Characterization of Detergent-Induced Barrier Alterations – Effect of Barrier Cream on Irritation

Manigé Fartasch, Esther Schnetz, and Thomas L. Diepgen
Department of Dermatology, University of Erlangen/Nürnberg, Erlangen, Germany

To gain a better understanding of the interaction of the model detergent sodium lauryl sulfate (SLS) with the stratum corneum, we investigated systematically the ultrastructural changes of the epidermal barrier and the nucleated parts of the epidermis after the occluded application of different concentrations of SLS in human. Different application models were investigated. Two of the three irritation procedures (long duration exposure and the repetitive exposure for 3 d) provoked damage of the nucleated parts of the epidermis and alterations of the lower parts of the stratum corneum. Here, the extrusion and transformation of lamellar body derived lipids into lamellar lipid bilayers were disturbed; however, the upper portions of stratum corneum displayed intact intercellular lipid layers that contradict the long-standing belief that surfactants damage the skin by delipidization. Furthermore, we investigated ultrastructurally and by measurement of transepidermal water loss the influence and protective capacity of a lipophilic barrier cream on acute irritant contact dermatitis. The irritant contact dermatitis was induced by the standardized cumulative short application model with two SLS concentrations (0.5% and 0.75%). The cumulative type of exposure simulates daily living more realistically. Because most of the previous tests have been performed on the human forearm or back, we analyzed whether the pattern of response was similar on both sites. The back showed a higher level of irritant reaction, but the pattern of irritant response proved to be similar to the forearm. Application of the barrier cream before and during irritation showed a decrease of transepidermal water loss enhancement with 0.5% SLS by 58% (back) and 49% (arm) and after irritation with 0.75% SLS by 56% (back) and 43% (arm). Because the experimental result correlated with the clinical experience, the development of the cumulative short exposure model might help to predict and to discriminate the efficacy of barrier creams. ***Key words: epidermal lipids/SLS/TEWL/ultrastructure. Journal of Investigative Dermatology Symposium Proceedings 3:121–127, 1998***

One of the most important functions of the stratum corneum (SC) is to serve as a barrier (Blank, 1965) that protects against the penetration of irritants and prevents transepidermal water loss (TEWL) through the skin. Irritation of the skin is accompanied by a complex array of epidermal and dermal metabolic responses. The role of the epidermal barrier and its repair seems to be especially crucial for the epidermal responses because recent studies have shown that the epidermal keratinocyte actively participates in the modulation of inflammatory reactions induced by the injuring agent (Wood *et al*, 1992; Nickoloff and Naidu, 1994; Tsai *et al*, 1994; von den Driesch *et al*, 1995) and the pathway of the irritant into the nucleated (viable) parts of the epidermis. Additionally, there seems to be a precise relationship between barrier function (after barrier disruption) and cytokine expression (Nickoloff and Naidu, 1994; Wilmer *et al*, 1994). The primary response to the irritation with the resulting barrier disruption is the attempt to restore the protective barrier so that penetration of environmental hazards and further alteration of the keratinocytes is prevented. The modulating and initiating effects of the keratinocytes are influenced by the reaction of the barrier and the pathway of the irritant into the nucleated (viable) parts of the epidermis.

Reprint requests to: Prof. Dr. Manigé Fartasch, Department of Dermatology, University of Erlangen, Hartmannstr.14, 91052 Erlangen, Germany.

Abbreviations: LB, lamellar body; SC, stratum corneum; SLS, sodium lauryl sulfate; TC, test cream; TEWL, transepidermal water loss.

Furthermore, it has been shown that the mechanisms by which irritants impair the skin differ widely depending on the type of substance (Willis *et al*, 1991; Fartasch, 1995, 1997; Yang *et al*, 1995).

Sodium lauryl sulfate (SLS), an anionic surfactant, is frequently used in the induction of experimental irritant contact dermatitis in animals and humans (Tupker *et al*, 1997), and characteristically induces a dose-related increase in TEWL (Agner and Serup, 1990; Di Nardo *et al*, 1996). Also, it is known that the most sensitive parameter and best index to monitor barrier alterations induced by SLS is the measurement of TEWL (Frosch *et al*, 1993). SLS irritation has also been used to test efficacy and value of barrier creams, moisturizers (Hannuksela and Kinnunen, 1992; Frosch and Kurte, 1994), and lipid mixtures (Yang *et al*, 1995).

Recently, it has been shown that the mechanism of SLS induced barrier disruption might be different from that of other irritants, especially from the solvent induced alteration (Yang *et al*, 1995). Yang *et al* (1995) have shown that SLS induced barrier perturbation did not improve by the application of the optimal lipid mixtures.

In spite of the fact that the use of purebred laboratory animals in current irritation test systems seems to ensure a uniformity of response (Patil *et al*, 1995) that cannot be expected in human subjects (Judge *et al*, 1996), the degree and the time course of irritation and barrier recovery for animals and humans seem to be different (Mortz *et al*, 1997). Therefore, we have performed studies on human skin *in vivo*. But the situation in humans is complicated by the fact that the factors governing individual responses to irritants are still poorly characterized. Thought also has to be given to the possibility of intrinsic variation in response to irritants (Basketter *et al*, 1996; Judge *et al*, 1996). It is likely

1087-0024/98/$10.50

that barrier function, which is mainly related to epidermal lipid composition, and one's genetically determined potential for epidermal cytokine release, both play an important role in an individual's susceptibility and response (Judge *et al*, 1996; York *et al*, 1996).

When studying the effect of a model irritant like SLS on human epidermal barrier *in vivo*, several variables have to be taken into account, such as the vehicle used in testing, the duration and number of applications, the type of occlusion, the site used, etc. Therefore, to understand the cellular mechanisms involved, we performed the following experiments.

(i) We first analyzed systematically the SLS-induced structural changes of the epidermal barrier and the nucleated parts of the epidermis reacting to different concentrations and different exposure times of SLS after a single application modus in human.

(ii) Because of the real-life exposure, where "cumulative" contacts with low concentrated irritants seem to induce contact dermatitis in most of the cases (Huener *et al*, 1994), we finally chose a standardized test procedure with multiple short application times and low concentrations of the irritant. In assessing the appropriate test concentration in the occluded patch and repetitive test system, it was important to choose a cumulative short exposure procedure that permits the development of a "positive," but not "severe," irritant response. This helps to avoid that too many subjects may have to discontinue the study due to a severe degree of reaction. Then we evaluated ultrastructurally and by measurement of TEWL, as a functional index for barrier alteration and repair, the irritating effect of the repeated short-term SLS exposure on the barrier and how the mechanisms and kinetics of the irritations were influenced by a model barrier cream.

(iii) Another aspect was to analyze the site of exposure (forearm or back). In most of the previous studies the forearm has been used as a convenient site for irritancy testing and the evaluation of barrier repair (Frosch *et al*, 1993; Mao-Qiang *et al*, 1996; Zhai and Maibach, 1996; Loden, 1997). Recently the back has been proposed because of the larger skin area (Frosch *et al*, 1994; Wigger-Alberti and Elsner, 1997). Because it is known that different anatomic localizations have shown different susceptibility to irritation, it is crucial to prove that the pattern of response is identical in both localizations.

MATERIALS AND METHODS

Biophysical noninvasive measurements The quantitative measurements of TEWL were carried out using the Tewameter TM 210 (Courage and Khazaka, Cologne, Germany). The TEWL is calculated automatically and expressed in g per $m^2 \times h$. The measurements were made according to the guidelines of Pinnagoda *et al* (1990). Measurements were carried out in an air-conditioned room after a rest of 30 min for equilibration (room temperature 20–22°C, relative humidity between 30% and 45%). The Tewameter probe was used in a holding device to avoid heating of the probe. The probe was rested on the skin and TEWL was continuously recorded for a period of 3 min. Mean values were obtained from three successive recordings for every test site.

Clinical examination Visual scoring was performed according to the following scale: 0, no reaction; 0.5, slight scaling or very weak erythema, smooth surface; 1, weak erythema, possibly slight infiltration, slight roughness, slight scaling, mild edema, fine fissures; 2, erythema, more roughness, scaling and edema, fissures; 3, pronounced erythema, extensive scaling, pronounced edema, possibly vesicles, bullae, pustules, and/or pronounced crusting.

Morphologic methods Punch biopsies were divided in half for transmission electron microscopy and processed for visualization of routine morphology and for epidermal lipids characterization. For routine electron microscopy, tissue was double fixed with 2.5% glutaraldehyde and OsO_4 and embedded in Epon 812. To study the epidermal lipids, the other half was fixed in acrolein vapor and 2.5% glutaraldehyde for 2 h, rinsed in buffer for 3 h, postfixed with 0.5% RuO_4 (Polyscience, Warrington, PA) with 0.25% potassium ferrocyanide (pH 6.2) for 1 h in darkness at 4°C, and embedded in Spurr's resin (Fartasch *et al*, 1993; Fartasch and Ponec, 1994). All tissues were sectioned and viewed on a Jeol 100 CX transmission electron microscope. Thin sections were examined before and after double staining with uranyl acetate and lead citrate. Semi-thin sections were partly examined unstained by light microscopy to control penetration of RuO_4 (dark staining). Serial sections were stained with 1% methylenblue.

Statistical analyses Data of clinical scoring are presented as means ± SEM. The distribution of ΔTEWL (equal to difference between the individual's baseline TEWL and increased TEWL after irritation) are presented as box plots. Box plots show the median, the twenty-fifth percentile, the seventy-fifth percentile, and values that are far removed from the rest. The lower boundary of the box is the twenty-fifth percentile, the upper boundary the seventy-fifth percentile; the line inside the box represents the median. Lines are drawn from the ends of the box to the largest and smallest observed values that are not outliers. Cases with values that are between 1.5 and 3 box lengths from the upper or lower edge of the box are called outliers (designated with the letter o). Cases with values that are more than three box lengths from the upper or lower edge of the box are called extreme values (designated by *).

The Wilcoxon test was used for statistical analysis. The correlation between the reaction pattern of the arm and the forearm was analyzed by the Spearman correlation coefficient (R). All the analyses were conducted with SPSS/PC+ Version 5.0 statistical software. All statistical tests were two-tailed, and a p value of less than 0.05 was considered to indicate statistical significance.

Ultrastructural studies on SLS irritation To ascertain the structural basis and cellular mechanism responsible for barrier disruption by SLS and the influence of test creams on the process of irritation and recovery, we studied the effect of different SLS exposures on human skin.

Experimental protocols Long-term exposure (24 h): nine human volunteers with no history of skin disease were tested after having given informed consent. SLS, minimum 99% purity (Merck, Darmstadt), were used at dilutions in water of 0.5% and 1% (wt/vol). Patch tests [Large Finn Chambers (inner diameter 12 mm) with filter paper discs, fixed on Scanpor tape (Scanpore tape; Hermal, Reinbeck, Germany)] were saturated with 50 μl of the SLS solution.

Each SLS-treated subject had five test areas on the volar aspects of both forearms (left arm: 0.5% SLS, 1.0% SLS, control with water; right arm: 0.5%, 1% SLS). In three of the seven subjects, biopsies of two test sites (0.5% and 1.0% SLS) were performed 30 min after the 24 h patch test. In the other six subjects the biopsies were performed after the maximum TEWL had been reached upon application of both concentrations (enhancement of TEWL as sign of an impaired barrier function; 24–48 h after the patches have been removed).

The TEWL measurements in the recovery phase were performed 2 h after the removal of patches and then every 6 h for the first 3 d. After the 3 d measurements were performed daily until baseline TEWL values were achieved. The experiments were performed simultaneously on both volar forearms and biopsies were only taken from the test sites of the left arm.

Short-term exposure (application times: 0.5 h, 2 h): four human volunteers were patch tested with 0.5% SLS for 0.5 h and 2 h on the forearms. The biopsies were performed 24 h after the removal of the patch tests. TEWL measurements were performed 30 min, 2 h, 6 h, 12 h, and 24 h after the removal of the patches. No measurable changes in TEWL values were detectable.

Short-term cumulative exposure (2 × 0.5% SLS) with and without test cream (TC): in two volunteers, SLS 0.5% (dissolved in water) was applied with large Finn chambers (12 mm, filling volume 0.05 ml) on two areas of the forearms. One test site was pretreated with the TC 10 min before each irritation. The chambers were removed after 30 min of exposure. A second exposure and pretreatment were performed at the same day after 3.5 h. Using this application scheme, the volunteers were treated for 3 d. To compare the morphologic changes of the irritated but with TC pretreated *versus* irritated skin alone, two 3 mm punch biopsies were performed in each panelist on day 3, 2 h after the last exposure.

Development of a repetitive irritation test in humans: determination of the threshold tolerance In assessing the appropriate test concentration in an occluded patch and repetitive test system, a continuous approach to testing was adopted.

In the first experiment (n = 8) we have chosen different concentrations of SLS (0.1%, 0.2%, 0.3%; 0.5%, 0.75%) for the above-mentioned cumulative exposure. In a preliminary study we had also included the concentration 1% of SLS in another four volunteers; but this concentration had to be canceled due to the strong clinical reactions in three of them.

Subjects Eight healthy subjects (three female, five male, mean age 30.1 y, range 26–53 y) with no history of atopic eczema or other skin diseases participated in the study. Informed consent was obtained from all of them.

Application of SLS The different concentrations of SLS (0.1%, 0.2%, 0.3%; 0.5%, 0.75% SLS dissolved in water) were applied with large Finn chambers (12 mm, filling volume 0.05 ml) and fixed with an overlapping sheet of Fixomull stretch tape (Beiersdorf, Hamburg, Germany). The chambers were removed after 30 min of exposure. A second exposure was performed at the

same day after 3.5 h. Using this scheme of application, the volunteers were treated for 3 d.

Comparison of anatomic sites The forearm and the back were used as sites for testing the efficacy of TC. As there can be intra-individual variation between different sites in their threshold tolerance of irritants (Judge *et al*, 1996), we compared the pattern of response in the forearm (A) and the back (B) in all of our subjects.

Site A: The volar side of the right forearm. Because of the instability of TEWL at the wrist and the enhancement of irritancy on proximal parts of the arm (Van der Valk and Maibach, 1989; Panisset *et al*, 1992), the five areas were located in the middle part of the forearms, 10 cm above the wrist skinfold. Two further test sites served as controls (treated with water or remaining untreated).

Site B: The paravertebral skins of the mid-back, and the test fields (two vertical rows with four chambers each) were randomized. The designation of SLS concentrations and controls to the test sites was randomized among the panelists.

Studying the effect of TC on irritation in a cumulative model (n = 13) In 13 volunteers (six female, seven males) the test fields on the forearm and the back were pretreated with the above-mentioned TC 10 min before the two different concentrations of SLS (0.75% and 0.5% SLS) were applied. Two further test sites on the back and forearm remained only exposed to the irritant. Water served as control. Regarding the pretreatment a standardized amount (0.05 ml) of the TC was subsequently applied to the allotted areas of 2 cm^2 on the forearms and back for 1 min with a gloved finger. In the case of remnants of the product being on the skin surface they were removed with absorbent paper tissue before irritation via different SLS concentrations was performed.

The chambers were removed after 30 min of exposure. A second exposure and pretreatment were performed at the same day after 3.5 h. Using this scheme of application (see above), the volunteers were treated for 3 d. TEWL measurements were performed at day 1 before the first application and at day 3 2 h after the last application. In six of the volunteers the TEWL was additionally recorded 2 h after the second exposure on day 2 (**Fig 7**). TEWL measurements were continued daily until baseline values of TEWL were reached by the test field irritated with 0.5% SLS and pretreated with the TC.

Test cream: Lindesa (Faweco, Darmstadt, Germany) with beeswax. Further constituents: water, glyceryl stearate SE, paraffinum liqidum, stearic acid, fragrance, methyl parabene, and allantoine.

The above-mentioned barrier cream had been selected due to clinical experience and observations in a population of hairdressers and metal workers, where it has been proven to reduce the frequency of irritant contact dermatitis.

Because there were no scientific data on the optimal timing of the application of barrier creams regarding the contact with the irritant, we have chosen an application time of 10 min before exposure to the irritant.

RESULTS

Structural basis of SLS irritation

SLS-treated skin after 24 h exposure Visual scoring: clinical grading of the 0.5% SLS test sites showed scaling (1+) in five of seven subjects. One additional subject also showed erythema (1+). With 1% SLS, five of seven subjects showed intense redness with scaling (2+) and one with edema, fissures, and vesicles (3+). Monitoring of the nonbiopsied test areas showed a normalization of TEWL after 10–12 d.

In the subjects exhibiting dry skin (a scaly surface) induced by 0.5% SLS, a one layered transit-cell zone, a sign of premature keratinization, and lipid droplets (L) were found in the matrix of horny cells (**Fig 1*a***). The epidermal lipids showed a lamellar arrangement (**Fig 1*b***). With 1% SLS, and to lesser degree with 0.5% SLS, the intercellular spaces showed focal intercellular edema. Disturbance of the rearrangement of lamellar body (LB) sheets into parallel lipid layers appeared in the lower SC (stratum compactum) as demonstrated by routine fixation of the 1% SLS-irritated tissue. The cornified envelope displayed broadening of its electron-dense margin as a sign of structural alteration of the horny cell as demonstrated by routine fixation (**Fig 2**). In some areas, especially in the stratum granulosum and the upper stratum spinosum of the epidermis, paranuclear vacuoles (V), indenting one side of the nuclei of the keratinocytes, were present. Simultaneously, widening of epidermal intercellular spaces partially filled with a homogeneous gray granular substance was present (**Fig 3**).

After short-term exposure Biopsies that were performed a few hours after short-term exposure did not show parakeratosis. There were no

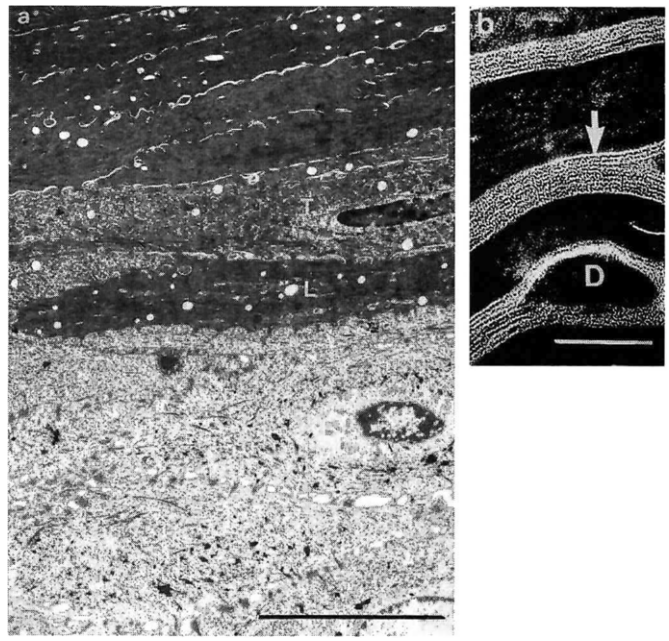

Figure 1. SC alterations induced by SLS. (*a*) Lipid droplets (L) in the matrix of the corneocytes and a transit cell (T) can be seen (RuO_4; *scale bar*, 10 μm). (*b*) Regular lamellar arrangement of epidermal lipids (*white arrows*) in the upper regions of SC in spite of SLS exposure. (D, desmosome; RuO_4; *scale bar*, 0.1 μm.)

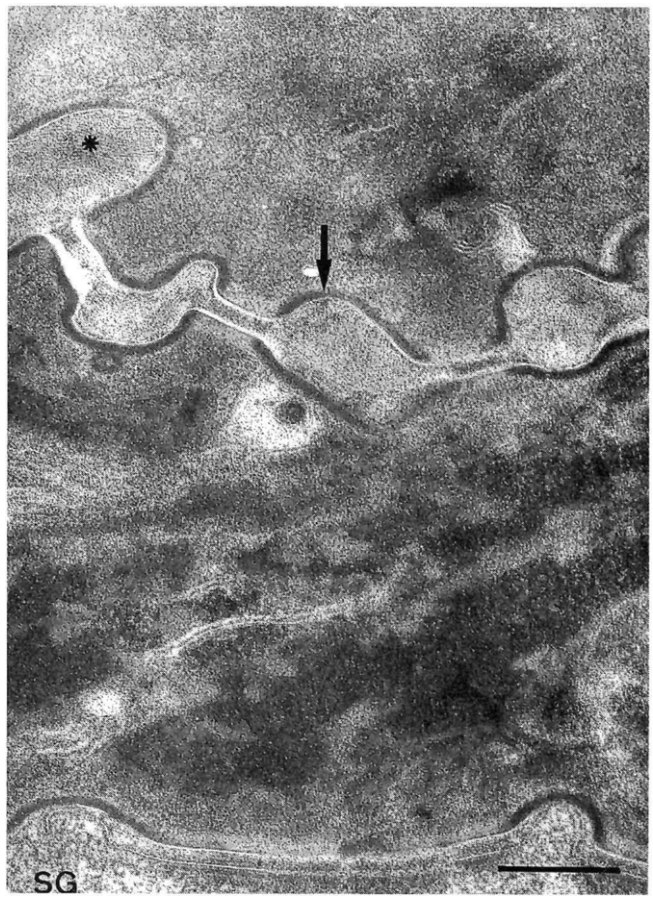

Figure 2. SC–stratum granulosum interface: the intercellular spaces showed widening. Instead of regular lamellar body-lipids (sheets), the intercellular spaces of the lower SC (stratum compactum) revealed granular material (*). The cornified envelope displayed broadening of its electron-dense margin (*arrow*) as a sign of structural alteration of the horny cell. (SG, stratum granulosum; OsO_4; *scale bar*, 1 μm.)

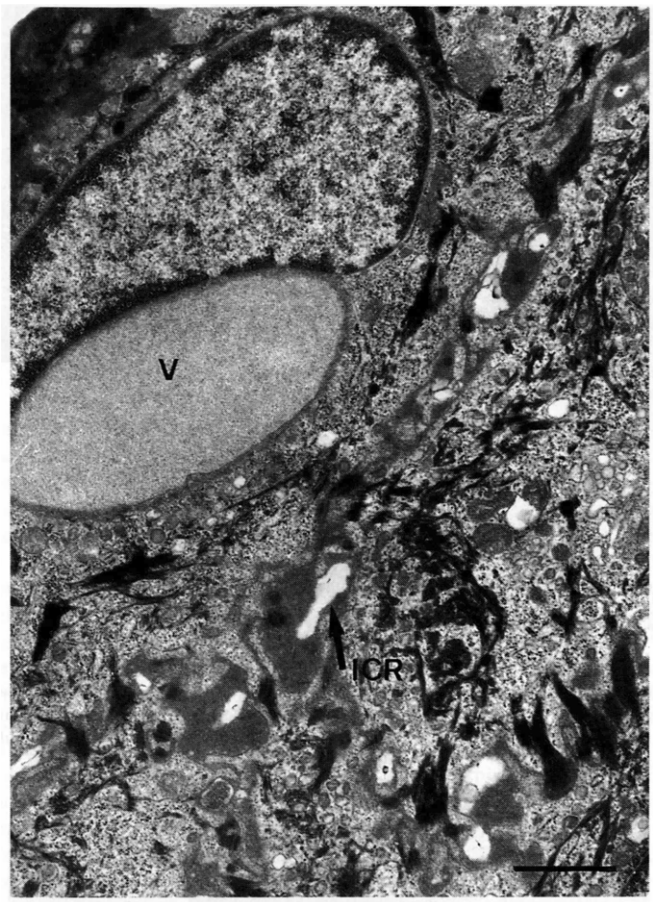

Figure 3. Paranuclear vacuoles (V), indenting one side of the nuclei of the keratinocytes, were present especially in the stratum granulosum and the upper stratum spinosum of the epidermis. Widening of epidermal intercellular rooms (ICR) partially filled with a homogeneous gray granular substance was present (OsO_4; *scale bar*, 1 μm).

signs of alteration regarding the barrier structures or the nucleated parts of the epidermis.

Cumulative short-term exposure with and without TC The observed changes were comparable with the long-term exposure and showed lipid droplets located in the corneocytes. Marked intercellular edema was seen in the nucleated parts of the epidermis. Influx of the granular substance into the intercellular spaces of the epidermis appeared in all regions of the epidermis. With RuO_4 staining a disturbance of LB lipid transformation into regular arranged epidermal lipids was seen, whereas the upper regions of stratum disjunctum still showed regular lamellar lipid structures. The pretreated areas showed similar alterations but the observed changes where less pronounced, with only a few vacuoles in the nucleated parts of the epidermis. There were only focal areas with alterations of LB sheet formation, and in most of the parts of the stratum compactum lamellar arrangement of epidermal lipids could be found.

SLS susceptibility of the back compared with the forearm Determination of the threshold tolerance to irritants: the back showed significantly higher clinical scorings than the forearm (**Fig 4*a*, *b***). On the forearm clinical signs of irritation were not visible on day 1. Only a moderate response was seen on day 3. On the back clinical responses were already visible on the first day, and stronger reactions on day 2 and 3.

The increase of TEWL values (ΔTEWL), the difference between an individual's baseline TEWL (pre-exposure on day 1) and increased TEWL after irritation (on day 3), was more pronounced with higher concentrations of SLS (**Fig 5*a*, *b***). The distributions of ΔTEWL are shown as box plots. There was a statistically significant difference between the applied SLS concentrations and the control sites ($p < 0.02$).

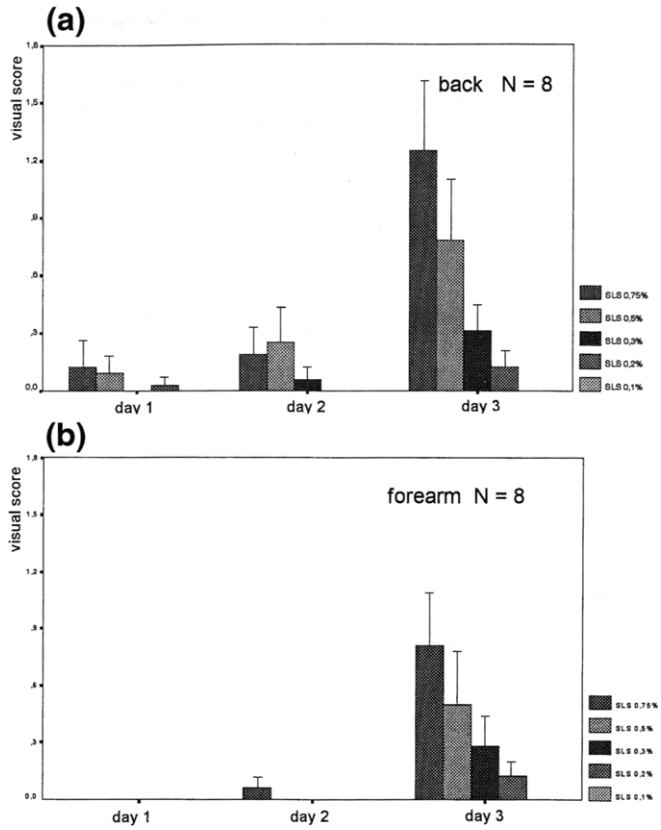

Figure 4. Clinical score (mean ± SEM) after irritation with different concentrations of SLS at the back (*a*) and forearm (*b*). The back showed significantly higher clinical scorings than the forearm. On day 1, clinical signs of irritation were not visible on the forearm, but were on the back.

But the irritation with 0.1% SLS showed no statistical difference compared with the water control. The variability of ΔTEWL values increased with increasing SLS concentrations (see length of box and of whiskers).

The back showed higher TEWL values than the forearm. Additionally, the ΔTEWL of the back showed a higher interindividual variability of values. There was a statistically significant correlation between the reaction pattern of the arm compared with the forearm (Spearman correlation coefficient $R > 0.7$).

Because concentrations of 0.1%, 0.2%, and 0.3% of SLS in some of the volunteers induced only a small increase in TEWL regardless of the test sites, 0.5% and 0.75% SLS have been chosen for the efficacy studies.

The irritant response in a cumulative model diminished significantly after the application of the TC The study suggested that irritation of the skin with 0.5% and 0.75% SLS would show sufficient positive reaction in the panelists. The intensity of irritation was diminished by the pretreatment with a cream. This was shown by clinical scoring and by the decrease of TEWL (**Fig 6*a*, *b***). Comparison with the untreated but irritant exposed sites (0.5% SLS) showed a decrease of TEWL enhancement by 58% (back) and 49% (forearm) (**Table I**). After irritation with 0.75% SLS the TEWL was diminished by 56% (back) and 43% (forearm). Monitoring of TEWL showed an increase of ΔTEWL regardless of the fact that there has been a pretreatment on day 1. On day 2 a plateau was reached due to the treatment with TC (see, for example, **Fig 7*a*, *b***, where in six volunteers TEWL had been measured on day 2 as well).

DISCUSSION

To study the reaction of the skin barrier to detergents, subjects have been exposed to different concentrations of the anionic surfactant SLS. In "every day" life the contact is usually cumulative and of short

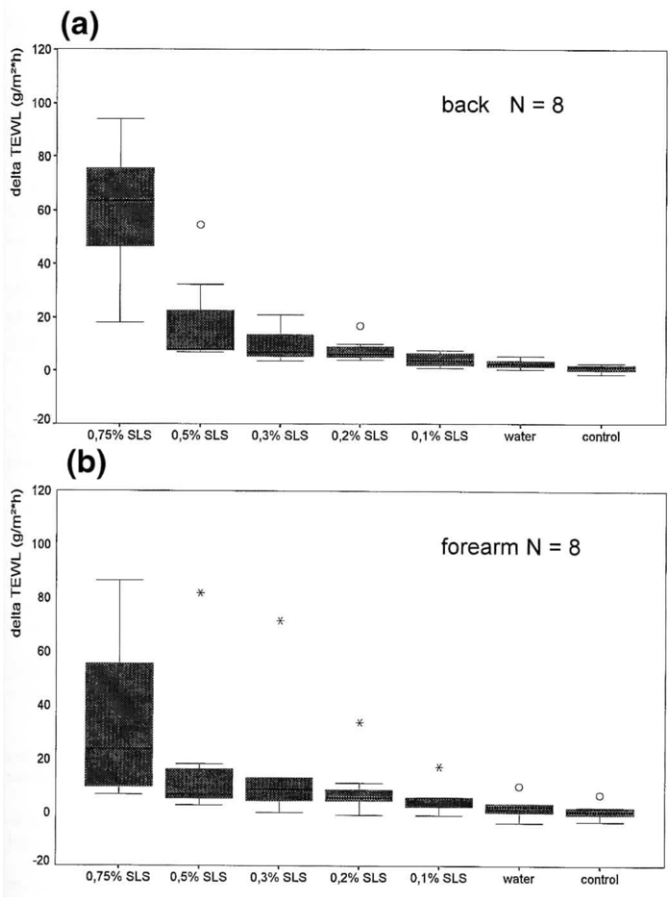

Figure 5. Distributions of ΔTEWL values. ΔTEWL is the difference between an individual's baseline TEWL (pre-exposure on day 1) and increased TEWL after irritationm (on day 3)] after irritation with different concentrations of SLS on the back (*a*) and forearm (*b*). Data are shown as box plots. The irritation was more pronounced with higher concentrations of SLS and was higher on the back compared with the forearm.

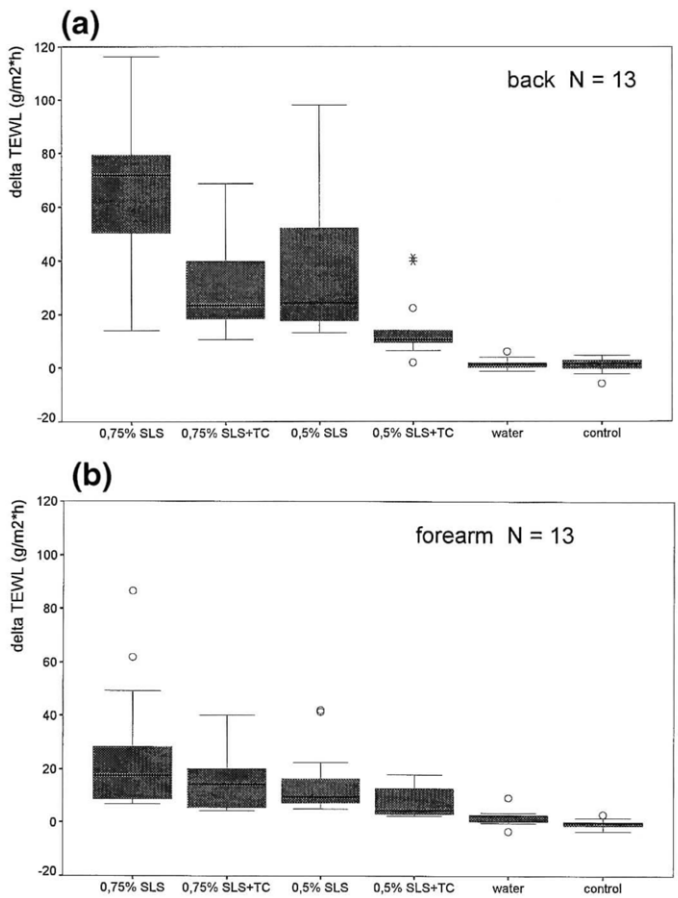

Figure 6. Distributions of ΔTEWL values after 3 d irritation with different concentrations of SLS (0.75%, respectively, 0.5%) with and without application of TC compared with irritation with water and with the control site. Data are shown as box plots. The irritation was more pronounced on the back compared with the forearm.

duration. This was the reason why a cumulative model was chosen for our studies on humans.

Studying the irritation by SLS ultrastructurally and functionally, we could show that the topical treatment with an anionic detergent such as SLS seems to alter the permeability barrier by mechanisms that were different from the process induced by an organic solvent. Whereas with acetone the extraction of SC lipids seems to be the main cause of barrier disruption (Yang *et al*, 1995; Fartasch, 1997) and barrier recovery seems to improve very quickly, the situation with detergents appears to be more complex (Yang *et al*, 1995). It has been suggested that detergents, such as SLS, may possibly cause more extensive damage, e.g., the denaturation of proteins, which would result in alterations that cannot be ameliorated by providing externally applied lipid mixtures alone. It has been suggested that SLS might penetrate and damage the deeper nucleated layers of the epidermis (Yang *et al*, 1995).

Our ultrastructural studies have shown that SLS at the concentration applied did not extract lipids like has been shown after application of acetone (Fartasch, 1995, 1997), but rather caused extensive damage to the deeper nucleated layers of the epidermis. A predominant feature of cutaneous response to the detergent was parakeratosis, the retention of nuclei within the SC and lipid droplets (Willis *et al*, 1989). Such an appearance may have a number of causes, including increased epidermal cell proliferation, accelerated keratinization, or direct cytotoxic injuries (Willis *et al*, 1991). In some areas the epidermal intercellular spaces were filled with a granular substance similar to that described by Tovell *et al* (1974), and probably representing serum influx into the epidermis.

The supposition that surfactants degrease the skin (e.g., a selective depletion of the lipids from the intercellular spaces; Proksch *et al*, 1991) has persisted in the literature (Imokawa *et al*, 1989). In this study, the upper SC showed regular lamellar arrangements of lipids after exposure to SLS for 24 h. This corroborates biochemical studies that have shown that in experimentally SLS treated skin the amount of ceramides did not differ from that in healthy control skin, with only a 4–7% removal of lipids (Froebe *et al*, 1990). This contradicts the long-standing belief that surfactants damage the skin by delipidization (Froebe *et al*, 1990). Our study corroborates the findings of Fulmer and Kramer (1986), who observed changes in lipid composition that did not reflect the immediate consequences of surfactant exposure. Ultrastructurally, the surfactant in low concentration did not seem to alter the existing lipid structure, but rather the processing into new epidermal lipids by: (i)

Table I. Reduction of the increase of TEWL due to the application of TC

	TEWL reduction[a] (p value)[b]
Back: Irritation with SLS concentration of 0.75% after application of TC compared with no TC application	56% ($p < 0002$)
Back: Irritation with SLS concentration of 0.5% after application of TC compared with no TC application	58% ($p < 0002$)
Forearm: Irritation with SLS concentration of 0.75% after application of TC compared with no TC application	43% ($p < 0002$)
Forearm: Irritation with SLS concentration of 0.5% after application of TC compared with no TC application	49% ($p < 0005$)

[a]Data are expressed as percentage of maximum water loss, i.e., 100% is ΔTEWL without TC.
[b]Wilcoxon test.

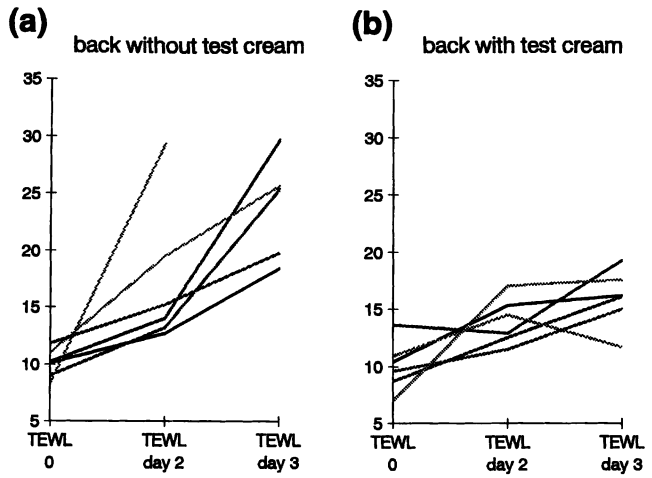

Figure 7. Monitoring of TEWL showed an increase of ΔTEWL regardless of the fact that there was a pretreatment on day 1. On day 2 a plateau was reached due to the treatment with TC (*b*), whereas the nontreated irritated areas show a steep increase of TEWL (*a*).

the direct alteration of the LB-secretory system of the stratum granulosum cells; (ii) disturbing the processing of LB-derived sheets (glucosylceramides) into bilayer structures in the intercellular spaces of the SC; and (iii) indirectly inducing a disturbed differentiation by alterating the viable epidermis (intracellular vacuoles, cytoplasmic edema, and widening of intercellular spaces). All these changes might explain the lasting TEWL enhancement since normalization of barrier function first occured after 10–12 d.

The short-term exposure in the above-mentioned cumulative model showed similar ultrastructural changes after the 3 d (but to a lesser degree with the TC). This can be explained by the tendency of SLS to accumulate in the epidermis. Studies on rat skin have shown that traces of SLS persist in tissues 7 d after a single 24 h exposure of 1% SLS – with higher levels in epidermis than in dermis (Patil *et al*, 1995; Szolar-Platzer *et al*, 1996). Therefore, it is possible that accumulation of SLS in the epidermis after and during the cumulative exposure with low concentrations which normally would not have evoked irritative reactions in single exposure finally induces irritation.

Results of animal experiments may not be valid for humans, particularly when dealing with irritants, in view of their complex action mechanisms and the high interindividual variability in susceptibility of human skin (Zhai and Maibach, 1996). *In vivo* methods (the biophysical techniques, like TEWL measurement) in human are based on the assessment of the reduction in the induced irritant and inflammatory changes in the skin when a barrier cream is used before application of an irritant. To study the efficacy of barrier creams to prevent irritation by SLS and whether the barrier recovery is influenced by TC, we had first to determine the degree of irritation. The concentrations of 0.5% and 0.75% SLS seemed to be optimal for our model. As expected, the clinical tissue response to SLS exposure was quite varied in the different volunteers (Judge *et al*, 1996). The back proved to be much more susceptible to irritation than the forearm, especially when concentrations of 0.75% were applied; however, the back showed the same pattern of response, with a retarded response to SLS than the forearm. This difference was also visible when the TC had been applied.

Pretreatment and treatment during the cumulative irritant exposure influenced the barrier properties of surfactant-damaged skin. The irritant response to SLS became significantly less pronounced after treatment of normal skin with the cream. The effect was more pronounced after the second and third day. The mechanism of these beneficial effects is not yet fully understood. The benefit of barrier creams in SLS-damaged skin seems not to be in resubstituting extracted lipids, as has been shown in previous studies (Yang *et al*, 1995; Mao-Qiang *et al*, 1996). Also, the internal structures of the lamellar bodies seemed not to be influenced by the TC, as it has been shown when applying so-called physiologic lipid mixtures.

But lipophilic barrier creams, like our TC, might alter the penetration of substances into the skin by interaction between the barrier cream and the substance or interaction between the barrier cream and SC (De Fine Olivarius *et al*, 1996). An important aspect might be that the lipophilic TC acts comparably with petrolatum (Ghadially *et al*, 1992; Mao-Qiang *et al*, 1995; Wigger-Alberti and Elsner, 1997), where repair of the barrier occurs by forming a bulk hydrophobic phase in the SC interstices (Mao-Qiang *et al*, 1996). The interaction of the cream with components of the barrier might influence and stabilize barrier function so that cytokine expression and the resulting induction of dermal inflammatory reaction are diminished.

We have to take into account, however, that the data of the cumulative test were generated with a model surfactant; it remains to be determined whether similar responses will be noted with chemicals of different physicochemical properties.

The study was supported by Ministry of Science and Technology (BMBF) grant 01HK9195.

REFERENCES

Agner TT, Serup TJ: Sodium lauryl sulfate for irritant patch testing – A dose–response study using bioengineering methods for determination of skin irritation. *J Invest Dermatol* 95:543–547, 1990

Basketter TDA, Griffiths THA, Wang TXM, Wilhelm TKP, McFadden TJ: Individual, ethnic and seasonal variability in irritant susceptibility of skin: the implications for a predictive human patch test. *Contact Dermatitis* 35:208–213, 1996

Blank TIH: Cutaneous barriers. *J Invest Dermatol* 45:249–256, 1965

De Fine Olivarius TF, Hansen TAB, Karlsmark TT, Wulf THC: Water protective effect of barrier creams and moisturizing creams: a new in vivo test method. *Contact Dermatitis* 35:219–225, 1996

Di Nardo TA, Sugino TK, Wertz TP, Ademola TJ, Maibach THI: Sodium lauryl sulfate (SLS) induced irritant contact dermatitis: a correlation study between ceramides and in vivo parameters of irritation. *Contact Dermatitis* 35:86–91, 1996

von den Driesch TP, Fartasch TM, Huner TA, Ponec TM: Expression of integrin receptors and ICAM-1 on keratinocytes in vivo and in an in vitro reconstructed epidermis: effect of sodium dodecyl sulfate. *Arch Dermatol Res* 287:249–253, 1995

Fartasch TM: Human barrier formation and reaction to irritation. *Curr Probl Dermatol* 23:95–103, 1995

Fartasch TM: Ultrastructure of the epidermal barrier after irritation. *Microsc Res Tech* 37:193–199, 1997

Fartasch M, Bassukas ID, Diepgen TL: Structural relationship between epidermal lipid lamellae, lamellar bodies and desmosomes in human epidermis: An ultrastructural study. *Br J Dermatol* 128:1–9, 1993

Fartasch M, Ponec M: Improved barrier structure formation in air-exposed human keratinoctye culture systems. *J Invest Dermatol* 102:366–374, 1994

Froebe TCL, Simion TFA, Rhein TLD, Cagan TRH, Kligman TA: Stratum corneum lipid removal by surfactants: relation to in vivo irritation. *Dermatologica* 181:277–283, 1990

Frosch TPJ, Kurte TA: Efficacy of skin barrier creams (IV). The repetitive irritation test (RIT) with a set of 4 standard irritants. *Contact Dermatitis* 31:161–168, 1994

Frosch TPJ, Kurte TA, Pilz TB: Efficacy of skin barrier creams (III). The repetitive irritation test (RIT) in humans. *Contact Dermatitis* 29:113–118, 1993

Fulmer TAW, Kramer TGJ: Stratum corneum lipid abnormalities in surfactant-induced dry scaly skin. *J Invest Dermatol* 86:598–602, 1986

Ghadially TR, Halkier-Sorensen TL, Elias TPM: Effects of petrolatum on stratum corneum structure and function. *J Am Acad Dermatol* 26:387–396, 1992

Hannuksela TA, Kinnunen TT: Moisturizers prevent irritant dermatitis. *Acta Derm Venereol (Stockh)* 72:42–44, 1992

Huener TA, Fartasch TM, Hornstein TOP, Diepgen TTL: The irritant effect of different metalworking fluids. *Contact Dermatitis* 31:220–225, 1994

Imokawa TG, Akasaki TS, Minematsu TY, Kawai TM: Importance of intercellular lipids on water retention properties properties of the stratum corneum: Induction and recovery study of surfactant dry skin. *Arch Dermatol Res* 281:45–51, 1989

Judge TMR, Griffiths THA, Basketter TDA, White TIR, Rycroft TRJ, McFadden TJP: Variation in response of human skin to irritant challenge. *Contact Dermatitis* 34:115–117, 1996

Loden TM: Barrier recovery and influence of irritant stimuli in skin treated with a moisturizing cream. *Contact Dermatitis* 36:256–260, 1997

Mao-Qiang TM, Brown TBE, Wu-Pong TS, Feingold TKR, Elias TPM: Exogenous nonphysiologic vs physiologic lipids. divergent mechanisms for correction of permeability barrier dysfunction. *Arch Dermatol* 131:809–816, 1995

Mao-Qiang TM, Feingold TKR, Thornfeldt TCR, Elias TPM: Optimization of physiological lipid mixtures for barrier repair. *J Invest Dermatol* 106:1096–1101, 1996

Mortz TCG, Andersen TKE, Halkier-Sorensen TL: The efficacy of different moisturizers on barrier recovery in hairless mice evaluated by non-invasive bioengineering methods. *Contact Dermatitis* 36:297–301, 1997

Nickoloff TBJ, Naidu TY: Perturbation of epidermal barrier function correlates with initiation of cytokine cascade in human skin. *J Am Acad Dermatol* 30:535–546, 1994

Panisset TF, Treffel TP, Faivre TB, Brunet Lecomte TP, Agache TP: Transepidermal water loss related to volar forearm sites in humans. *Acta Derm Venereol (Stockh)* 72:4–5, 1992

Patil TS, Singh TP, Sarasour TK, Maibach TH: Quantification of sodium lauryl sulfate penetration into the skin and underlying tissue after topical application – pharmacological and toxicological implications. *J Pharm Sci* 84:1240–1244, 1995

Pinnagoda J, Tupker RA, Agner T, Serup J: Guidelines for transepidermal water loss (TEWL) measurement. *Contact Dermatitis* 22:164–178, 1990

Proksch TE, Feingold TKR, Qiang TM, Elias TPM: Barrier function regulates epidermal DNA synthesis. *J Clin Invest* 87:1668–1673, 1991

Szolar-Platzer TC, Patil TS, Maibach THI: Effect of topical laurocapram (azone) on the in vitro percutaneous permeation of sodium lauryl sulfate using human skin. *Acta Derm Venereol* 76:182–185, 1996

Tovell TPWA, Weaver TAC, Hope TJ, Sprott TWE: The action of sodium lauryl sulfate on rat skin – An ultrastructural study. *Br J Dermatol* 90:501–506, 1974

Treffel TP, Gabard TB, Juch TR: Evaluation of barrier creams: an in vitro technique on human skin. *Acta Derm Venereol (Stockh)* 74:7–11, 1994

Tsai TJC, Feingold TKR, Crumrine TD, Wood TLC, Grunfeld TC, Elias TPM: Permeability barrier disruption alters the localization and expression of TNFalpha protein in the epidermis. *Arch Dermatol Res* 286:242–248, 1994

Tupker RA, Willis E, Berardesca E, Lee CH, Fartasch M, Agner T, Serup J: Guidelines on sodium lauryl sulfate (SLS) exposure tests. *Contact Dermatitis* 37:53–69, 1997

Van der Valk TPG, Maibach THI: Potential for irritation increases from the wrist to the cubital fossa. *Br J Dermatol* 121:709–712, 1989

Wigger-Alberti TW, Elsner TP: Petrolatum prevents irritation in a human exposure model in vivo. *Dermatology* 194:247–250, 1997

Willis TCM, Stephens TCJM, Wilkinson TJD: Epidermal damage induced by irritants in man: A light and electron microscopic study. *J Invest Dermatol* 93:695–699, 1989

Willis TCM, Stephens TCJM, Wilkinson TJD: Selective expression of immune-associated surface antigens by keratinocytes in irritant contact dermatitis. *J Invest Dermatol* 96:505–511, 1991

Wilmer TJL, Burleson TFG, Kayama TF, Kanno TJ, Luster TMI: Cytokine induction in human epidermal keratinocytes exposed to contact irritants and its relation to chemical-induced inflammation in mouse skin. *J Invest Dermatol* 102:915–922, 1994

Wood TLC, Jackson TSM, Elias TPM, Grunfeld TC, Feingold TKR: Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice. *J Clin Invest* 90:482–487, 1992

Yang TL, Mao-Qiang TM, Taljebini TM, Elias TPM, Feingold TKR: Topical stratum corneum lipids accelerate barrier repair after tape stripping, solvent treatment and some but not all types of detergent treatment. *Br J Dermatol* 133:679–685, 1995

York TM, Griffiths THA, Whittle TE, Basketter TDA: Evaluation of a human patch test for the identification and classification of skin irritation potential. *Contact Dermatitis* 34:204–212, 1996

Zhai TH, Maibach THI: Effect of barrier creams: human skin in vivo. *Contact Dermatitis* 35:92–96, 1996