Reconstructed Epidermis and Full-Thickness Skin for Absorption Testing: Influence of the Vehicles used on Steroid Permeation

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Summary — A protocol for percutaneous absorption studies has been validated, based on the use of reconstructed human epidermis (RHE) and aqueous solutions of test substances. However, it is often the case that it is more-complex formulations of drugs or chemicals which will make contact with the skin surface. To investigate whether RHE and the reconstructed full-thickness skin model (FT-model) can be used to predict uptake from formulations, we compared the permeation of hydrocortisone and testosterone when applied in emulsion form and as a solution containing the penetration enhancer, ethanol. Human and pig skin and a non-cornified alveolar model served as references. The results were compared with steroid release from the formulations. The permeation rates of the steroids were ranked as: alveolar model >> RHE > FT-model, pig skin > human skin. In accordance with the rapid hydrocortisone release from the formulations, the permeation rates of this steroid exceeded those of testosterone. Only minor differences were observed when comparing the testosterone formulations, in terms of release and permeation. However, the ranking of the permeation of the hydrocortisone formulations was: solution > w/o emulsion > o/w emulsion, which permitted the elucidation of penetration enhancing effects, which is not possible with drug release studies. Differences in penetration were most obvious with native skin and reconstructed tissues, which exhibited a well-developed penetration barrier. In conclusion, RHE and skin preparations may be useful in the development of topical dermatics, and in the framework of hazard analysis of toxic compounds and their various formulations.

Key words: *alveolar tissue, formulation effects, human skin models, reconstructed full-thickness skin, reconstructed human epidermis, skin absorption.*

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Introduction

Toxicological testing and hazard analysis are of great importance in the protection of human health and the environment. However, animal welfare is also a major concern. Therefore, validated alternatives to animal experiments are constantly being sought. For regulatory purposes, reconstructed human epidermis (RHE) has been introduced for studying adverse skin effects such as corrosion (1–5) and irritation (6–9). Recently, genotoxicity (10–12) and skin sensitisation (13–15) have also become major considerations in *in vitro* test development.

When procedures have been validated, test guidelines (TGs) are adopted by the Organisation for Economic Cooperation and Development (OECD). For hazard analysis by percutaneous absorption, the OECD Member Countries accepted TG 428 (16), which is accompanied by Guidance Document (GD) 28 (17), for testing cutaneous uptake *in vitro* by using human and animal skin. This is of major importance, since, after the gut and the lung, the skin is the third biggest absorption organ, and is particularly relevant for pesticide intoxication (18, 19). According to GD 28, RHE can also be used, given that comparable results are obtained with RHE and the established skin preparations. To overcome the shortage of viable human skin for toxicity testing, a validation study was performed to establish a test procedure for percutaneous absorption with RHE. These experiments were exclusively based on aqueous solutions (20–22).

To further test the experimental protocol, the influence on substance uptake of semi-solid vehicles and penetration enhancers was investigated. For both hazard prediction in toxicology, and for the estimation of the availability of the active agent in drug development, vehicle effects need to be reflected correctly by the test membrane used. However, this issue has been studied only rarely, with conventional application methods (23–25) and with particulate carrier systems (26). Drug release studies performed on a routine basis with artificial

membranes can reveal the effects of vehicles, but they may be less predictive of the effects of product formulations containing penetration enhancers (25, 27). As a result, they may fail to elucidate the intrinsic penetration potential of the drug (27).

For a more-detailed insight into the influence of the barrier function of the tissue, we compared permeation through commercially-available RHEs, the FT-model and an alveolar model, taking drug release into consideration. While the non-cornified alveolar tissue should be more permeable than RHE, the FT-model might be less permeable (28). This hypothesis was tested by using a representative RHE (21), as well as a commercially-available FT-model and an alveolar model — the latter being of particular interest in relation to anti-asthmatic drugs. Moreover, FT-models appear to be the most potentially interesting models for use in genotoxicity studies, since they are constructed from two differentiated cell types which are well known to differ in drug metabolism capacity (26, 29). The impact of skin metabolism on the effects of drugs and chemicals has only recently begun to be understood in detail (24, 30, 31).

Materials and Methods

Chemicals

Hydrocortisone (logP = 1.43; MW = 362; CAS No. 50-23-7) and testosterone (logP = 3.32; MW = 288; CAS No. 58-22-0) were selected as model drugs, because of their relatively similar structures, but different (medium and high, respectively) lipophilicities. These substances, as well as ethanol (CAS No. 64-17-5), miglyol 812 and the ingredients of the receptor fluid (5% [w/v] bovine serum albumin in phosphate buffered saline, pH 7.4; PBS/ BSA), were obtained from Sigma (St. Louis, MO, USA). ^{3}H-hydrocortisone (66.0Ci/mmol) and 2,4,6,7-^{3}H-testosterone (100Ci/mmol) were purchased from Amersham (Freiburg, Germany), and purity was checked by thin-layer chromatography before use. Stock solutions (at 100mg/ml) in ethanol, were stable at 4°C for at least 4 weeks. The scintillation cocktail (Optiphase Supermix) was purchased from Wallac (Turku, Finland). Biopsy punches (with diameters of 4mm and 8mm) were obtained from Stiefel (Offenbach, Germany). Emulsifiers (Tween-80, Span-20) were obtained from Caelo (Hilden, Germany).

Test formulations

Three formulations, each containing 0.1% (w/v) testosterone or hydrocortisone (1μCi/500μl), were prepared. For the ethanol/miglyol (E/M) solution, 50mg of the drug were initially dissolved in 5ml of ethanol. Before use, a 500μl aliquot of this solution, spiked with 10μCi of the radiolabelled drug, was diluted to 5ml with miglyol and mixed thoroughly for 10–15 seconds. The resulting drug concentration in the E/M solution was 0.1% (w/v). For the preparation of a semi-solid o/w emulsion (which was stable for approximately 48 hours), 4mg of testosterone or hydrocortisone were dissolved in 2ml miglyol, with vortexing for 15 minutes. Following the addition of 0.1g Tween-80 (approximately equivalent to 2.5% [v/v]) and 8μCi of the labelled steroid, an o/w emulsion was formed by mixing with 2ml of distilled water, with the aid of a rotor-stator mixer. Replacing the Tween-80 in part by Span-20 (0.08g) as the emulsifying agent, resulted in a w/o emulsion which was stable for approximately 5 days.

Reconstructed skin models and skin

RHE (SkinEthic® RHE/L/17: Reconstituted Human Epidermis, large; age: day 17; 4.00cm^{2}) was obtained from Laboratoire SkinEthic (Nice, France); EpiAirway™ (Air-606; alveolar model) and EpiDermFT™ (EFT-306; FT-model) were purchased from MatTek Corp. (Ashland, MA, USA). The tissues were incubated overnight at 37°C, in the growth medium recommended by the manufacturers, with 5% (v/v) CO_2 and saturated humidity.

Human skin (abdomen or breast) was obtained (with permission) from females aged 20–62 years, who were undergoing cosmetic surgery. Pig skin (Deutsche Landrasse breed) was obtained from a local abattoir, after specific instruction that the cadaver should not undergo soaking in boiling water. The skin was wrapped in ice-cold cloth and transferred to the laboratory immediately, thus avoiding contamination of the surface by subcutaneous lipids. In the laboratory, the skin, trimmed of subcutaneous fat and connective tissue, was subjected to cryopreservation at –25°C for at least 24 hours, and 6 months maximum. Immediately before use in an experiment, the skin was thawed and full thickness skin was prepared from human skin (1000 ± 100μm) as well as from the pig skin, by using a Dermatome™ (Aesculap, Tuttlingen, Germany; 21).

The test protocol

The protocol followed was according to OECD TG 28 (17) and the rules established in the validation study (21, 22), and had previously been used for a primary evaluation of drug metabolism and formulation effects (24). Briefly, following a visual check of tissue integrity, skin (rehydrated in PBS for 30 minutes) and reconstructed tissues were mounted into Franz cells (15mm diameter; 12cm^{3} receiving compartment volume; PermeGear, Bethlehem, PA,

USA), with the horny layer of the skin, RHE and the FT-model facing the air. The supporting membrane or dermis was kept in contact with the receptor fluid, which was maintained at a constant temperature of 37 ± 1°C and stirred with a magnetic bar at 500rpm. The integrity of the skin samples was monitored throughout the experiment and any air bubbles were removed. After equilibration of the Franz cell for 30 minutes, 500µl of the donor preparation (1µCi labelled steroid) were applied to the skin surface and left in place for the entire experiment. The opening of the Franz cell was covered by Parafilm® for the duration of the experiment. The concentrations of the test compounds in the donor solutions were chosen to ensure that the concentrations in the receptor fluid during the experiment did not exceed of 10% of the saturation solubility. With the most permeable alveolar model, the maximum concentration of testosterone in the BSA-containing receptor medium in the study was quantified as 4.6µg/ml, which represents 14.7% of the saturation solubility of testosterone (31.31µg/ml at pH 7.4 in PBS; 21). In the case of the other biological matrices (Table 1), maximum testosterone concentrations were clearly lower. Considering the solubility-enhancing effect of the addition of BSA by virtue of the binding of the substance to the protein, the measured concentration of the test substance should have been significantly below the permitted limit.

Drug release

Testosterone and hydrocortisone release data were derived from permeation measurements through polycarbonate membrane (pore size 0.4µm; Nunc, Wiesbaden, Germany) and cellulose nitrate mem-

Table 1: Summary of permeation data

Formulation		Human skin	Pig skin	RHE	FT-model	Alveolar model
Hydrocortisone						
E/M	Lag time	1.63 ± 0.15	2.77 ± 0.95	3.44 ± 0.67	1.53 ± 0.52	0
	$P_{app} \times 10^{-8}$	15.89 ± 2.20	35.85 ± 4.06	42.58 ± 1.47	34.07 ± 9.17	256.64 ± 48.36
	Amount	2.51 ± 0.23	6.19 ± 0.17	6.61 ± 2.20	5.69 ± 1.65	19.23 ± 1.16
o/w	Lag time	2.79 ± 1.57	3.06 ± 0.52	3.93 ± 0.28	2.21 ± 1.60	0
	$P_{app} \times 10^{-8}$	5.37 ± 0.83	18.67 ± 2.64	21.32 ± 4.12	17.53 ± 11.50	224.94 ± 59.26
	Amount	0.84 ± 0.06	3.11 ± 0.20	3.27 ± 0.61	2.70 ± 0.77	18.45 ± 2.00
w/o	Lag time	1.71 ± 0.96	3.04 ± 0.73	3.46 ± 0.10	3.55 ± 0.13	0
	$P_{app} \times 10^{-8}$	8.59 ± 3.27	28.30 ± 4.75	34.30 ± 11.51	25.82 ± 13.86	234.92 ± 44.64
	Amount	1.42 ± 0.14	4.80 ± 0.67	5.41 ± 1.49	4.19 ± 1.65	19.17 ± 3.70
Testosterone						
E/M	Lag time	0	0.05 ± 0.47	0.04 ± 0.03	0.13 ± 1.60	0
	$P_{app} \times 10^{-8}$	4.68 ± 0.82	8.92 ± 3.60	25.93 ± 0.84	11.24 ± 2.48	83.19 ± 32.79
	Amount	0.82 ± 0.08	1.53 ± 0.40	4.43 ± 0.16	2.17 ± 0.42	9.32 ± 1.86
o/w	Lag time	0.42 ± 0.34	0.48 ± 0.99	0.05 ± 0.04	0.05 ± 0.02	0
	$P_{app} \times 10^{-8}$	9.21 ± 1.01	8.56 ± 1.54	29.27 ± 2.83	17.67 ± 4.06	84.70 ± 36.52
	Amount	1.26 ± 0.01	1.69 ± 0.34	5.15 ± 0.47	2.87 ± 0.30	10.61 ± 3.10
w/o	Lag time	0.48 ± 0.25	0.13 ± 1.19	0.03 ± 0.17	0.44 ± 1.27	0
	$P_{app} \times 10^{-8}$	8.19 ± 1.06	8.36 ± 2.63	30.96 ± 3.38	14.31 ± 1.01	75.58 ± 42.22
	Amount	1.02 ± 0.15	1.72 ± 0.39	5.50 ± 0.54	2.48 ± 0.42	9.40 ± 4.70

P_{app} *values (×10⁻⁸; cm/s), lag time (h), and drug amount permeated into the acceptor medium (%) after a 26-hour incubation, for 0.1% (w/v) hydrocortisone and testosterone preparations. E/M = ethanol/miglyol solution; o/w = oil-in-water emulsion; w/o = water-in-oil emulsion. Test substances were applied to human skin, pig skin, RHE, an FT-model, and an alveolar model.*

brane (pore size 0.1μm; Schleicher & Schuell, MicroScience, Dassel, Germany) (27, 32, 33). The procedure was perfomed in accordance with the Franz cell protocol, as described above.

Drug quantification

Uptake was determined by radiochemical measurement of the hydrocortisone and testosterone amounts in the receptor fluid, which was sampled hourly for 6 hours, then at 8, 10, 12, then 22, 24, and 26 hours; the volume removed (400μl) was replaced with fresh receptor fluid. The samples were subjected to scintillation counting (Microbeta Plus; Wallac, Turku, Finland), as described in Schreiber *et al.* (22). To overcome the quenching effect of BSA, calibration curves were obtained by dissolving the radiolabelled drug in PBS/BSA. The limit of detection was 0.1μg/ml, and linearity ranged from 0.5 to 500μg/ml for hydrocortisone and from 0.2 to 5000μg/ml for testosterone.

Data analysis procedure/biostatistical methods

Human skin and pig skin were each obtained from three donors, and the reconstructed tissues were from three batches. With each skin/skin model, two parallel experiments were performed with each drug. Therefore, six tests in total were performed for each skin/skin model. The data are presented as arithmetic mean values ± SD. Permeation is expressed as the drug amount in the receptor medium after a 6-hour incubation, normalised for the area of the exposed skin surface (in $\mu g/cm^2$), and also as the apparent permeability coefficient P_{app} (= $[V/A*C_i]*dC_A/dt$), which takes the exposed surface area A (1.768cm^2; 21) into account within the calculation. Volume V was 12cm^3 in all the experiments; C_i gives the initial concentration of the applied substance in $\mu g/cm^3$; dC_A/dt represents the increasing concentration of the substances in the receptor medium with increasing time. The P_{app} values and lag times (intersection of the linear part of the regression line with the x-axis) are calculated for each donor or batch, by using the spread sheets and an algorithm developed previously (34).

For drug release experiments, mean cumulative steroid amounts in the receptor medium were plotted *versus* the square root of time (Equation 1; 35, 36):

$$\mathbf{Q_t = K*t^{(1/2)}} \qquad \text{(Equation 1)}$$

Where: Q_t = the cumulative drug amount recovered in the receiving compartment (μg); K = the kinetic constant indicative of the release rate ($\mu g/h^{(1/2)}$); and $t^{(1/2)}$ = the square root of time ($h^{(1/2)}$).

The kinetic constant K (slope of the plot) and the release lag-time (x-axis intercept = lag-$time_{rel}$) were calculated by linear regression.

Results

According to the results of a recent validation study based on a comparison of the permeabilities of substances applied in aqueous solutions, through three commercially-available RHE models, it is evident that these models may be regarded suitable for *in vitro* testing (21, 37–39). To evaluate formulation effects in the present study, we compared the permeation of hydrocortisone and testosterone through reconstructed tissues and skin, when applying two semi-solid formulations (o/w and w/o emulsions) and a solution (E/M; Figure 1). In addition to a representative RHE, the study included human skin, pig skin (21), a reconstructed full thickness skin model, and an alveolar model. Drug release from the preparations was also determined and taken into consideration.

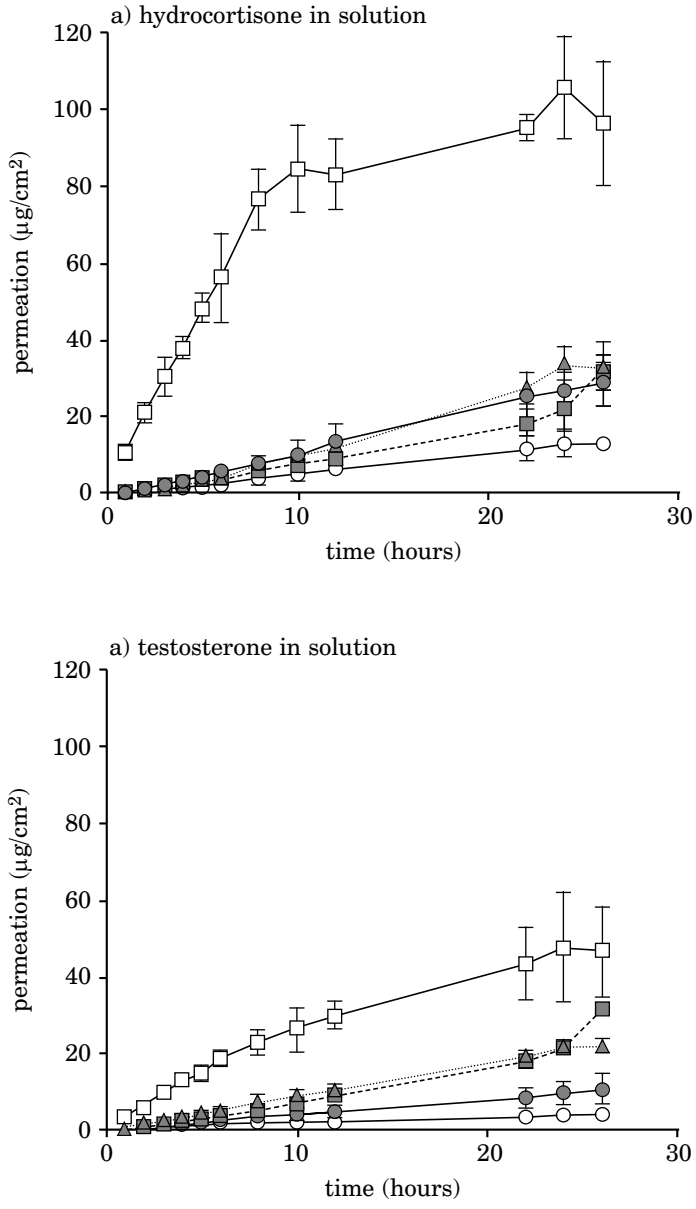
Influences of test matrices

With the exception of the alveolar model, it was evident that less than 10% of the applied steroids permeated through the sample and into the receptor fluid (Table 1). With the alveolar model, however, the total testosterone and hydrocortisone amounts which permeated into the receptor fluid were approximately 10% and almost 20% of the applied doses, respectively. Thus, sink conditions were not achieved with the non-cornified tissue, which is also reflected by the decreasing permeation rates (Figure 1). Correspondingly, the algorithm for the P_{app} value calculation (34) from the linear increase in drug levels in the receptor medium accepted only the values obtained for 6 hours, when studying the alveolar model. With the RHE and FT-model, however, drug levels in the receptor medium for 8–12 hours were accepted for the P_{app} value calculation and the linear increase of drug levels with pig skin even permitted data obtained for 22 hours to be used for the calculation.

It was apparent from the P_{app} values and total amounts (% of dose) permeated (Table 1) that, in general, hydrocortisone permeation exceeded testosterone permeation, irrespective of the formulation applied. The permeation rates of hydrocortisone and testosterone (P_{app} values) through human skin appeared to be less than those through pig skin (both 1000 ± 100μm; Table 1). The P_{app} values calculated for the FT-model and for pig skin were similar for each of the hydrocortisone formulations, whilst testosterone permeation through the FT-model was more rapid. As was to be expected, both steroids permeated through the RHE faster than

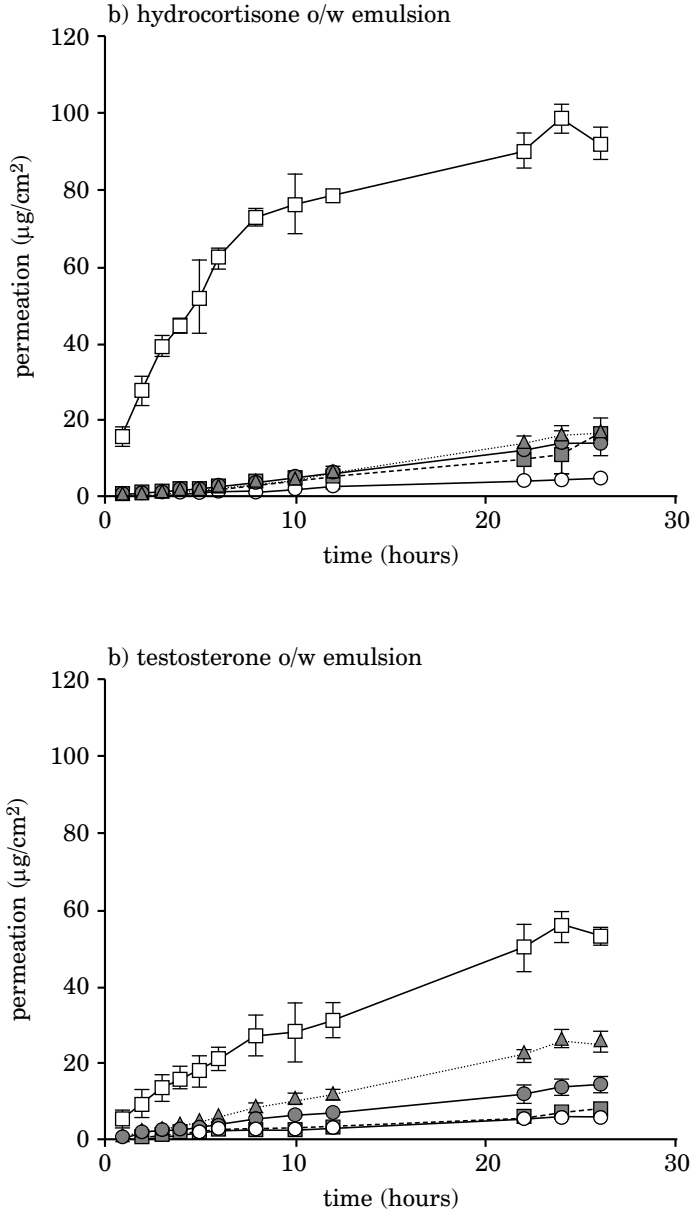
through native skin (about 4-fold faster in the case of human skin) or the FT-model. Permeation through the alveolar model was much more rapid than through the other preparations tested. In fact, P_{app} values calculated for the alveolar model exceeded those of RHE by 6-fold to 10-fold for hydrocortisone and by about 3-fold for testosterone. The respective ratios for the permeation through the alveolar model, with respect to the FT-model were 8-fold to 13-fold for hydrocortisone and 5-fold to 8-fold for testosterone, while the permeability of the alveolar model as compared to human skin, was even more enhanced. The non-cornified tissue exhibited a more-pronounced overestimation of cornified tissue permeability in the case of hydrocortisone as compared to testosterone. That the overestimation was lower in the case of testosterone reflects the lipophilic properties of the barriers

Figure 1: Permeation of hydrocortisone and testosterone, both in ethanol/miglyol solution and in emulsion form, through human skin, pig skin, RHE, an FT-model, and an alveolar model



○ = *human skin;* ■ = *pig skin;* ▲ = *RHE;* ● = *FT-model;* □ = *alveolar model.*

Figure 1: continued

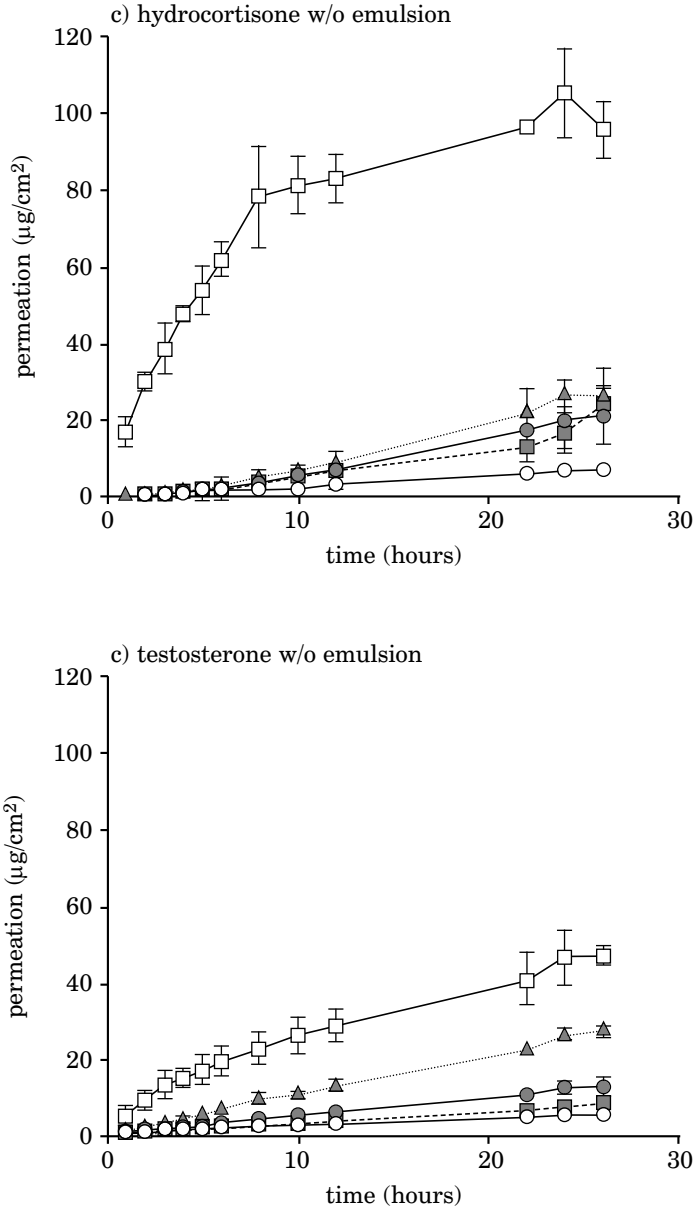


○ = *human skin;* ■ = *pig skin;* ▲ = *RHE;* ● = *FT-model;* □ = *alveolar model. o/w = oil-in-water.*

formed by native skin, RHE, and the FT-model. Nevertheless, as depicted in Figure 2, there is a close correlation between the permeation coefficients of the various test matrices and those of human skin. This holds true even for the alveolar model. Taken together, the permeation of the test preparations was according to the rank order predicted from tissue morphology and the related barrier properties.

In view of the number of relevant differences in the experimental protocol (i.e. supplementation of the receptor fluid with BSA, testosterone concentration, and vehicle variations), any comparison with the previous data must be conducted with great caution. When BSA 5% (w/v) was added to the receptor fluid to improve steroid solubility, the permeation of testosterone increased by about two-fold (22). When the 0.1% (w/v) testosterone ethanol solution and the corresponding o/w and w/o emulsions were applied to pig skin, the P_{app} values (Table 1) were within the range of those obtained when applying an 0.004% (w/v) aqueous solution (non-BSA supplemented

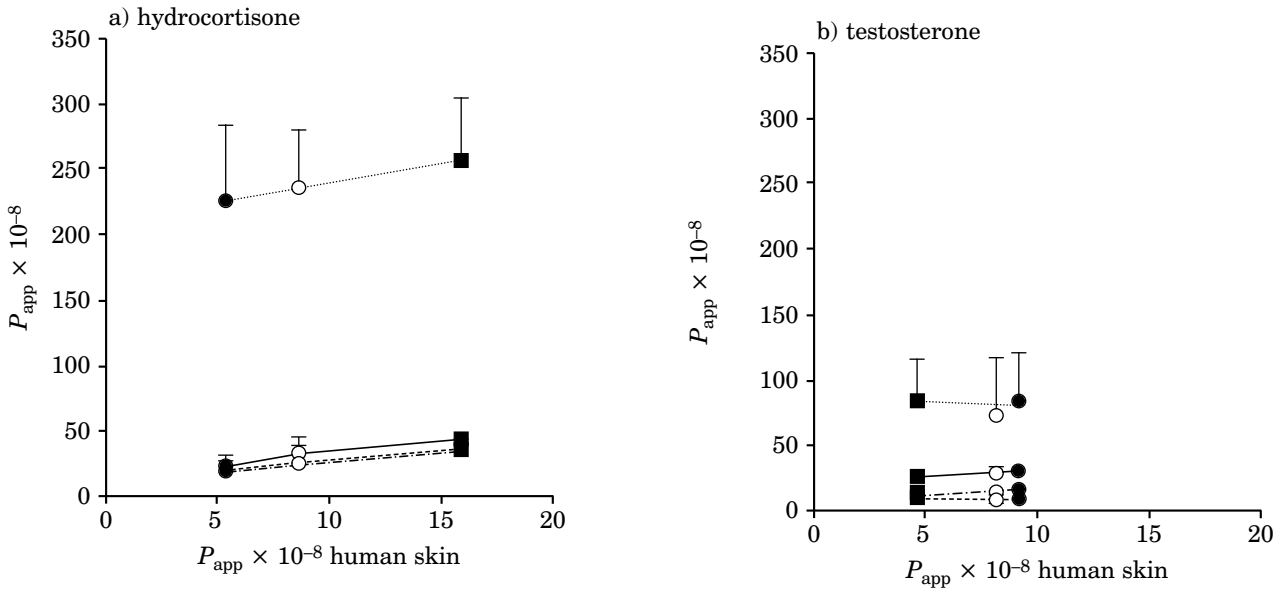
Figure 1: continued



○ = *human skin;* ■ = *pig skin;* ▲ = *RHE;* ● = *FT-model;* □ = *alveolar model. w/o = water-in-oil.*

receptor fluid; P_{app} = 8 × 10^{-8}cm/s; 21); a somewhat higher permeation of 51 × 10^{-8}cm/s was described in another study (40). In contrast to the current results (Table 1), a previous study showed that the permeation of testosterone through human epidermis sheets exceeded its permeation through pig skin (21). A direct comparison of testosterone permeation through epidermis sheets and dermatomed skin (as used in this study) from identical human donors, did not indicate any differences in permeation (22) — a finding that was also reported previously by Cross *et al.* and Diembeck *et al.* (41, 42). RHE samples used here, however, were less permeable, as observed previously with batches from the same supplier (P_{app} = 600 × 10^{-8}cm/s; 21). While no significant lag-time was apparent for testosterone permeation, lag-times of 1.63 to 3.44 hours were apparent for the permeation of hydrocortisone, except in the case of the alveolar model. In fact, variable delay in testosterone permeation has been described (39, 21), which can be due to the respective donor animal and the experimental protocol.

Figure 2: Comparison of the permeation of hydrocortisone and testosterone, both in solution and in emulsion form, through reconstructed matrices, pig skin and human skin



The permeation (P_{app} [cm/s]; mean ± SD) of a) hydrocortisone and b) testosterone through reconstructed skin (——— = RHE; -·-·-·-·- = FT-model; ····· = alveolar model) and pig skin (----------), is shown in relation to their permeation through human skin. The steroids were applied as oil-in-water (o/w; ●) and water-in-oil (w/o; ○) emulsions, or dissolved in ethanol/miglyol (■). Experiments were performed with the skin of two donors and with two batches of each reconstructed tissue, each in triplicate.

Biological *versus* artificial membranes

Steroid permeation through the polycarbonate membrane was more rapid than that through the cellulose nitrate membrane (Table 2). This is in accordance with the pore sizes, 0.4μm and 0.1μm, respectively. Uptake was very rapid with both membranes , and no significant lag-times were observed. This is in contrast to skin, RHE and the FT-model, where significant lag-times were obtained when testing all the hydrocortisone preparations (Table 1), and when applying testosterone solutions (21, 39). Moreover, the more-rapid steroid permeation through both types of artificial membrane (K), which was ascertained in order to quantify drug release for comparison with the permeability (P_{app} values) of the cornified biological matrices, highlights the functional biological barriers inherent in the RHE and in the FT-model. Interestingly, the SkinEthic RHE is constructed by using the highly-permeable polycarbonate membrane as a support.

Formulation effects

Subsequently, whether the higher permeability of hydrocortisone as compared to testosterone (Table 1) is linked to drug release, was investigated. In fact, there was a more-rapid hydrocortisone release from all the preparations as compared to testosterone release, which favours hydrocortisone uptake by the skin (Table 2). Hydrocortisone release was ranked as follows: w/o emulsion > o/w emulsion > E/M solution, when using the cellulose nitrate membrane, while differences between the emulsions were less obvious when using the polycarbonate membrane for the drug release studies. However, a clearly different formulation-dependency of hydrocortisone permeation was observed for all of the biological membranes (including the alveolar model), where the permeation was ranked as follows: E/M solution > w/o emulsion > o/w emulsion. It is possible that the well-known penetration-enhancing effect of ethanol (for reviews, see 43, 44) caused the increase in P_{app} values for the hydrocortisone solution. In addition, this rather hydrophilic steroid partitions more favourably into the *stratum corneum* from an external lipid phase as compared to an external water phase, which does not hold true with the more lipophilic testosterone. Testosterone release data were similar for each of the formulations (Table 2), and the P_{app} values for testosterone permeation for all the formulations were not different either (Table 1). Thus, it was

Table 2: Hydrocortisone and testosterone release data

Membrane	Formulation	K (μg/h$^{(1/2)}$)	Lag–time$_{rel}$ (h$^{(1/2)}$)	R^2
Hydrocortisone				
Cellulose nitrate	Solution	27.39 ± 0.92	–1.46 ± 0.29	0.9702
	o/w emulsion	31.71 ± 0.04	–2.36 ± 0.05	0.9182
	w/o emulsion	37.91 ± 2.99	–1.71 ± 0.66	0.9307
Polycarbonate	Solution	112.50 ± 10.10	0.44 ± 0.08	0.9861
	o/w emulsion	97.16 ± 21.80	–0.38 ± 0.51	0.9592
	w/o emulsion	103.24 ± 14.18	–0.57 ± 0.14	0.9811
Testosterone				
Cellulose nitrate	Solution	4.76 ± 1.53	–0.49 ± 0.13	0.9941
	o/w emulsion	5.52 ± 0.40	–1.14 ± 0.31	0.9869
	w/o emulsion	8.13 ± 0.18	–1.61 ± 0.06	0.9907
Polycarbonate	Solution	29.54 ± 4.05	0.45 ± 0.08	0.9921
	o/w emulsion	29.03 ± 10.57	–0.11 ± 0.32	0.9849
	w/o emulsion	14.51 ± 2.21	–0.21 ± 0.44	0.9730

Lag-time$_{rel}$ and slope (K) are given as mean values ± SD; n = 2. R^2 values are calculated from the average profiles. o/w = oil-in-water; w/o = water-in-oil.

concluded that ethanol did not enhance testosterone penetration to a significant extent.

Discussion

During the last decade, major progress has been made in the replacement of animal experiments by approved *in vitro* approaches; more *in vitro* approaches are on the horizon. This is most welcome progress, not least because of the EU REACH initiative, which requires detailed toxicological testing of many existing chemicals, for which documented toxicological profiles are not available, but also because of the ban on animal experiments by the 7th Amendment to the EU Cosmetics Directive, starting in 2009. Following successive improvements in the morphology and the lipid profile (21, 37, 38), RHE is particularly attractive for skin corrosivity and irritancy testing, because of its close resemblance to human epidermis (1–9). *In vitro* testing for skin sensitisation and genotoxicity, which is also under development, requires information on skin uptake and on local biotransformation — both of which are essential for the assessment of these hazards. Ideally, all the data should be generated with similar tissue preparations, which meet defined structural and performance criteria. An *in vitro* protocol for skin uptake testing has been developed and validated, which allows the quantification of the amount of a chemical penetrating (e.g. for sensitisation and genotoxicity testing) and permeating (for systemic toxicity testing) the skin (21, 22), but this protocol describes the use of solutions solely in aqueous form.

The current experiments extend the database, both with respect to biological preparations and applied formulations, and followed the discriminative infinite dose approach. In fact, we observed the anticipated differences in the permeability of the preparations, the alveolar model being the most permeable (Figure 1). The high permeability of the alveolar model is explained by its lack of cornification. However, we cannot exclude that mechanical stress due to the Franz cell experimental set-up (22), may further increase the apparent permeability. With both the drugs and all the formulations tested, the permeability of the reconstructed tissues and the pig skin was closely correlated with permeation through human skin. The rank order was the same in all cases, and it was only the absolute permeability which differed (Table 1). Interestingly, even a penetration enhancer effect was correctly identified with the reconstructed tissues, while release experiments failed to elucidate any effects of ethanol on the absorption of hydrocortisone (Table 2). Given that this will potentially prove to be true in future experiments, this *in vitro* approach is not only suitable for risk analysis, but also for use in drug development, thus reducing the need for animal experimentation and likely to decrease the risks to patients receiving a new drug or new formulation. Recent *in vitro* experiments based on RHE did not only identify formulation effects, but

also highlighted the influence of skin biotransformation on the skin penetration and permeation of a glucocorticoid diester (24). Due to pronounced differences in the metabolic capacities of the keratinocytes, which form the epidermis, and the fibroblasts, which dominate within the dermis (29, 45), the potential value of the FT-model also deserves further investigation.

Conclusions

The current study, in which formulation effects on percutaneous absorption were investigated, supports the results of a formal validation study which demonstrated that RHE should be suitable for use for hazard assessment in regulatory toxicology. Moreover, the RHE and full-thickness skin models may be suitable for the development of topical dermatics and to study genotoxicity and skin sensitisation caused, for example, by cosmetic formulations.

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