast to the FD-C, the SB-M showed only very small amounts of penetrated 5(6)-carboxyfluorescein, although the marker compound was used at a 10× higher concentration.

DISCUSSION

The main difference between the FD-C and the SB-M is the presence of a phosphate buffer at the basolateral side of the skin specimen in the FD-C. Although no flufenamic acid was found in this buffer, it can be presumed that the observed effects described previously are caused by this buffer and could be the reason for the different results obtained with both systems. Studies with limited amounts of applied drug preparation resulted in a total drug recovery of 95.53 ± 4.81%, and therefore indicated that the analytical method is sensitive enough and not the reason for the different data.

The following explanations are conceivable. During the FD-C experiments, water and drug molecules penetrate into the deeper skin layers from opposite directions. They may hinder each other and act as a barrier, which may explain the slower penetration rate during the first hour in the FD-C. Later, the hydration of the skin leads to an increase of the fluidity of the membrane bilayers in the SC (20,21). Furthermore, once the buffer substances penetrate into the SC, a change of the dissociation degree of the fatty acids in the SC may take place, which influences the solubility of the penetrating drug. A change in the buffer capacity of the skin and its pH can also occur. Depending on the drug properties, its solubility could increase and changes in the distribution balance may take place as a result of the dissociation of the drug molecules. This results in a higher diffusion rate of the applied drug through the SC and higher amounts of this drug in the tissue.

The measurements of the thickness of the skin before and after the end of the experiments indicate that in the FD-C experiments a water uptake occurs at up to 3 hours, which is not observed in the SB-M. The data suggest that the skin is fully hydrated after 3 hours, which is in accordance with the very slow penetration rate at the short incubation times (hindrance by the water), but the penetration rate rises with longer incubation times (diffusion without any counter current). The penetration experiments with the hydrophilic marker compound (5(6)-carboxyfluorescein) confirm this water uptake from the basolateral side of the skin (Fig. 4).

The nonlinear curves of the drug concentration–SC depth–profiles (Fig. 3), which have been observed by the use of both *in vitro* test systems as well as under analogous *in vivo* conditions, may be caused by the heterogeneous anatomical (22) and chemical structure of the SC. The anatomical structure can be divided into the SC disjunctum and the SC conjunctum. The membranes are looser up in the SC disjunctum than in the SC conjunctum, e.g. measurable over the corneocyte adhesion (23). The chemical heterogeneity of the SC is supported by data, such as the changing amounts and composition of the lipid components in dependence of the SC depth, which have been investigated in our laboratories (9,24) as well as in others (25). This could explain why the drug

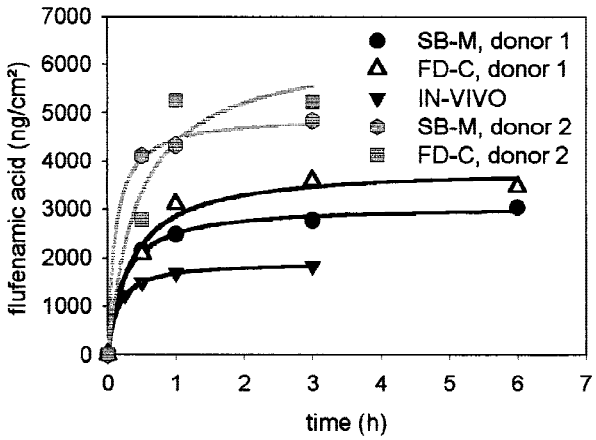

Fig. 5. 'Best fit' calculation of the means of the detected flufenamic acid amounts (ng/cm^2) in the stratum corneum against the incubation time (h) [n = 2 to 5 (*in vitro*) and n = 12 (*in vivo*)].

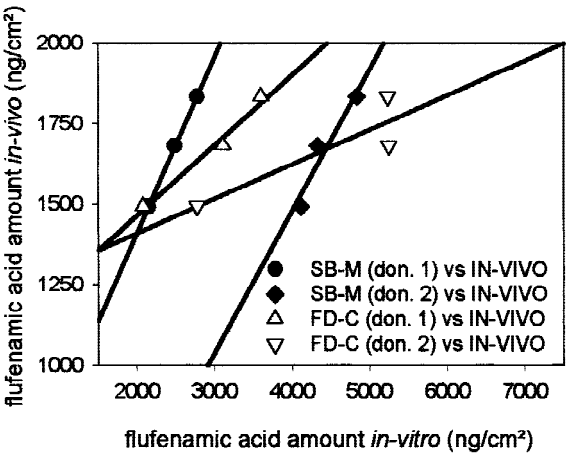

Fig. 6. Mean of the *in vitro–in vivo* correlation (ng/cm^2) [n = 2 to 5 (*in vitro*) and n = 12 (*in vivo*)].

penetration is easier and therefore faster in the SC disjunctum. These approaches are in contrast to the ones proposed by others (26) who assume a homogeneous transport in the SC.

The gradient in the outermost layer of the SC obtained using the SB-M may be explained by the higher pressure, which was caused by the application of the ointment in this model compared to the FD-C or the *in vivo* application. This may increase the penetration rate of the ingredients of the wool alcohols ointment into the skin under the conditions of the SB-M and therefore influence the solubility and distribution of flufenamic acid in the different skin layers.

The *in vivo* experiments yielded smaller drug amounts in the SC compared to both *in vitro* test systems. The different skin areas which were used in this study, (abdomen for *in vitro,* forearm for *in vivo*) could influence the drug penetration (27–29) as well as the metabolism and the transport of the drug in the bloodstream, which is of importance concerning the results of the experiments. Under *in vivo* conditions there is a high tissue clearance by the bloodstream. Therefore, higher drug amounts cannot be built up in the SC and the deeper skin layers. Using the FD-C, this clearance might be simulated by the buffer. This is why the SC profiles of the FD-C resemble those of *in vivo* conditions more than the SB-M. In the latter, no further clearance takes place, since only the tissue acts as a receptor. The differences in the detected drug amounts between the *in vivo* experiments and the FD-C may be caused by the different clearance rates.

Permeation experiments, where the drug is detected in the basolateral receptor compartment, usually lead to parameters like flux and different coefficients, e.g., for diffusion, permeation, and partition concerning the permeated drug (7,30). In contrast to permeation studies, our approaches allow to fit the detected drug amounts against the incubation times. A 'best fit' calculation (Sigma Plot 4.01) is shown in Fig. 5, based on a Michaelis–Menten kinetic, which is applicable to many saturable processes:

$$m_{act} = \frac{m_{max} \times t_{inc}}{t_{max/2} + t_{inc}}$$

Here, m_{max} is the drug amount of flufenamic acid (ng/cm^2) present in the SC after a steady state is established. The incubation time, after which half of the maximal drug amount in the steady state is reached, is given by $t_{max/2}$ (h). t_{inc} represents the incubation time (h) and m_{act} the actual drug amounts (ng/cm^2) at time t_{inc}. Fitting the data to this equation allows the comparison of different ointment bases as well as of the results from the skin of several donors. In both *in vitro* test systems, a higher m_{max} was found for the skin of donor 2 compared to that of donor 1. Nevertheless, the relation between the m_{max} values of the FD-C and the SB-M were nearly the same: 1.26 (donor 1) and 1.29 (donor 2). All m_{max} values determined *in vitro* are clearly higher than those detected *in vivo.* In contrast to the varied m_{max} values the values obtained for $t_{max/2}$ are more or less the same in all experiments.

The very good correspondence between the SC data for both *in vitro* test systems and the *in vivo* conditions is also evident by a direct linear correlation (Fig. 6) of the drug amount time data (Sigma Plot 4.01), resulting in the following correlation coefficients:

1.) The FD-C versus *in vivo:* $r = 0.990$ (donor 1) and $r = 0.892$ (donor 2)

2.) The SB-M versus *in vivo:* $r = 0.999$ (donor 1) and $r = 0.958$ (donor 2)

Both investigated dermal test systems have their advantages and disadvantages and are only models, which try to imitate the real situation *in vivo.* The disadvantage of both test systems is the absence of the blood flow. Decisive for the drug penetration using the SB-M is only the solubility in the different skin layers. The washing out is limited due to the absence of an acceptor medium or the blood flow. Therefore, the time at which an ointment can be applied to the skin's surface is also limited (in dependence of the ointment base and the characteristics of the drug), because there will be a drug buildup from the deepest skin layers to the SC (sink conditions do not exist further). The FD-C is a model which is already established for percutaneous absorption studies. The acceptor medium beneath the skin may help to simulate the blood flow by maintaining sink conditions in the skin during the whole experimental time. The success of this practice depends on the solubility of the drug in the acceptor medium. At the same time, there will always be an interaction

Table I. Mean of the Coefficients Obtained from the 'Best Fit' Calculations of the Detected Flufenamic Acid Amounts (ng/cm^2) in the Stratum Corneum Against the Incubation Time (min) ± s.e. [n = 2 to 5 (*in vitro*) and n = 12 (*in vivo*)]

	donor 1		donor 2		
	FD-C	SB-M	FD-C	SB-M	*In Vivo*
m_{max} (ng/cm^2)	3877.53 ± 225.86	3077.43 ± 66.15	6382.80 ± 1330.78	4947.34 ± 136.36	1918.49 ± 2.69
$t_{max/2}$ (min)	21.0 ± 6.0	13.8 ± 1.8	27.0 ± 19.8	6.6 ± 1.8	8.4 ± 0.06
r	0.9917	0.9987	0.9679	0.9992	0.9999

between the acceptor medium and the skin, which may effect its barrier properties.

The *in vitro–in vivo* correlation obtained with both models indicates that both test systems have the potential to predict drug penetration into human skin *in vitro,* at least as the SC is concerned. For the deeper skin layers further investigations regarding correlation between *in vivo* and *in vitro* are currently in progress.

ACKNOWLEDGMENT

The Fonds der Chemischen Industrie is thanked for financial support. The authors would like to express gratitude to Professor emerit. H. Loth for his helpful advice and critical discussions.

REFERENCES

1. S. A. Akhter and B. W. Barry. Permeation of drugs through human skin: method and design of diffusion cells for in vitro use. In: R. Marks and G. Plewig, *Skin models,* Springer Verlag, New York, 1986 p. 358–370.
2. R. L. Bronaugh. Diffusion cell design. In: V. P. Shah and H. I. Maibach, *Topical drug bioavailability, bioequivalence and penetration,* Plenum Press, New York/London, 1993, pp. 117–125.
3. T. J. Franz. Percutaneous absorption—on the relevance of in vitro data. J. Invest. Dermatol. **64**:190–195 (1975).
4. M. Hailer. Freisetzung von Salicylsaeure aus Suspensionssalben: Liberation im Membranmodell im Vergleich zur Wirkstoffabgabe an excidierter Haut. Dissertation Saarbruecken 1981.
5. S. Blasius. Sorptionsvermittler—Einfluss auf die Liberation von Indomethacin aus Salben and auf die Arzneistoffaufnahme durch excidierte Haut. Dissertation Saarbruecken 1985.
6. D. Borchert. Methoden zur Untersuchung der simultanen Penetration von Arzneistoffen and Vehikelbestandteilen aus Salben in exzidierter Humanhaut. Dissertation Saarbrücken 1994.
7. T. J. Franz, P. A. Lehman, S. F. Franz, H. North-Root, J. L. Demetrulias, C. K. Kelling, S. J. Moloney, and S. D. Gettings. Percutaneous penetration of N-nitrosodiethanolamine through human skin (in vitro): Comparison of finite and infinite dose applications from cosmetic vehicles. *Fundam. Applied Toxiccol.* **21**:213–221 (1993).
8. T. Wild. Einfluss der physikochemischen Eigenschaften von Arzneistoffen and Vehikeln auf die Permeabilitaet der menschlichen Hornschicht. Dissertation Saarbruecken 1988.
9. F. Theobald. In-vitro Methoden zur biopharmazeutischen Qualitaetspruefung von Dermatika unter Berucksichtigung der Lipidzusammensetzung des Stratum corneum. Dissertation Saarbruecken 1998.
10. U. Schaefer and H. Loth. An ex-vivo model for the study of drug penetration into human skin. *Pharm. Res.* **13**(Suppl.):366 (1996).
11. R. L. Bronaugh, R. F. Stewart, and M. Simon. Methods for in-vitro percutaneous absorption studies VII: use of excised human skin. J. Pharm. Sci. **75**:1094–1097 (1986).
12. S. M. Harrison, B. W. Barry, and P. H. Dugard. Effects of freezing on human skin permeability. *J. Pharm. Pharmacol.* **36**:261–262 (1984).
13. R. G. van der Molen, F. Spies, J. M. van 't Noordende, E. Boelsma, A. M. Mommaas, and H. K. Koerten. Tape stripping of human stratum corneum yields cell layers that originate from various depths because of furrows in the skin. *Arch. Dermatol. Res.* **289**:514–518 (1997).
14. V. P. Shah, G. L. Flynn, A. Yacobi, H. I. Maibach, C. Bon, N. M. Fleischer, T. J. Franz, S. A. Kaplan, J. Kawamoto, L. J. Lesko, J.-P. Marty, L. K. Pershing, H. Schaefer, J. A. Sequeira, S. P. Shrivastava, J. Wilkin, and R. L. Williams. Bioequivalance of topical dermatological dosage forms—methods of evaluation of bioequivalance. *Pharm. Res.* **15**:167–171 (1998).
15. L. K. Pershing, J. Corlett, and C. Jorgensen. In vivo pharmacokinetics and pharmacodynamics of topical ketoconazole and miconazole in human stratum comeum. *Antimicrob. Agents Chemother.* **38**:90–95 (1994).
16. W. A. Ritschel, A. Sabouni, and A. S. Hussain. Percutaneous absorption of coumarin, griseofulvin and propanolol across human scalp and abdominal skin. *Meth. And Find. Exp. Clin. Pharmacol.* **11**:643–646 (1989).
17. R. Panchagnula, K. Stemmer, and W. A. Ritschel. Animal models for transdermal drug delivery. *Methods Find. Exp. Clin. Pharmacol.* **19**:335–341 (1997).
18. T. Kimura, N. Nagahara, K. Hirabayashi, Y. Kurosaki, and T. Nakayama. Enhanced percutaneous penetration of flufenamic acid using lipid disperse systems containing glycosylceramides. *Chem. Pharm. Bull.* **37**:454–457 (1989).
19. Y. Kurosaki, N. Nagahara, T. Tanizawa, H. Nishimura, T. Nakayama, and T. Kimura. Use of lipid disperse systems in transdermal drug delivery: Comparative study of flufenamic acid permeation among rat abdominal skin, silicon rubber membrane and stratum corneum sheet isolated from hamster cheek pouch. *Int. J. Pharmaceut.* **67**:1–9 (1991).
20. A. Alonso, N. C. Meirelles, V. E. Yushmanov, and M. Tabak. Water increases the fluidity of intercellular membranes of stratum corneum: correlation with water permeability, elastic and electrical resistance properties. *J. Invest. Dermatol.* **106**:1064–1069 (1996).
21. T. S. Wiedmann. Influence of hydration on epidermal tissue. *J. Pharm. Sci.* **77**:1037–1041 (1988).
22. C. S. King, S. P. Barton, S. Nicholls, and R. Marks. The change in properties of the stratum corneum as a function of depth. *Br. J. Dermatol.* **100**:165–172 (1979).
23. S. J. Chapman, A. Walsh, S. M. Jackson, and P. S. Friedmann. Lipids, proteins and corneocyte adhesion. *Arch. Dermatol. Res.* **283**:167–173 (1991).
24. G. Hauck. Hornschichtlipide: Methoden zu ihrer Bestimmung sowie ihr Einfluß auf die Penetration von Flufenaminsäure in das Stratum Corneum. Dissertation Saarbruecken 1994.
25. F. Bonte, A. Saunois, P. Pinguet, and A. Meybeck. Existence of a lipid gradient in the upper stratum corneum and its possible biological significance. *Arch. Dermatol. Res.* **289**:78–82 (1997).
26. Y. N. Kalia, F. Pirot, and R. H. Guy. Homogeneous transport in a heterogeneous membrane: water diffusion across human stratum corneum in vivo. *Biophys. J.* **71**:2692–2700 (1996).
27. A. Rougier, D. Dupuis, C. Lotte, R. Roguet, R. C. Wester, and H. I. Maibach. Regional variation in percutaneous absorption in man: Measurement by the stripping method. *Arch. Dermatol. Res.* **278**:465–469 (1986).
28. M. A. Lampe, A. L. Burlingame, J. A. Whitney, M. L. Williams, B. E. Brown, E. Roidman, and P. M. Elias. Human stratum corneum lipids: characterization and regional variation. *J. Lipid. Res.* **24**:120–130 (1983).
29. P. M. Elias, E. R. Cooper, A. Korc, and B. E. Brown. Percutaneous transport in relation to stratum corneum structure and lipid composition. *J. Invest. Dermatol.* **76**:297–301 (1981).
30. Y. Seta, A. H. Ghanem, W. I. Higuchi, S. Borsadia, C. R. Behl, and A. W. Malick. Physical model approach to understanding finite dose transport and uptake of hydrocortisone in hairless guinea-pig skin. *Int. J. Pharm.* **81**:89–99 (1992).