Effect of Selective Lipid Extraction from Different Body Regions on Epidermal Barrier Function

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Purpose. To assess the effects of selective lipid extraction and tape stripping on transepidermal water loss (TEWL) at three body regions in the pig.

Methods. Lipids were extracted from the abdominal, inguinal, and back regions using three different solvent extraction procedures or cellophane tape stripping (15×) on Yorkshire pigs. Three solvent extraction stages were I, cyclohexane (5 ml for three, 1-min extractions); II, cyclohexane/ethanol (4:1) (5 ml for three, 1-min extractions); and III, cyclohexane/ethanol (1:4) (5 ml for three, 3-min extractions) extracted as follows: Site A, Stage I; Site B, Stage I and II; Site C, Stage I, II and III. Erythema, edema, and TEWL were assessed in control, tape-stripped, and extracted sites at 0, 6, and 24 h. The extracted lipids were analyzed by thin layer chromatography and quantified by densitometry for ceramide, cholesterol, cholesterol esters, fatty acids, and triglycerides.

Results. The change in TEWL (Δ TEWL) in 14 of the 15 sites was the highest at 24 h and generally increased with each additional extraction. The greatest changes were present in the back. Each extraction stage removed specific lipids in reproducible quantities that caused the Δ TEWL to increase from 0 to 24 h. Lipid removal was verified by transmission electron microscopy. The mean total lipid concentration depended on extraction solvents and body region, and was reproducible across sites and regions at equivalent stages of lipid extraction. Relative proportions of individual lipids extracted were similar across all body regions. Higher concentrations of total lipids were extracted from the back.

Conclusions. These studies demonstrate that extraction of lipids increased the Δ TEWL to a level similar to repeated tape stripping at all body sites in the pig. This study suggested that strategies that could biochemically alter epidermal lipid composition may increase absorption of simultaneously administered topical compounds and may be useful to enhance drug delivery.

KEY WORDS: epidermal lipids; ceramides; lipid extraction; tape stripping, transepidermal water loss (TEWL) ; electron microscopy; membrane lipids.

INTRODUCTION

It is widely accepted that the primary barrier to topical drug and chemical exposure is the outermost layer of skin, the stratum corneum. Numerous techniques have been applied to modulate skin permeability by altering the lipid composition of the stratum corneum (1–4). This approach to penetration enhancer development would be facilitated if the specific lipids involved in maintaining barrier function could be identified. Those responsible could then be targeted to enhance drug penetration. However, there are few studies specifically relating systematic lipid compositional changes to barrier function.

Epidermal lipids, specifically those within the intercellular spaces of the stratum corneum, maintain the integrity of the epidermal barrier and water diffusion through the skin. The lipid within mammalian stratum corneum is primarily composed of 40%–50% ceramides, 20%–27% cholesterol, 10% cholesterol esters but only 1%–2% in pigs, and 10%–12% free fatty acids (5,6). Triglycerides make up only 1%–2% of total epidermal lipids (7). Other investigators have found that the concentration of ceramides, fatty acids, cholesterol, and cholesterol ester was inversely proportional to epidermal depth, whereas phospholipid concentration increased with depth (8). It has been known for some time that epidermal lipids of the pig were in the same class as those obtained from human epidermis (9). This similarity and the general morphological similarity between human and pig skin support its use as a preferred animal model for human percutaneous absorption and transdermal delivery (10–12).

Transepidermal water loss (TEWL), a noninvasive method to measure water loss across the skin, has been used to quantitate the efficiency of the barrier function of the stratum corneum lipids. The removal of the lipids in the stratum corneum not only causes an increase in TEWL, but also allows an increase in the penetration of compounds into the skin. This enhancement in compound penetration is the basis for the more efficient delivery of therapeutic compounds across the skin barrier. Extraction with organic solvents and tape stripping the stratum corneum removes the lipid barrier to facilitate an increase in the penetration of lipophilic and lipophobic compounds. Bligh and Dyer (13) used a chloroform/methanol solution *in vitro* to extract stratum corneum lipids that caused necrosis of the skin, proving it unsuitable for *in vivo* studies (2). Topical methods for the *in vivo* extraction of stratum corneum lipids were described by Imokawa et al. (14,15) and Lavrijsen et al. (2) using a solution of acetone/diethylether.

Hexane/isopropanol (16) and cyclohexane/ethanol (17,18) have been used in healthy human volunteers to extract epidermal lipids with minimum irritation. However, neither these studies nor other extraction protocols evaluated assessed resultant changes in barrier function nor compared it to tape-stripping techniques.

The purpose of this study was to correlate the total concentration of lipids extracted from the three regions of the pig with TEWL and to assess the irritation potential of each individual extraction procedure. Three body regions were studied because of the known differences in morphology and physiology (19) as well as chemical absorption across body sites (20). A multistage approach to lipid extraction, coupled with TEWL assessment after each stage, increased the efficiency of these studies. Finally, tape stripping was used as a positive control for removing the stratum corneum barrier. These studies should further assess whether TEWL is a relevant measurement to assess barrier function.

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MATERIALS AND METHODS

Weanling Yorkshire pigs (female) weighing 20 to 30 kg were purchased commercially and acclimated for 1 week prior to the study. The pigs were housed in a temperature- and light-regulated facility on elevated pen floors and provided *ad libitum* water and food. The back and abdomen of each pig were carefully clipped to remove the hair 24 h prior to the start of the experiment. (Animals used were in accordance with the Principles of Laboratory Animal Care [NIH publication #85-23, revised 1985]). On Day 1, the pigs were anesthetized with ketamine/xylazine/telazol and five random sites within each of three body regions (abdominal, inguinal, and middle back) demarcated as follows: Site A, Stage I extraction; Site B, Stage I and II extraction; Site C, Stage I, II, and III extraction; Site D, tape stripped; and Site E, control. TEWL was measured with a Tewameter® TM 210 (Courage + Khazaka, Cologne, Germany) at each site to determine the normal TEWL baseline values. Anesthetized pigs were placed under a fume hood, and a glass well having an area of 3.14 cm^2 was held firmly against the site for the extraction procedure: Stage I, cyclohexane (100%), 5 ml was applied for 1 min, three repetitions; Stage II, cyclohexane/ethanol (4:1), 5 ml was applied for 1 min, three repetitions; and Stage III, cyclohexane/ethanol (1:4), 5 ml was applied for 3 min, three repetitions. Following each extraction, the solvent was aspirated from the well, placed in a scintillation vial, dried under a stream of dry nitrogen, and stored at –20°C for analysis. Tape stripping was carried out using Scotch Magic™ Tape (3M) 15 times on the surface of the skin.

Skin irritation marked by erythema and edema was scored according to the method of Draize (21). TEWL measurements were taken before treatment, 0 h, 6 h, and 24 h to determine the effects of the extractions and tape stripping on TEWL. The 0 h time was actually taken at 5 min after treatment to allow for degassing of volatile solvents. The average TEWL (the mean TEWL of each previous 20 s measurement ± standard deviation; the value with the smallest SD over the entire 2 min measurement window) calculated by the Tewameter was used to determine the change in TEWL (Δ TEWL = average post-treatment TEWL ÷ average pretreatment TEWL) for a site at a specific posttreatment time point. The mean Δ TEWL was generated and the significant difference ($p<0.05$) between treatments determined using LSD in the ANOVA procedure of PC SAS (version 6.12).

Following the 24-h TEWL measurements, skin biopsies were taken with one-half of the biopsy fixed in 10% neutral buffered formalin, processed through graded ethanols, and embedded in paraffin. Paraffin sections, approximately 6 μm thick, were mounted on slides and stained with hematoxylin and eosin (H&E). The other half of the biopsy was embedded and frozen in an isopentane well cooled by liquid nitrogen. Twenty micron sections were mounted and fixed in Trump's fixative. Sections were postfixed in 0.25% phosphate-buffered ruthenium tetroxide for 45 min at 4°C to preserve and stain the intercellular lipids. Sections were then dehydrated through graded ethanols, cleared in acetone, and infiltrated and embedded in Spurr's resin. Thin sections (800–1000 Å) were mounted on copper grids and examined on a Philips EM208S transmission electron microscope operating at an accelerating voltage of 80 KV.

Lipid Analysis

The dried lipid samples were transferred to 16 × 12 mm culture tubes with two 3 ml rinses of chloroform:methanol (2:1). The combined rinses from each sample were dried under a nitrogen stream, capped and stored at –20°C. Glass plates (20 × 20cm) coated with 0.25 mm thick silica gel G (Adsorbosil-plus-1; Alltech Associates, Deerfield, IL) were washed with chloroform/methanol (2:1), activated in a 110°C oven, and the adsorbent scored into 6 mm wide lanes. Each sample was dissolved in 100 μl of chloroform/methanol (2:1), and the samples were applied 2 to 3 cm from the bottom edge of the plate with calibrated glass capillaries. Ten microliters of sample was used for analysis of the nonpolar lipids (cholesterol and cholesterol esters) and 25 μl for analysis of polar lipids. Standards were used to establish quantitation curves. The chromatograms were developed according to the following: nonpolar lipids—chromatograms were developed to 20 cm with hexane, followed by toluene to 20 cm, and hexane/ethyl ether/acetic acid (70:30:1) to 12 cm; polar lipids—the chromatograms were developed to 3 cm with chloroform/methanol/acetic acid/water (40:10:1:1), followed by chloroform/methanol/acetic acid (190:9:1) to 20 cm, and by hexane/ethyl ether/acetic acid (70:30:1) to 20 cm.

Following development, the chromatograms were air dried, sprayed with 50% sulfuric acid, and heated to 220°C on an aluminum slab placed on a hot plate. After 2 h, charring was complete and the chromatograms were scanned with a photodensitometer. For each lipid class a standard curve was established and used for conversion of peak areas into lipid weights. Correction was made for the portions of the total samples used for analysis and results were expressed as total micrograms of each lipid per sample.

RESULTS

No edema was noted in any region of the pig during the extraction process, but the intensity of erythema usually increased with each additional extraction. The erythema completely disappeared after 6 h. The mean normal pretreatment baseline values for TEWL at the three body regions of the pig were mean ± SEM ($g/m^2/h$) for: back 13.60 ± 0.41, abdominal 15.71 ± 0.76, and inguinal 16.46 ± 0.45. The lowest TEWL readings were recorded in the back, followed by the abdomen, and then the inguinal region. In the abdominal region of the pig, the 24 h mean change (Δ) in TEWL increased with each additional extraction. The mean Δ TEWL in the abdominal area declined from 0 to 6 h, and then increased at 24 h. In the inguinal and back regions, the mean Δ TEWL generally peaked at 24 h and was progressively higher with each additional extraction. Sites A, B, D, and E exhibited the greatest terminal mean Δ TEWL values in the back, whereas Site C had the greatest value in the inguinal region. The mean values in the control sites were similar to the B sites (Stage I and Stage II extraction); the terminal values of the Stage I extraction were lower than those of the controls. Table I shows significant statistical differences ($p<0.05$) within the inguinal and back regions: inguinal sites A, B, and control (E), 0 h and 6 h significantly lower than 24 h mean Δ TEWL; the back, Site B, 0 h significantly lower than 24 h and in back tape stripped (D) and control (E), 0 h and 6 h significantly lower than 24 h mean Δ TEWL. In general, the back had the

Table I. Mean TEWL Change for Three Body Regions Following Extraction in Sites A (Stage I), B (Stage I and II), C (Stage I, II, and III), (D) Tape Stripped, and (E) Control

Site	Time (h)	Mean TEWL change ± SEM		
		Abdominal	Inguinal	Back
A	0	1.12 ± 0.12	0.83 ± 0.10[a]	0.87 ± 0.11
	6	0.97 ± 0.15	0.84 ± 0.03[a]	1.09 ± 0.14
	24	1.02 ± 0.14	1.21 ± 0.07[b]	1.23 ± 0.04
B	0	1.03 ± 0.08	0.80 ± 0.08[c]	0.92 ± 0.04[g]
	6	0.89 ± 0.06	0.84 ± 0.02[c]	1.09 ± 0.15
	24	1.25 ± 0.13	1.24 ± 0.13[d]	1.39 ± 0.06[h]
C	0	1.09 ± 0.20	1.30 ± 0.42	1.08 ± 0.28
	6	0.99 ± 0.04	1.02 ± 0.12	1.24 ± 0.18
	24	1.37 ± 0.14	1.53 ± 0.22	1.46 ± 0.07
D	0	1.08 ± 0.09	0.98 ± 0.17	1.16 ± 0.09[i]
	6	0.96 ± 0.08	0.99 ± 0.06	1.15 ± 0.14[i]
	24	1.41 ± 0.20	1.30 ± 0.14	1.50 ± 0.05[j]
E	0	1.04 ± 0.16	0.88 ± 0.14[e]	0.94 ± 0.07[k]
	6	0.88 ± 0.04	0.85 ± 0.05[e]	0.87 ± 0.06[k]
	24	1.29 ± 0.11	1.23 ± 0.09[f]	1.32 ± 0.02[l]

Note. ([a] and [b]), ([c] and [d]), ([e] and [f]), ([g] and [h]), ([i] and [j]), ([k] and [l]) are significantly different ($p < 0.05$).

highest Δ TEWL with each treatment, followed by the abdominal and the inguinal regions. No significant differences ($p<0.05$) were found across the regions within each postextraction time point.

Morphological Observations

The histology of the extracted, tape-stripped, and control sites exhibited slight to moderate intracellular epidermal edema within the abdomen and inguinal regions of the pig. Moderate intracellular epidermal edema was present in the back, control, tape-stripped, and extracted sites. The stratum corneum of the extracted and tape-stripped sites was less compact and had a basket weave appearance compared to the compact control sites. In addition, the tape-stripped sites had focal areas completely devoid of stratum corneum layers.

Transmission electron microscopy (TEM) of skin from the back region of the pig revealed intercellular lipid in each of the five sites. The control and tape-stripped sites contained lipid that was homogeneous and compact (Fig. 1). Focal areas of lipid lamellae detachment or disruption were evident within Sites D and E. Alternatively, some areas within the extracted sites were completely devoid of intercellular lipid or contained areas of noncompact intercellular lipid lamellae (Figs. 2 and 3).

Lipid Analysis

Stage I extraction of Sites A, B, and C within the three body regions of the four pigs removed ceramide (CER) 1 (mean range 1.9–3.6 μg), CER 2 (mean range 3.9–9.1 μg), cholesterol (CH) (mean range 37.7–44.7 μg), fatty acid (FA) (mean range 18.4–28.7 μg), triglycerides (TG) (mean range 7.7–15.7 μg), and cholesterol esters (CE) (mean range 4.7–26.6 μg) (Fig. 4). Although CER 3, 4, 5, and 6 typically were not extracted during Stage I, small amounts (1.1–1.6 μg) were found in the abdomen of one pig. The mean concentration of CH, FA, TG, and CE extracted from Sites A, B, and C during Stage I extraction were highest in the back, usually followed by the inguinal and abdominal areas. This trend also appeared with CER 1 and 2 in Sites B and C. In addition, mean CE concentration was more than double the inguinal and abdominal values. Stage II extraction of Sites B and C removed CER 1 (mean range 4.5–7.0 μg), CER 2 (mean range 23.2–25.7 μg), CER 3 (mean range 7.0–9.5 μg), CER 4 (mean range 3.3–5.0 μg), CER 5 (mean range 3.8–5.0 μg), CER 6 (mean range 6.2–10.5 μg), CH (mean range 30.3–34.2 μg), FA (mean range 34.7–40.6 μg), TG (mean range 4.1–5.5 μg), and CE (mean range 3.2–8.1 μg) (Fig. 5). The mean concentrations of CER 4, TG, and CE extracted from Sites B and C during Stage II extraction were greatest in the back, whereas CER 1 was greatest in the abdomen. Stage II extracted a higher amount of the polar CER 1, CER 2, and nonpolar FA in the three regions of the pig than the Stage I extraction. Stage III extraction of Site C removed CER 1 (mean range 3.5–4.8 μg), CER 2 (mean range 11.0–15.8 μg), CER 3 (mean range 3.4–5.2 μg), CER 4 (mean range 1.7–2.4 μg), CER 5 (mean range 2.9–3.9 μg), CER 6 (mean range 3.4–5.3 μg), CH (mean range 33.2–36.8 μg), FA (mean range 27.0–29.0 μg), TG (mean range 3.7–5.4 μg), and CE (mean range 3.3–4.1 μg) (Fig. 6). The mean concentrations of CER 1–6 (total ceramide), CH, and FA were greater in the abdominal than the inguinal or back. The concentration of lipids extracted in Stage III was generally less than or marginally higher than those extracted in Stage II, with the exception of CH.

Each solvent extracted a specific ratio of lipids relative to each region of the pig. Stage I (cyclohexane) and Stage III

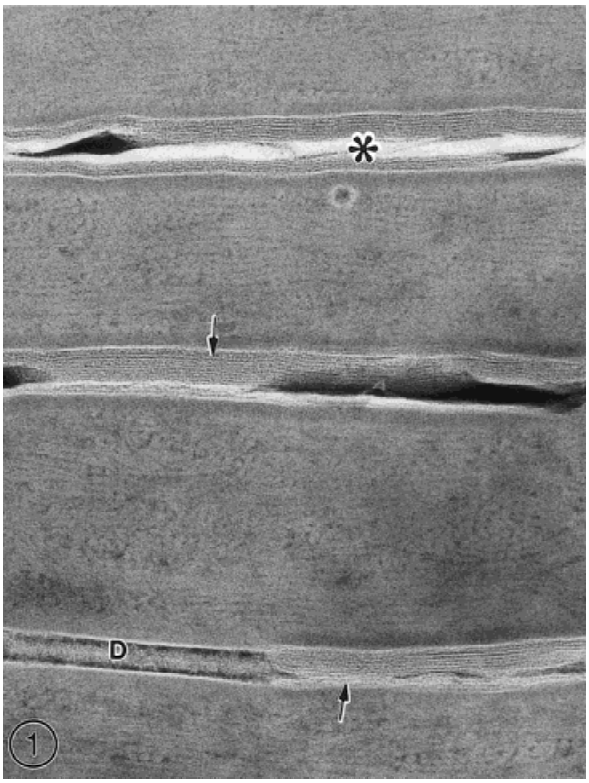

Fig. 1. Electron micrograph depicting compact stratum corneum (SC) with intercellular lipid lamellae (arrows), and desmosomes (D). Note areas devoid of lipid and lamellae detachment (*). Tape stripped, back (Site D). ×156,600.

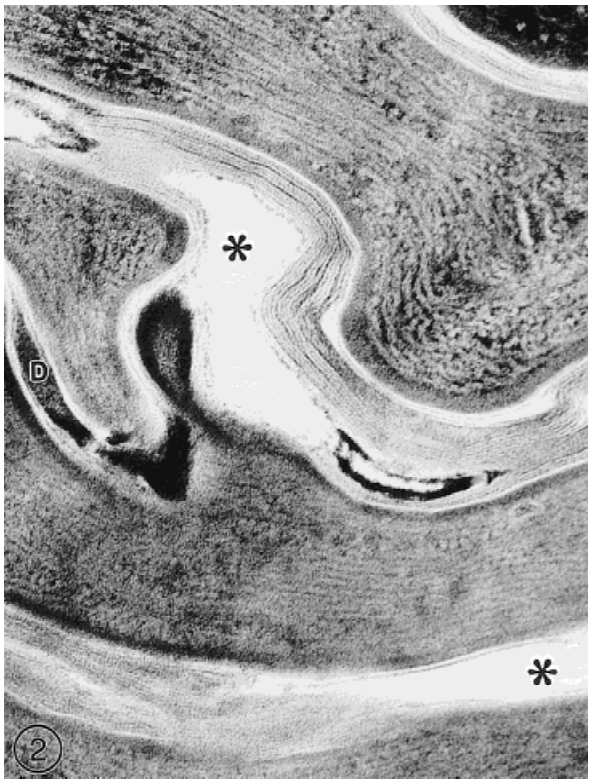

Fig. 2. Electron micrograph depicting areas devoid of intercellular lipid (*) between the stratum corneum layers. Note desmosome attachment (D). Stage I extracted from back (Site A). ×140,300.

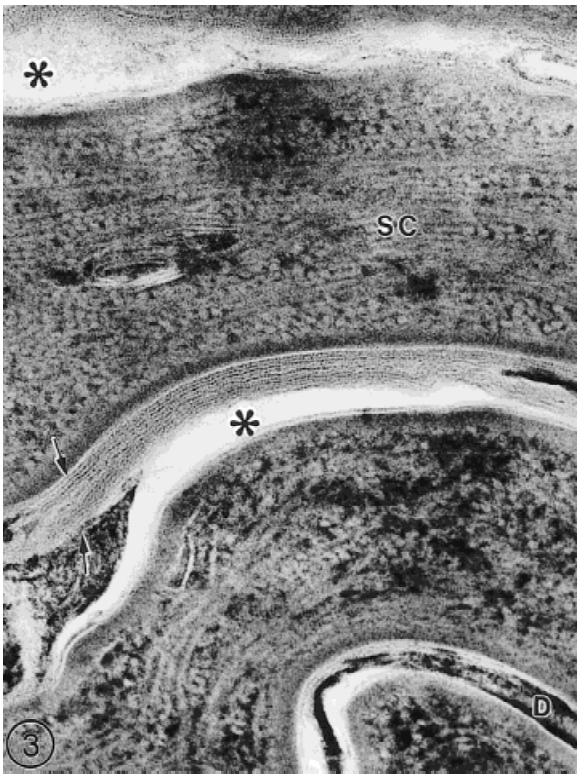

Fig. 3. Electron micrograph of stratum corneum layers (SC) showing the intercellular space filled with compact lipid lamellae (arrows), and desmosome attachment (D). Note areas completely lacking lipid lamellae (*). Stage II extracted from back (Site B). ×140,300.

(cyclohexane/ethanol; 1:4) removed a greater percentage of CH, whereas Stage II (cyclohexane/ethanol; 4:1) removed a greater percentage of total ceramide. Stage I extracted more CER 1–6 and CH, and less CE from the abdominal and inguinal regions. In all three regions, Stage I extracted a higher percentage of TG. Table II depicts lipid analysis by site showing a dramatic increase in percentage weight of total extracted ceramide between Site A and Site B, with a slight increase in Site C. The percentage weights of stratum corneum lipids in Site C across body regions were 30% ceramides, 32% cholesterol, 26% fatty acids, and 6% triglycerides. Also, the percentage weight of TG steadily decreased by site C < B < A. In Sites A, B, and C, the greatest concentration of total lipid was extracted from the back.

DISCUSSION

Transepidermal water loss has been an important parameter in assessing epidermal barrier function, the effect of occlusion, and irritant dermatitis on the skin. In this study we measured the water evaporation gradient at the skin surface of the pig across three body regions. The TEWL probe consists of an open chamber with an inner diameter of 10 mm containing two humidity and two temperature sensors. The instrument measures the TEWL at any given moment, the mean TEWL ± standard deviation over a previous window of time (20 s), the temperature of the skin, and the humidity of the skin. Studies in humans have shown an immediate increase in TEWL following the removal of occlusive patches, occlusive patches containing water, and occlusive patches containing sodium lauryl sulfate (22–24). This increase in TEWL is caused by a functional or structural change in the stratum corneum barrier (22). In addition, cutaneous application of organic solvents *in vitro* (1,25) and *in vivo* (26–27) have caused TEWL to increase.

Several factors, including room temperature and relative humidity, affect TEWL measurements. The period of time required for skin equalization has been reported from 20 (23) to 30 min (23,24) and as long as 3 h (26) following the removal of the occluded dose prior to TEWL measurement. During this study we determined that 10 min appeared adequate to balance water diffusion between the stratum corneum and the ambient air with the onset of repair mechanisms. This also would allow any residual solvent to evaporate prior to determining TEWL. Some investigators found that the TEWL measurements could be carried out with a 30-s to 60-s probe application once the probe reached temperature equilibrium with the skin. We increased the measurement of the TEWL to 2 min to ensure consistent assessments. Inconsistencies of the TEWL data may be explained, at least partially, by time constraints due to methodology. The extraction procedure and subsequent TEWL measurements were time-consuming and carried out on a total of 15 sites per pig (Sites A, B, C, D, and E for three body regions). This required each pig to be anesthetized for a minimum of 3 h, which induced a slight cooling of body temperature.

The *in vivo* extraction of lipids should require a method that does not alter epidermal morphology. However, severe morphological damage was evident in all layers of the epidermis following chloroform/methanol extraction, but no damage was found in skin extracted with acetone/ether (2). How-

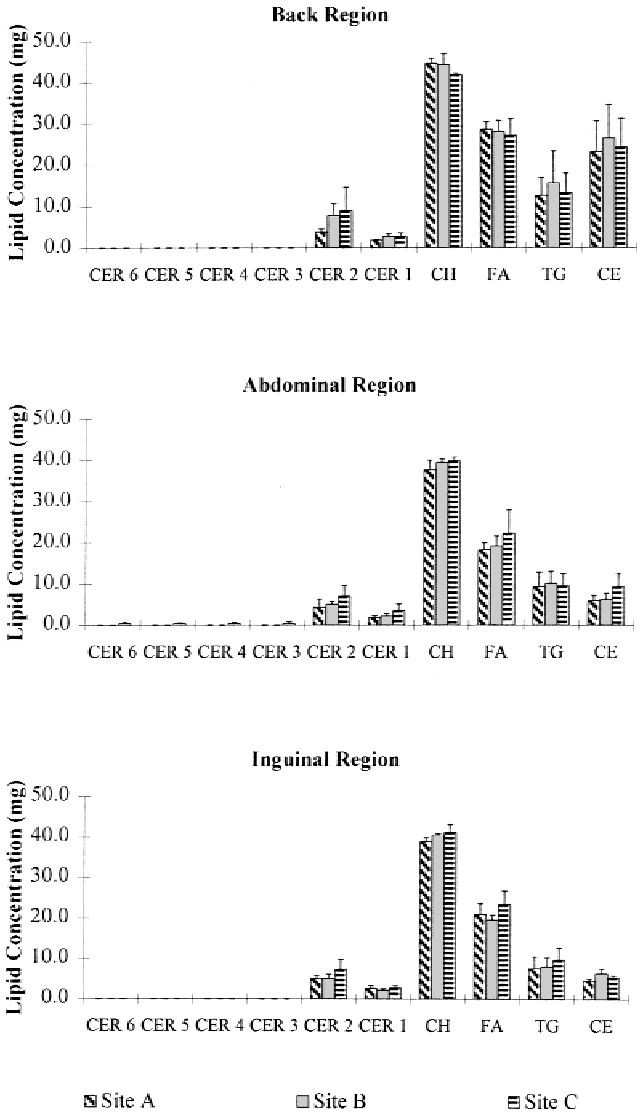

Fig. 4. Mean concentration of lipid extracted in Stage I within each body region of the pig.

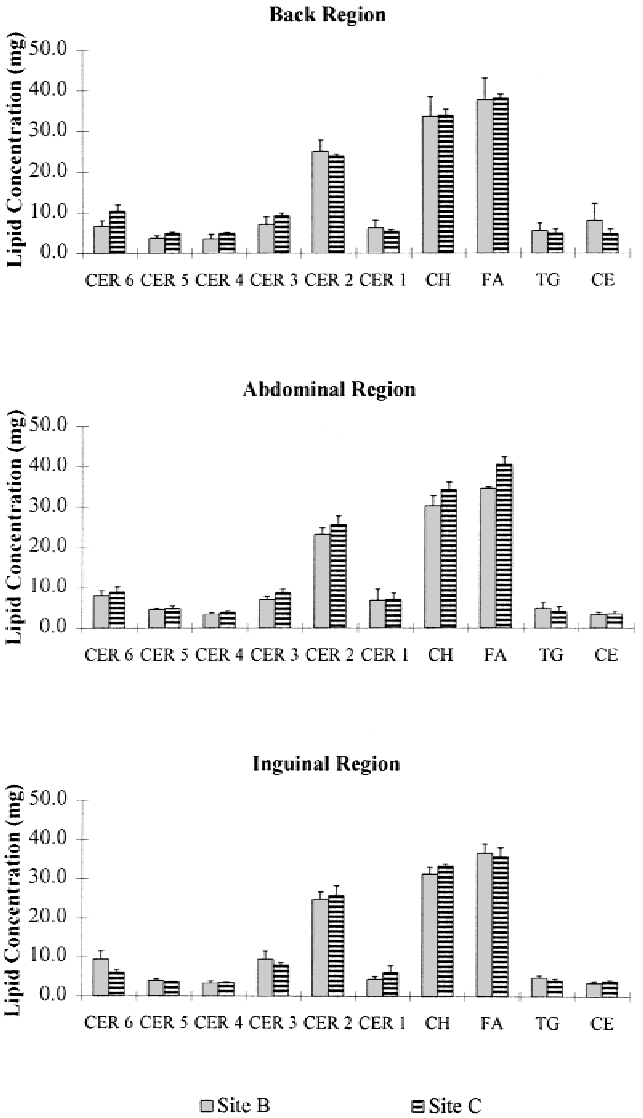

Fig. 5. Mean concentration of lipid extracted in Stage II within each body region of the pig.

ever, some investigators have reported that acetone/ether applied to the human forearm for 5 to 20 min induced a chapped, scaly reaction that lasted more than 4 days (14). A solution of hexane and methanol caused extreme skin irritation (17) but a cyclohexane/ethanol extraction was not irritating in humans (17,18). Therefore, this procedure and extraction solution was utilized in this study. Although some immediate erythema was noted in the pig following extraction, the effect on the epidermal morphology was minimal and consisted of a loss of stratum corneum compactness and slight to moderate intracellular epidermal edema. The intensity of the erythema did increase with additional extraction, but had disappeared by 6 h following the treatment.

TEM of normal porcine skin postfixed in ruthenium tetroxide has shown extensive lipid lamellae in the intercellular spaces of the stratum corneum (28). Lipid extraction of isolated porcine corneum has revealed that the lipid was removed. We found that the lipid was partially removed during the *in vivo* extraction of Sites A, B, and C. Other investigators have determined that topical extraction procedures probably do not remove significant amounts of lipids from the lower layers of the stratum corneum (2,18) nor completely extract the lipids from the upper layers. In addition, we found that the stratum corneum in the extracted sites was less compact and often separated into a basket weave appearance and therefore made it difficult to analyze by TEM. The intercellular lipid probably increases cohesion between adjacent layers of the stratum corneum (29).

The Stage I extraction fraction in Site C assayed for small amounts of CER 3–6 in the abdomen of a single pig. Although CER 3–6 represents about 70% of the total stratum corneum ceramides (6), absolute cyclohexane did not extract these polar lipids from the stratum corneum in other treatment sites or body regions. Therefore, these ceramides are probably contaminants. The three extraction stages worked for the extraction of the nonpolar lipids (CH, FA, and CE 2). Stage I was more effective in the extraction of TG and CE. In previous studies investigators (7,30) found no measurable triglyceride in stratum corneum prepared from clean young pigs. Since the percentage weight of TG was less with each

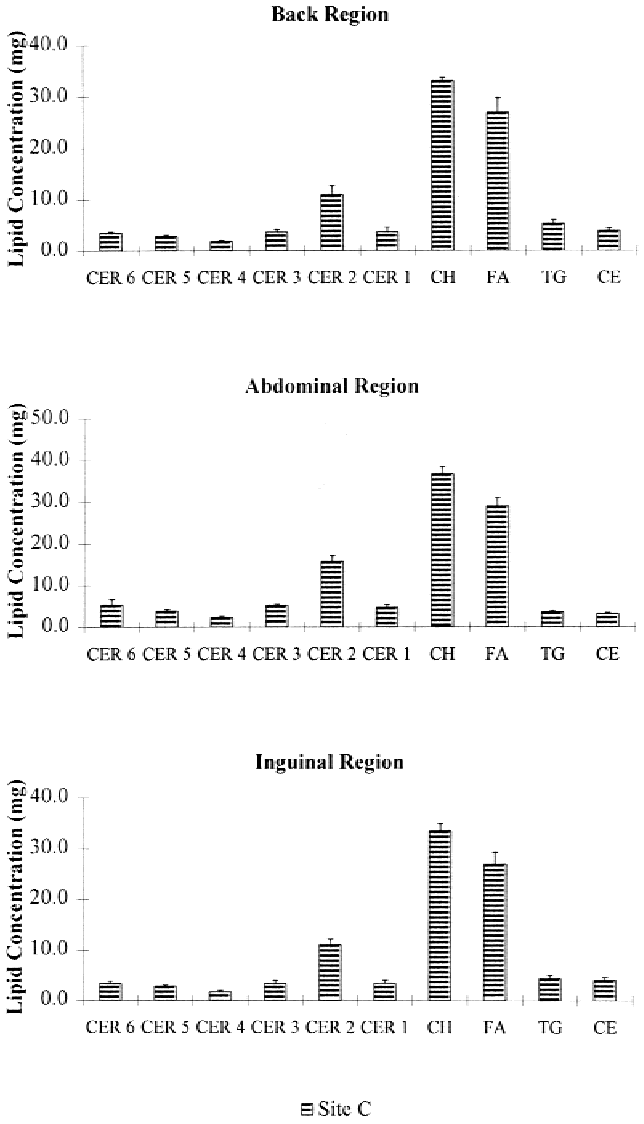

Fig. 6. Mean concentration of lipid extracted in Stage III within each body region of the pig.

Table II. Mean Percentage Weight of Total Lipid Extracted from Each Site in Three Body Regions

		Percentage weight		
	Lipid[a]	Abdominal	Inguinal	Back
Site A	CER 6	0	0	0
Stage I	CER 5	0	0	0
	CER 4	0	0	0
	CER 3	0	0	0
	CER 2	5.6	6.4	3.4
	CER 1	2.4	3.3	1.6
	CER 1–6	**8.0**	**9.7**	**5.0**
	CH	48.5	48.6	38.8
	FA	23.6	26.2	24.9
	TG	12.2	9.6	11.0
	CE	7.8	5.8	20.2
Site B	CER 6	3.8	4.5	2.5
Stage I and II	CER 5	2.2	1.9	1.4
	CER 4	1.6	1.5	1.3
	CER 3	3.3	4.5	2.7
	CER 2	13.6	14.0	12.5
	CER 1	4.3	3.1	3.4
	CER 1–6	**28.9**	**29.5**	**24.0**
	CH	33.5	33.6	29.7
	FA	25.8	26.3	25.1
	TG	7.2	6.0	8.1
	CE	4.6	4.6	13.2
Site C	CER 6	4.2	3.0	3.9
Stage I, II, and III	CER 5	2.6	2.1	2.2
	CER 4	1.9	1.6	1.9
	CER 3	4.2	3.6	3.6
	CER 2	14.1	14.0	12.4
	CER 1	4.5	3.9	3.4
	CER 1–6	**31.4**	**28.4**	**27.4**
	CH	32.2	34.2	30.6
	FA	26.7	27.3	26.0
	TG	5.0	5.8	6.7
	CE	4.7	4.3	9.4

[a] CER, ceramide; CH, cholesterol; FA, fatty acids, TG, triglycerides; CE, cholesterol esters.

additional extraction step, it is reasonable to assume that surface contaminants account for these results. Stage II and Stage III were more effective than Stage I for the removal of the ceramides, CH, and FA. The relative amounts of lipid extracted from the pigs in this study correlate well with other porcine profiles (7). Others have found greater relative amounts of ceramides and less cholesterol (30). In addition, Bonte et al. (17) had poor lipid recovery using cyclohexane and ethanol, which probably indicates that their extraction procedure was not as efficient as other methods.

The Stage I extraction of CER 1, CER 2, CH, FA, TG, and CE from Site A using cyclohexane depressed the initial mean ΔTEWL values in the back and inguinal to about 87% and 83% of preextraction values. The mean ΔTEWL value in the back increased to about 109% at 6 h causing minimal erythema. The mean ΔTEWL values steadily increased to a 24-h value of 123% in the back, decreased at 6 h, then increased to 102% at 24 h in the abdomen, and remained steady through 6 h to peak at 121% after 24 h in the inguinal region. Site B extraction by Stage I and Stage II (CER 1–6, CH, FA, TG, and CE) resulted in mean ΔTEWL that generally approximated the Stage I values. The mean ΔTEWL values were approximately the same as the Stage I alone at 6 h, whereas the 24 h mean values were moderately (back and abdomen) to slightly (inguinal) higher. Therefore, the removal of the additional lipids had little effect on the mean ΔTEWL until 24 h following the extraction. The cyclohexane/ethanol in the Stage II extracted higher concentrations of CER 1 and FA (approximately two times), and CER 2 (approximately four times) than the cyclohexane alone. Again, FA must be considered a contaminant. The Stage I, Stage II, and Stage III (CER 1–6, CH, FA, TG, and CE) extraction in Site C resulted in mean ΔTEWL values that were moderately higher in the back and abdomen than the Site B mean ΔTEWL values. In the inguinal region of the pig, the mean change in the Site C TEWL is much higher than in Sites A and B. The removal of the additional lipids by cyclohexane/ethanol (1:4) caused in an increased loss of barrier function in the three regions of the pig, which resulted in an increase in mean ΔTEWL at 0 h, 6 h, and 24 h postextraction.

In Sites A and B, the increase in TEWL by region on the pig can be correlated with the removal of total lipid from the

skin. In both sites, the higher ΔTEWL is consistent with the increase in lipid extracted from the back. In Site C, however, the greatest concentration of lipid was removed from the back, whereas the greatest ΔTEWL was in the inguinal region. Other investigators found no significant and consistent correlation between the removal of stratum corneum lipids and the TEWL response (1).

In summary, our data suggest that a correlation does exist between the presence of total lipid and TEWL and that this response is different across different body sites. Regional differences have also been seen in both skin morphology and cutaneous blood flow assessed by laser Doppler velocimetry studies (19). This is consistent with differences in drug penetration across body sites of the pig (20).

Each extraction procedure was repeatable from animal to animal and from one anatomic site to another. Each stage removed specific lipids that generally caused the mean ΔTEWL to increase from 0 through 24 h. It is significant that the extraction of lipids across all body sites of the pig increases TEWL to a level similar to that seen with repeated tape stripping. However, it is not known how an increase in TEWL correlates to increased drug delivery because drug absorption is dependent on the physiological properties of the drug. These studies confirm that strategies such as lipid extraction, inhibition of lipid synthesis, and penetration enhancers that biochemically alter epidermal lipid composition alter epidermal barrier function in a manner that may be useful to enhance drug delivery.

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