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The Isolated Perfused Bovine Udder as an in vitro Model of Percutaneous Drug Absorption
Skin Viability and Percutaneous Absorption of Dexamethasone, Benzoyl Peroxide, and Etofenamate

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Using udders from slaughtered cows as a new in vitro model of percutaneous drug absorption, the tissue viability and the percutaneous absorption of dexamethasone, benzoyl peroxide, and etofenamate were studied. The organ was perfused with gassed tyrode solution for up to 6 hr. As shown by measurement of glucose consumption, lactate production, lactate dehydrogenase activity, and pH in the perfusate, the tissue was viable over a 6-hr period. This was confirmed by a histological examination. Determination of the udder skin-fold thickness demonstrated that no edema developed within the perfusion period. A maximum skin penetration of dexamethasone was found after administration of dexamethasone dissolved in acetone with dimethyl sulfoxide, followed by ointment with salicylic acid, ointment without salicylic acid, and acetone solution. Experiments with benzoyl peroxide and etofenamate demonstrated that the perfused udder skin was capable of metabolizing drugs in vitro. In conclusion, the isolated perfused bovine udder is a new in vitro model, which maintains bovine udder skin with an isolated vasculature in a viable state. Using this in vitro model, we note it is possible to compare the dermal penetration, metabolism, and absorption of substances after topical administration of different drug formulations.

Keywords: Skin penetration models; in vitro dermatopharmacology; Percutaneous absorption; Isolated perfused bovine udder; Dexamethasone; Benzoyl peroxide; Etofenamate

Introduction

Percutaneous absorption studies are performed in various in vivo and in vitro models to determine the rate of drug absorption via the skin. Such models are used to study the pharamcokinetics of drugs for topical treatment of skin disorders and for transdermal therapeutic systems, for example, in the case of estrogens or nitroglycerin. In addition, various drug formulations containing nonsteroidal antiphlogistics are in clinical use for transdermal treatment of joint diseases. The transdermal administration of systemically acting drugs is suitable to minimize systemic side effects. Also in toxicological research, percutaneous penetration studies are necessary (Riviere et al., 1986).

In vivo studies in humans and in animals are restricted by ethical considerations and animal protection (Pershing and Krueger, 1987). Therefore, in vitro models of percutaneous absorption are a necessity. Established in vitro models with perfused skin preparations include isolated rabbit ear, isolated dog leg, and isolated pig ear or pig abdominal skin flaps (Riviere et al., 1986; Pershing and Krueger, 1987; Monteiro-Riviere et al., 1987; Carver et al., 1989). However, at present, there is only one in vitro model of percutaneous drug absorption that can be obtained without sacrificing laboratory animals, that is, the use of the isolated perfused ears from slaughtered pigs (de Lange et al., 1992).

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We were interested in establishing a new model, namely, the isolated perfused bovine udder, which may overcome some of the limitations of previously used in vitro models. The study presented here determines parameters of skin viability as well as the dermal absorption of dexamethasone, benzoyl peroxide, and etofenamate in this new model.

Materials and Methods

Test Substances

Dexamethasone was obtained from Sigma (Deisenhofen, F.R.G.). The dexamethasone ointment used in the study was purchased from Pharmasal (Anemul, Gräfelfing, F.R.G.). Benzoyl peroxide and DMSO were from Merck (Darmstadt, F.R.G.). A benzoyl peroxide-containing shampoo was from Chassot (Peroxyderm, Ravensburg, F.R.G.). Etofenamate, flufenamic acid, and the etofenamate-containing drug Rheumon Lotio were obtained from Troponwerke (Köln, F.R.G.).

Perfusate

As perfusion medium, a gassed (95% oxygen, 5% carbon dioxide) tyrode solution (8 g/L NaCl, 200 mg/L KCl, 265 mg/L $CaCl_2 \cdot 2H_2O$, 213 mg/L $MgCl_2 \cdot 6H_2O$, 1 g/L $NaHCO_3$, 65 mg/L NaH_2PO_4, 1.1 g/L glucose) containing 5 mg heparin per liter was used. The temperature of the perfusion fluid was 38.5°C. Since possible edematous effects of the tyrode solution could impede drug absorption, dextran (60 g/L) was added to the perfusion fluid in an additional series of absorption studies with dexamethasone. Furthermore, the possibility of edema formation was studied in separate experiments (see below).

In Vitro Model

Bovine udders were obtained immediately after the slaughter of healthy cows. The udder was dissected, and heparinized tyrode solution (about 500 mL) was infused immediately via the arteria pudendalis externa to counteract clot formation. The udder was then transported to the laboratory, and the two right mamma complexes were perfused with heparinized tyrode solution as rapidly as possible by a peristaltic pump (Masterflex 7518-10; Cole-Parmer Instr., Chicago, IL, U.S.A.) with a perfusion pressure of 80–100 mmHg. The isolated perfused bovine udder is supplied by a separate arterial influx via the cannulated right and/or left arteries (arteria pudendalis externa). The udder consists of four separate glands. The glands of the right and left side are supplied by the arteria pudendalis externa dexter and sinister. The venous drainage takes place via the vena epigastrica cranialis superficialis (Schummer et al., 1976). The left side of the udder was not used in the experiments. The period of perfusion started within 30 min after slaughtering. The organ was supplied by the cannulated arteria pudendalis externa with a venous drainage via the vena epigastrica cranialis superficialis. During the perfusion period, the perfusate flux was recorded. The perfusate flux was between 60 and 100 mL per minute. This perfusate flux, which is lower than the physiological blood supply of the udder in vivo, was chosen on the basis of earlier studies demonstrating a sufficient dermal blood supply by the infusion with 1% trypan blue containing perfusion fluid (Kietzmann, M., unpublished experiments). Figure 1 shows a schematic diagram of the isolated perfused bovine udder. At the end of the perfusion periods, trypan blue solution (1%) was infused via the arteria pudendalis externa to prove if dermal perfusion was sufficient. The present study includes only organ preparations in which the stained dermal blood vessels were visible.

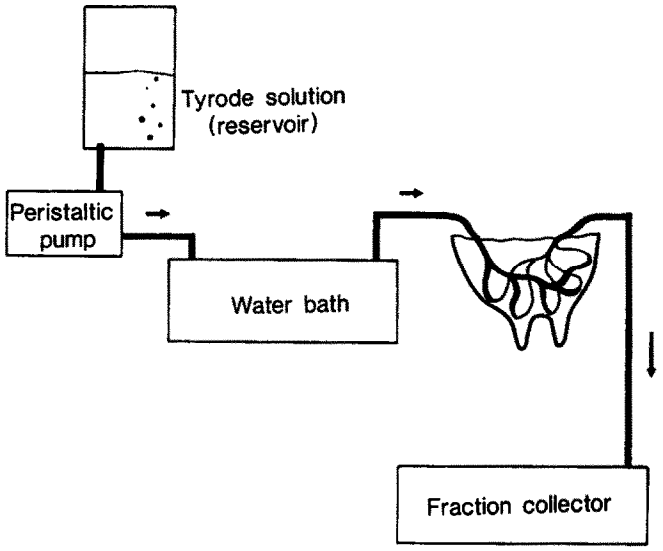

Figure 1. Schematic diagram of the isolated perfused bovine udder.

Edema Formation and Tissue Viability

Six udders were perfused with tyrode solution over a 6-hr period. To examine a possible edema formation, we registered the skin-fold thickness and the skin-surface temperature within this period. Furthermore, the udders were weighed before and after the perfusion period. The skin-fold thickness was measured using a cutimeter (Mitutoyo, Neuss, F.R.G.), and the skin surface temperature, by using a surface thermometer (Kleinfeld, Hannover, F.R.G.). Simultaneously, pH, lactate, and glucose concentration as well as lactate

dehydrogenase activity were determined in perfusate fractions collected every hour. For determination of the lactate and glucose concentration and the lactate dehydrogenase activity, we used commercial test kits (Boehringer, Mannheim, F.R.G.).

Dermal Absorption of Dexamethasone

At 15 min after starting the perfusion, dexamethasone formulations were administered topically on 100 cm^2 skin areas. The administered dose was 0.08 mg/cm^2 dexamethasone. The following drug formulations were used (n = the number of udder preparations per formulation):

1. dexamethasone solution in acetone, 0.8 mg/mL (n = 5);
2. dexamethasone solution in acetone, 0.8 mg/mL, with the addition of 10% DMSO (n = 6);
3. dexamethasone ointment, 0.8 mg/g (n = 6); and
4. dexamethasone ointment, 0.8 mg/g, with addition of 5 mg/g salicylic acid (n = 6).

For the preparations in acetone, the total volume (10 mL) was slowly administered on the skin within 5 min.

Dermal Metabolism and Absorption of Benzoyl Peroxide

At 15 minutes after starting the perfusion, benzoyl peroxide formulations were administered topically on skin areas of 100 cm^2. The administered dose was 5 mg/cm^2 benzoyl peroxide. The following drug formulations were used (n = the number of udder preparations):

1. benzoyl peroxide solution in acetone, 50 mg/mL (n = 6);
2. benzoyl peroxide solution in acetone, 50 mg/mL, with addition of 10% DMSO (n = 5); and
3. benzoyl peroxide shampoo, 25 mg/mL (n = 3).

Dermal Metabolism, and Absorption of Etofenamate

At 15 minutes after starting the perfusion, Rheumon Lotio was administered topically on skin areas of 100 cm^2 of six udders. The administered dose was 20 mg/cm^2 etofenamate.

Perfusate and Skin Samples for Studies of Drug Absorption

Perfusion fractions (50 mL) were collected 30 min after topical drug administration and then every 15 min up to 135 min. At the end of the perfusion period, skin punch biopsies (8 mm diameter) were sampled from the treated area after skin cleaning with a piece of wood-wool. To exclude contamination by the test substances remaining on the skin surface, we removed a superficial skin layer (20 μm) after freezing by use of a cryotome. The biopsies as well as the collected perfusate fractions were stored at −20°C.

Radioimmunoassay of Dexamethasone

The dexamethasone concentration was measured by a specific radioimmunoassay which used antiserum from Paesel (Frankfurt, F.R.G.). The glucocorticoid was extracted with ethyl acetate from both the perfusate and homogenized skin biopsies. The organic extract was evaporated to dryness at 37°C under nitrogen. Using this radioimmunological method, we achieved a detection limit of 62.5 pg dexamethasone/assay. The dexamethasone recovery from perfusate and skin was 87% and 81%, respectively.

Thin-Layer Chromatography of Benzoyl Peroxide and Benzoic Acid

Benzoyl peroxide and benzoic acid were extracted three times with ethyl ether from both perfusate aliquots and homogenized skin biopsies. The pooled organic extracts were evaporated to dryness at 37°C under nitrogen and reconstituted in 100 μL acetone. High-performance thin-layer chromatography (HPTLC) was performed on silica-gel HPTLC-plates (60 F-254, 10 × 10 cm, Merck, Darmstadt). Aliquots of 2.5 μL were applied to the plates and then developed with acetone/benzene (15:85). Quantitative determinations of benzoyl peroxide and benzoic acid were carried out with a Zeiss PMQ-3 TLC-scanner at a wavelength of 210 nm. Evaluation was carried out via peak areas. The linearity of the method ranged from 0.1 to 1.0 ng benzoyl peroxide and benzoic acid per microliter. A detection limit of 0.1 μg benzoyl peroxide and 0.02 μg benzoic acid per milliliter of perfusate was achieved (Kietzmann et al., 1989).

Thin-Layer Chromatography of Etofenamate and Flufenamic Acid

Skin samples were homogenized in a solution of sodium fluoride (0.4 mg/mL). By addition of sodium hydroxide, the pH of homogenates and perfusate fractions was adjusted at 12. Etofenamate was extracted three times with toluene from both perfusate aliquots and homogenized skin biopsies. After extraction of etofenamate, the pH of aqueous samples was acidified by addition of hydrochloric acid. Thereafter, flufenamic acid was extracted three times with toluene. The

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pooled organic extracts were evaporated to dryness at 37°C under nitrogen and reconstituted in 100 μL acetone. High-performance thin-layer chromatography was performed on silica-gel HPTLC-plates (60 F-254, 10 × 10 cm, Merck, Darmstadt). Aliquots of 5-μL were applied to the plates (cleaned with toluene) and then developed with cyclohexane/ethyl acetate/formic acid (80:40:5). Quantitative determinations of etofenamate and flufenamic acid were carried out with a Zeiss PMQ-3 TLC-scanner at a wavelength of 280 nm. Evaluation was carried out via peak areas. The linearity of the method ranged from 1 to 200 ng/μL etofenamate and flufenamic acid (Dell et al., 1990).

Statistics

A statistical analysis of the data of skin-fold thickness, skin temperature, perfusate pH, glucose and lactate concentration, and lactate dehydrogenase activity in the perfusate was carried out by analysis of variance for paired data and paired *t* test. The computer program SAS was used for this purpose.

The total amount of absorbed substances was calculated from the concentrations measured in the perfusate fractions. A statistical analysis of the data measured in the perfusate was performed by the Mann–Whitney test. The skin concentration which was measured after treatment with the various galenic formulations was compared using one-way analysis of variance followed by the Newman–Keuls test. These statistical calculations were performed with the computer program Pharm/PCS.

Histology

For histological examination, udder skin specimens fixed in buffered formaldehyde solution (4%) were embedded in paraffin. Preparations, (6–7 μm thick, sectioned exactly in the vertical direction) were stained with hemalaun-eosin.

Results

Model Characteristics

A photomicrograph of the bovine udder skin (Figure 2) shows histological similarities to human skin with respect to epidermal thickness, surface contours of dermal papillae, and folds. The in vitro model described in the present study maintains the bovine udder skin with an isolated vasculature perfused with a rate maintained between 60 and 100 mL/min. The perfusion pressure was between 80 and 100 mmHg. The physiologically intact organ perfused with oxygen-containing tyrode solution is maintained in a viable state. Thus, there were no significant changes of the pH, glucose concentration, or lactate dehydrogenase activity within a perfusion period of 6 hr (Figure 3). The lactate production decreased from an initial 1400 to 1000 mg/hr after 2 hr, but only slightly decreased thereafter.

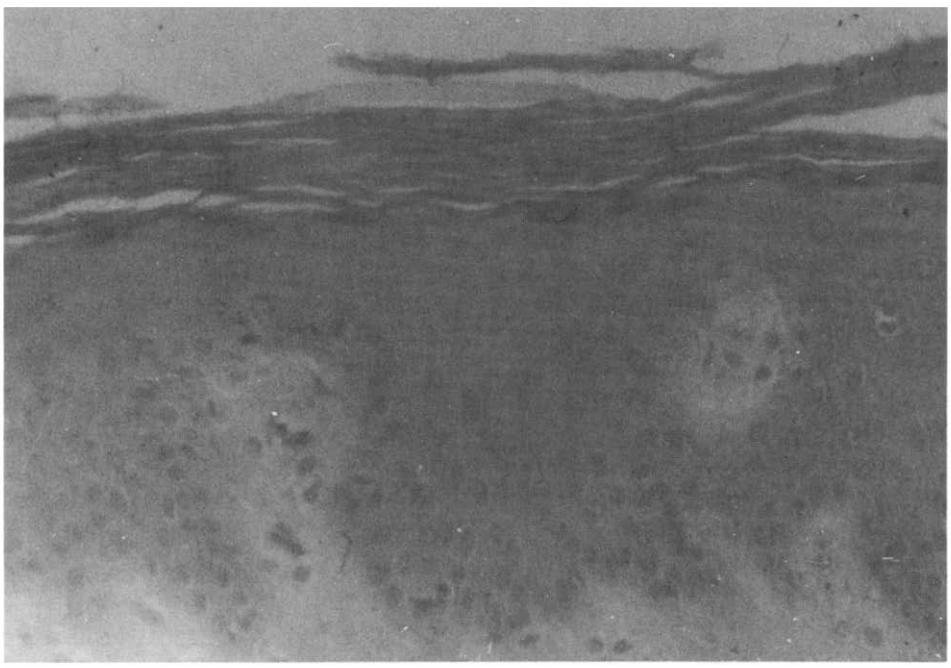

Figure 2. Photomicrograph of bovine udder skin. (HE stain, magnification, 60×).

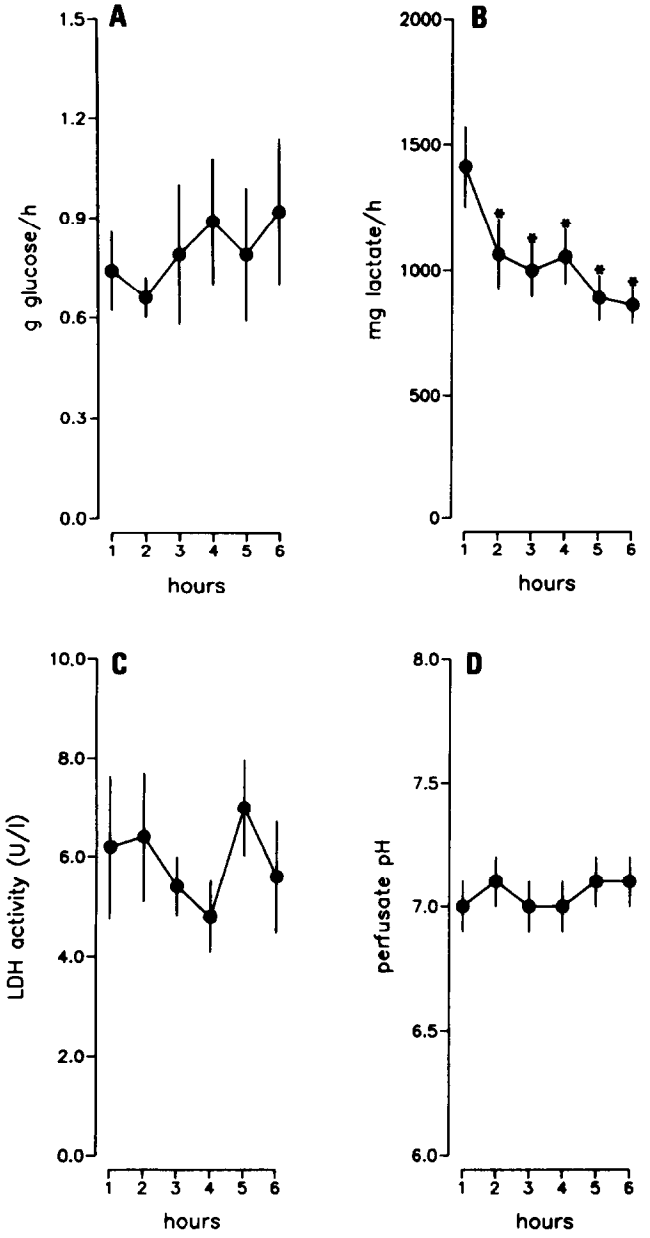

Figure 3. Glucose consumption (A), lactate production (B), lactate dehydrogenase activity (C), and perfusate pH (D) in the isolated perfused bovine udder. Perfusion over a period of 6 hr. Data are given as mean ± S.E. of six separate experiments. * = $p < 0.05$ (analysis of variance and t test) compared to the initial value. No significant differences were found between the values shown at 2–6 hr.

The initial high value of lactate may be caused by the anaerobic conditions during the transport of the udder without perfusion. Based on the not significantly changed perfusate glucose concentrations, the glucose consumption of the udder was not altered during perfusion. In addition, no degenerative histological changes were found by light microscopy.

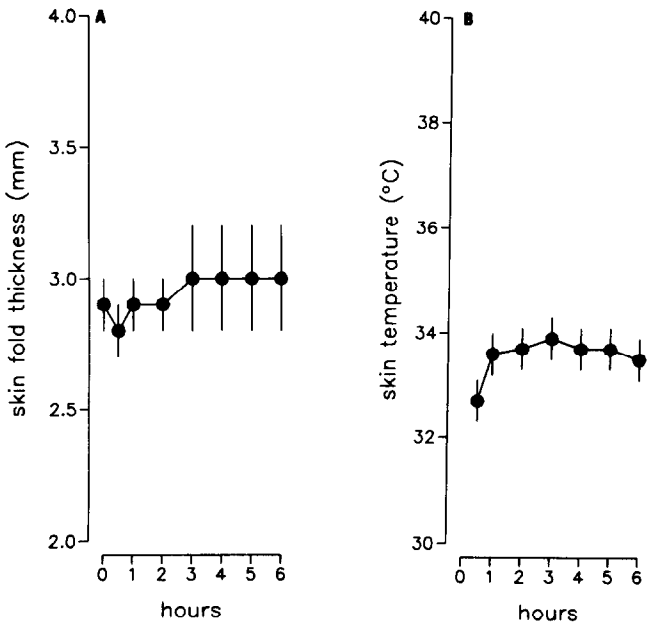

Figure 4. Skin-fold thickness (A) and skin-surface temperature (B) in the isolated perfused bovine udder. Perfusion over a period of 6 hr. Data are given as mean ± S.E. of six separate experiments. No significant differences were found (analysis of variance).

The development of dermal edema is a problem in studies using isolated perfused organs. Determining the degree of edema formation by weighing the total organ before and after the perfusion period is unsuitable due to the filling of the vasculature with the onset of perfusion. Moreover, a continuous artificial "milk" production was observed which would influence measurements of the organ weight. Thus, the relatively small (14%) difference in udder weight found between weight measurements before and after a 6-hr period of perfusion (12.5 ± 1.9 kg before and 14.3 ± 1.9 kg after perfusion; mean ± S.E. of six udders) would not necessarily exclude marked edema formation. Therefore, the skin-fold thickness was used as a more reliable parameter of edema formation instead of the organ weight. As shown in Figure 4, no significant changes in skin-fold thickness were observed during a 6-hr perfusion period. The skin temperature increased (nonsignificantly) initially to 33°C, but showed no further changes. Histologically, no edema development in the udder skin was found.

Dexamethasone Absorption

Within 135 min after topical administration of the dexamethasone solution (total dose 8 mg), average values of 334 ng dexamethasone were found in the perfusate. When 10% DMSO was included in the acetone

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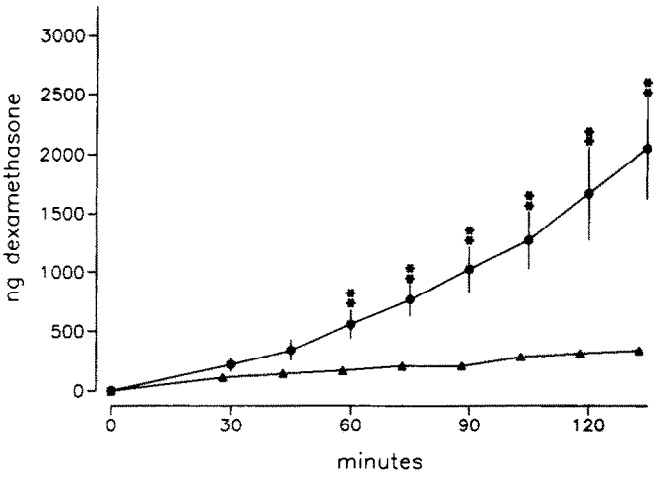

Figure 5. Absorption rate–time curve (cumulative) of dexamethasone in the isolated perfused bovine udder. The udder skin was perfused with tyrode solution. Dexamethasone was administered as a solution in acetone (triangles) and in acetone with addition of 10% DMSO (circles) on a treatment area of 100 cm^2. The administered dose was 8 mg/100 cm^2. Measurement of the dexamethasone concentration in the perfusate was carried out by radioimmunoassay. Concentrations in the perfusate are given as mean ± S.E. of five (acetone) and six (acetone with DMSO) separate experiments. In case of absence of S.E. bars, S.E. was smaller than the symbol used. As summarized in Table 2, a significantly increased dexamethasone absorption was found after treatment with the solution with DMSO; ** = $p < 0.01$ (Mann–Whitney test).

solution the dexamethasone concentration was significantly enhanced (Figure 5). After administration of dexamethasone ointments with and without salicylic acid, average values of only about 150 ng dexamethasone were measured in the perfusate over the perfusion period of 135 min. There were no significant differences between the ointment formulations. The dexamethasone concentration within skin was found to be in the range of 7–45 ng/cm^2 after application of different formulations. There were no significant differences of skin dexamethasone concentration between any of the formulations (Table 1).

To evaluate the influence of perfusion medium on drug absorption, we performed the udder perfusion using a tyrode solution with addition of dextran in a second test series. No significantly different absorption rates were found between the experiments with and without addition of dextran. The total amounts of absorbed dexamethasone and the results of the statistical analysis are summarized in Table 2.

Benzoyl Peroxide Absorption

The skin concentrations of benzoyl peroxide and its metabolite benzoic acid are summarized in Table 3. Whereas the skin concentration of benzoyl peroxide was 0.35 mg/cm^2, no benzoyl peroxide was detected in perfusate fractions after topical treatment with the acetone solution (limit of detection 0.1 μg/mL perfusate). Instead of benzoyl peroxide, a metabolite of benzoyl peroxide, benzoic acid, was found in the skin and also in perfusate fractions (Figure 6, Table 3). The average benzoic acid skin concentration was 0.47 mg/cm^2. The amount of absorbed benzoyl peroxide measured as benzoic acid in the perfusate was 22% of the administered dose. These data indicate that benzoyl peroxide is metabolized in the skin to benzoic acid which is rapidly absorbed. The addition of DMSO to the solution did not affect the penetration of benzoyl peroxide into the skin. Compared to the solution in acetone, the amount of benzoic acid in the perfusate and the skin concentration of benzoyl peroxide were significantly lower after administration of the shampoo. In contrast, the skin concentration of benzoic acid was comparable to the solution. The amount of absorbed benzoic acid was significantly lower than after treatment with the acetone solution (Figure 6).

Etofenamate Absorption

Following 135 min after application to six udders, the skin concentrations of etofenamate and its metabolite flufenamic acid were 350 (288–369) and 12 (7.5–15.7) μg/cm^2 skin. The detectable skin concentra-

Table 1. Concentration of Dexamethasone in the Udder Skin After Topical Treatment

Perfusion Medium		Tyrode Solution Without Dextran	Tyrode Solution with Dextran
Treatment with	Number of Udders	ng Dexamethasone/cm^2	
Acetone solution	5	22.7 (7.4–35.4)	15.6 (14.2–17.1)
Acetone solution with 10% DMSO	6	18.1 (14.9–20.1)	Not measured
Ointment	6	17.9 (15.2–19.9)	Not measured
Ointment with salicylic acid (5 mg/g)	6	22.7 (14.6–44.6)	Not measured

Note: The udder skin was perfused with tyrode solution with or without addition of dextran. Dexamethasone (8 mg/100 cm^2) was administered as acetone solution, acetone solution with addition of DMSO, as ointment, and as ointment with addition of salicylic acid on a treatment area of 100 cm^2. Measurement of the dexamethasone concentration in the skin was carried out by radioimmunoassay. Data are given as median and range of five to six udders. No significant difference of the dexamethasone concentration in the skin was found.

Table 2. Total Amount of Absorbed Dexamethasone[a]

A. Perfusate Medium Without Dextran

Treatment with	Amount of absorbed dexamethasone (ng)	Significant Difference to: Solution with DMSO	Ointment	Ointment with salicylic acid
Solution	334 (236–444)	**	**	N.S.
Solution with DMSO	2062 (800–3767)		**	**
Ointment	147 (84–241)	**		N.S.
Ointment with salicylic acid	151 (94–233)	**	N.S.	

B. Perfusate Medium with Dextran

Treatment with	Amount of absorbed dexamethasone (ng)	Significant Difference to Perfusate Medium I
Solution	273 (193–345)	N.S.

Abbreviations: N.S., not significant.

Note: Comparison of the total amount of dexamethasone found in the perfusate within the perfusion period of 135 min (Mann–Whitney test).

[a] Means and range of five to six udders.

** $p < 0.01$. The dose of dexamethasone was 8 mg/100 cm^2 in all experiments.

tion of flufenamic acid documents that etofenamate is partly metabolized in the skin. Whereas the total amount of absorbed etofenamate was between 246 and 645 μg (mean 452 μg), flufenamic acid was not found in the perfusate.

Discussion

In vivo and in vitro models of percutaneous absorption are a necessary tool of drug development for topical use (Schaefer et al., 1987). Because in vivo models are limited by ethical considerations, in vitro models are used predominantly to predict the transcutaneous penetration and the dermal absorption of drugs and drug formulations. The in vitro models which are currently used to measure the percutaneous absorption rate of topical administered substances have both advantages and limitations compared to in vivo models (Reifenrath et al., 1984; Pershing and Krueger, 1987; Priborsky and Mühlbachova, 1990; de Lange et al., 1992; Wasmus et al., 1992). The barrier and reservoir function of the horny layer can be estimated using diffusion chambers with skin or epidermal flaps (Dick and Scott, 1992). Isolated human or animal skin is used in diffusion cell systems to characterize the penetration via the stratum corneum and the viable skin layers (Schaefer et al., 1978; Dick and Scott, 1992). But, using these models, the dermal absorption rate is not measurable. Preparations with perfused skin are necessary for this purpose.

In order to evaluate the bioavailability of dermally administered drugs, metabolic reactions in the viable epidermal and dermal layers have to be considered (Kappus, 1989). Therefore, an in vitro system that is capable of predicting dermal absorption rates has to be constructed from functional, viable skin as it exists in situ with its own vascular supply (Pershing and Krueger, 1987). The perfusion rate, the skin temperature as well as the dermal generation of drug metabolites should be recorded (Riviere et al., 1986).

The study described here was performed to evaluate the isolated perfused bovine udder as an additional in

Table 3. Concentration of Benzoyl Peroxide and Benzoic Acid in the Udder Skin After Topical Treatment

Treatment With	Number of Udders	Benzoyl Peroxide (mg/cm^2)	Benzoic Acid (mg/cm^2)
Acetone solution	6	0.35 (0.30–0.46)**	0.47 (0.12–0.60)
Acetone solution with 10% DMSO	5	0.41 (0.34–0.58)**	0.48 (0.26–0.71)
Shampoo	3	0.04 (0.03–0.15)	0.36 (0.14–0.51)

Note: The udder skin was perfused with tyrode solution. Benzoyl peroxide (500 mg/100 cm^2) was administered as acetone solution, acetone solution with addition of DMSO, and as shampoo (Peroxyderm) on a treatment area of 100 cm^2. Measurement of the concentration of benzoyl peroxide and benzoic acid in the skin was carried out by thin-layer chromatography. Data are given as median and range of three to six udders.

** $p < 0.01$ compared to the shampoo (one-way analysis of variance and Newman–Keuls test).

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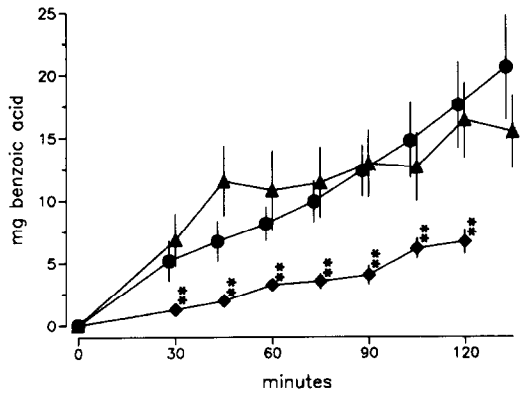

Figure 6. Absorption rate–time curve (cumulative) of benzoyl peroxide (measured as benzoic acid in perfusate fractions) in the isolated perfused bovine udder. The udder skin was perfused with tyrode solution. Benzoyl peroxide was administered as solution in acetone (triangles), in acetone with addition of 10% DMSO (circles) and as a shampoo (rectangles) on a treatment area of 100 cm^2. The administered dose was 500 mg/100 cm^2. No benzoyl peroxide could be measured in the perfusate but only its metabolite benzoic acid, indicating metabolism of the parent drug during skin penetration. Measurement of the benzoic acid concentration in the perfusate was carried out by high-performance thin-layer chromatography. Concentrations in the perfusate are given as mean ± S.E. of six (acetone), five (acetone with DMSO), and three (shampoo) separate experiments. In case of absence of S.E. bars, S.E. was smaller than the symbol used. Compared to the applied solutions with and without addition of DMSO, a significantly diminished absorption was found after treatment with the shampoo; ** $p < 0.01$ (Mann–Whitney test).

vitro model of percutaneous penetration and dermal absorption. In comparison to in vivo and in vitro animal models like the isolated perfused porcine skin flap or rabbit ear (Riviere et al., 1986; Pershing and Krueger, 1987), the isolated perfused bovine udder is not laboratory intensive. Within 30 min of slaughter, the udder can be prepared for a skin penetration study, provided the distance between slaughter house and laboratory is not too far.

The viability of the perfused udder skin was demonstrated by a nearly unchanged glucose consumption, an initially decreasing lactate production and a nearly unchanged lactate dehydrogenase activity. The initially measured high lactate concentration in perfusate samples may have been caused by the anaerobic conditions during transport of the udder from the slaughterhouse. Electron micrographs demonstrated that the bovine udder skin was structurally intact over the perfusion period (Kietzmann, M., unpublished results), since no changes in the capillaries or degeneration in the spinous, granular, and horny layer of epidermis were found. Therefore, there is no indication of a loss of function of the isolated perfused bovine udder skin within the maximum perfusion period of 6 hr. Also, it may be assumed that no significant edema developed within the perfusion period of 6 hr. From additional studies we know that a prolongation of the perfusion period to 8 hr is possible (Kietzmann et al., 1992). As shown by Hiernickel (1983) for perfused skin preparations, the results of the present experiments demonstrate that the udder perfusion with tyrode as an acellular solution is without negative effect on the functional integrity of the udder skin or the determined dermal drug absorption rates. Recent studies on transdermal delivery of isosorbide dinitrate substantiate that drug metabolism takes place in the perfused bovine udder skin, thus demonstrating the viability of the in vitro model (Kietzmann et al., 1992).

Concluding from the results reported here, the isolated bovine udder seems to be suitable as an in vitro model of dermal absorption because of specific anatomic conditions of this skin gland (Schummer et al., 1976). In contrast to other in vitro models, the isolated perfused udder is perfused by large amounts of perfusate. The perfusate flux chosen for the present experiments was 60–100 mL per minute, which is below the blood flow through the udder in vivo, but resulted in a sufficient skin vascularity. In our experiments, the treated skin area was 100 cm^2. Because of this large dosing area and the large sample volume (fractionated perfusate), test substances can be enriched by extraction from large sample volumes, thus reaching very low limits of detection with analytical methods.

The percutaneous penetration of dexamethasone, benzoyl peroxide, and etofenamate was measured to test the isolated perfused bovine udder as an in vitro model. In view of the low percutaneous absorption of glucocorticoids in vivo (Feldman and Maibach, 1969), dexamethasone was used to prove if the barrier function of the skin is maintained under the condition of the present in vitro model. Benzoyl peroxide is used frequently for the topical treatment of human acne. The nonsteroidal antiinflammatory agent etofenamate is used for systemic and topical therapy of inflammatory joint diseases. Benzoyl peroxide and etofenamate are known to be metabolized in the skin in vivo (Seubert et al., 1984; Dell et al., 1990) and were, therefore, used to examine if the metabolic capacity of the skin is maintained with the isolated perfused udder. Furthermore, both benzoyl peroxide and etofenamate are known to be percutaneously absorbed in vivo at a much higher extent than glucocorticoids (Seubert et al., 1984; Dell et al., 1990), so that by use of these drugs the predictive value of the udder model could be evaluated.

The present data substantiate that only a small amount of the applied dexamethasone dose is absorbed through the skin. Comparison with results of studies

on human skin is difficult because significant differences of dermal absorption exist between various body regions. Using various regions of human skin for drug administration, Feldmann and Maibach (1969) studied the dermal absorption of steroids in vivo. Calculated from the dexamethasone concentration measured in urine, the authors found an absorption of only 0.005% per hour after topical administration on the forearm skin. In our experiments, 0.001% to 0.0025% dexamethasone were absorbed per hour except in the experiments with addition of DMSO which increased the absorption to 0.012% per hour. The low percutaneous absorption of dexamethasone found in isolated perfused bovine udder preparations also demonstrates that the barrier function of the skin (i.e., stratum corneum) is maintained in vitro.

No significant differences in dermal dexamethasone absorption were found between application as a solution in acetone or as an ointment. An addition of 5 mg/g salicylic acid to the ointment remained without a significant effect on the dermal absorption of the glucocorticoid. Krochmal et al. (1989) showed, using diffusion cells, that salicylic acid induced an increase of the dermal absorption of hydrocortisone within nearly 20 hr. It can be concluded that the perfusion period of our study was too short to find such a salicylic acid effect. In contrast to salicylic acid, DMSO (10% in acetone) induced a significant increase in the percutaneous penetration.

Topically administered drugs are enriched first in the horny layer of the epidermis which acts as a reservoir (Dupuis et al., 1984). Thereafter, the drugs penetrate through the viable layers of the skin. Substances with a higher and a lower flux rate can be distinguished (Guy and Hadgraft, 1984). Based on the results of this study, it can be concluded that dexamethasone penetrates the viable epidermal and dermal layers slowly. In contrast, benzoyl peroxide rapidly penetrates into the skin and is metabolized to benzoic acid which is rapidly absorbed. This confirms a study of Seubert et al. (1984) with human skin in vivo and of Kietzmann et al. (1989) with canine skin in vitro. Compared to dexamethasone, a much larger amount of benzoyl peroxide penetrated into the skin. Benzoyl peroxide (35 mg) and benzoic acid (47 mg) were measured in the treated area of 100 cm^2 after administration of the benzoyl peroxide solution in acetone. This corresponds to 18% of the administered benzoyl peroxide dose. The amount of benzoic acid which was measured in the perfusate represents 4% of the administered dose of benzoyl peroxide. Therefore, up to 22% of the administered benzoyl peroxide penetrated into the skin within the perfusion period.

Compared to benzoyl peroxide, a smaller fraction of etofenamate is metabolized in the skin. Calculated from the perfusate concentrations, the absorbed amount of etofenamate is 2% of the administered dose within the perfusion period of 2 hr.

In conclusion, the isolated perfused bovine udder seems to be suited to compare the rate of percutaneous penetration and dermal absorption of various substances and drug formulations by measuring skin and perfusate concentrations. The first data on drug absorption and metabolism presented in this paper seem to indicate that the system yields data that can be extrapolated to humans, although more experiments with other drugs are needed to substantiate the validity of the model in this respect.

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References

Carver MP, Williams PL, Riviere JE (1989) The isolated perfused porcine skin flap. III. Percutaneous absorption, pharmacokinetics of organophosphates, steroids, benzoic acid, and caffeine. *Toxicol Appl Pharmacol* 97:324–337.

De Lange J, van Eck P, Elliott GR, de Kort WLAM, Wolthuis OL (1992) The isolated bolld-perfused pig ear: an inexpensive and animal saving model for skin penetration studies. *J Pharmacol Meth* 27:71–77.

Dell HD, Brons J, Fiedler J, Kamp R, Pelster B (1990) Tierexperimenteller Nachweis langanhaltender Freisetzung von Etofenamat unter Verlängerung der Halbwertszeit nach intramuskulärer Applikation. *Arzneim-Forsch (Drug Res)* 40:300–305.

Dick IP, Scott RC (1992) Pig ear skin as an in-vitro model for human skin permeability. *J Pharm Pharmacol* 44:640–645.

Dupuis D, Rougier A, Roguet R, Lotte C, Kalopissis G (1984) In vivo relationship between horny layer reservoir effect and percutaneous absorption in human and rat. *J Invest Dermatol* 82: 353–356.

Feldmann RJ, Maibach HI (1969) Percutaneous penetration of steroids in man. *J Invest Dermatol* 52:89–94.

Guy RH, Hadgraft J (1984) Prediction of drug disposition kinetics in skin and plasma following topical administration. *J Pharmaceut Sci* 73:883–887.

Hiernickel H (1983) Methode zur Gewinnung langlebiger supravitaler Hautpräparate. *Z Hautkr* 58:820–833.

Kappus H (1989) Drug metabolism in the skin. In: *Pharmacology of the skin. Handbuch experimentelle Pharmakologie* Volume 87/ 2. Eds. MW Greaves and S Shuster. Berlin: Springer-Verlag, pp. 123–163.

Kietzmann M, Bigler B, von Tscharner C, Heitmann HH, Müther T (1989) Untersuchungen zur Verträglichkeit, Wirkung im epidermalen Stoffwechsel und Metabolisierung von Benzoylperoxid beim Hund. *Kleintierpraxis* 35:31–34.

Kietzmann M, Arens D, Löscher W, Lubach D (1991) Studies on the percutaneous absorption of dexamethasone using a new in vitro model, the isolated perfused bovine udder. In: Prediction of Percutaneous Penetration. Eds., RC Scott, RH Guy, J Hadgraft, and HE Boddé. London: IBC Technical Services Ltd, pp. 519–526.

Kietzmann M, Wenzel B, Arens D, Blume B (1992) Percutaneous absorption of isosorbide dinitrate in the isolated perfusion bovine udder. *Naunyn Schmiedeberg's Arch Pharmacol* 345(Suppl): R23.

Krochmal L, Wang JCT, Patel B, Rodgers J (1989) Topical corticosteroid compounding: effects of physicochemical stability and skin penetration rate. *J Am Acad Dermatol* 21:979–984.

Monteiro-Riviere NA, Bowman KF, Scheidt VJ, Riviere JE (1987) The isolated perfused porcine skin flap (IPPSF). II. Ultrastructural and histological characterization of epidermal viability. *In Vitro Toxicol* 1:241–252.

Pershing LK, Krueger GG (1987) New animal models for bioavailability studies. In: *Pharmacology and the Skin,* Volume 1. Eds., B Shroot and H Schaefer. Basel: Karger Verlag, pp. 57–69.

Priborsky J, Mühlbachova E (1990) Evaluation of in-vitro percutaneous absorption across human skin and in animal models. *J Pharm Pharmacol* 42:468–472.

Reifenrath WG, Chellquist EM, Shipwash EA, Jederberg WW, Krueger GG (1984) Percutaneous penetration in the hairless dog, weanling pig and grafted athymic nude mouse: evaluation of models for predicting skin penetration in man. *Br J Dermatol* 111(Suppl):123–135.

Riviere JE, Bowman KF, Monteiro-Riviere NA, Dix LP, Carver MP (1986) The isolated perfused porcine skin flap. I. A novel animal model for percutaneous absorption and cutaneous toxicologic studies. *Fundam Appl Toxicol* 7:444–453.

Schaefer H, Stüttgen G, Zesch A, Schalla W, Gazith J (1978) Quantitative determination of percutaneous absorption of radiolabelled drugs in vitro and in vivo by human skin. *Curr Probl Dermatol* 7:70–94.

Schaefer H, Lambrey B, Caron D, Illel B, Renucci F (1987) Methods in skin pharmacokinetics: Introduction. In: *Pharmacology and the Skin,* Volume 1. Eds., B Shroot and H Schaefer. Basel: Karger Verlag. pp. 50–56.

Schummer A, Wilkens H, Vollmerhaus B, Habermehl KH (1976) *Lehrbuch der Anatomie der Haustiere,* III. *Kreislaufsystem, Haut und Hautorgane*. Berlin: Verlag Paul Parey.

Seubert S, Seubert A, Ippen H (1984) Untersuchung zur Penetration von Benzoylperoxid in die Haut. *Hautarzt* 35:455–458.

Wasmus G, Burkhard HJ, Schreiber G (1992) Entwicklung und Erprobung einer Methode zur Abschätzung der dermalen Exposition. Dortmund Schriftenreihe der Bundesanstalt für Arbeitsschutz.