The Temperature Effect on *In vitro* Penetration of Sodium Lauryl Sulfate and Nickel Chloride through Human Skin

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Irritant contact dermatitis is a major problem in dermatology. One important group of substances causing irritant dermatitis is detergents. Exposure of the skin to detergents is frequent in both work and domestic environments. In the present paper we have studied how the penetration through the skin, and thus the effect, of the detergent sodium lauryl sulfate (SLS) is altered when the temperature is raised from 22°C to 40°C or 60°C. We found that the penetration of sodium lauryl sulfate increased with increasing temperature. When comparing the increased penetration of sodium lauryl sulfate with the change in NiCl penetration at the same temperatures, we found that the increase in penetration was more pronounced for the detergent. This implies that the detergent also had a different effect on the structure and function of the epidermal barrier itself. The results underline the importance of chosing the right (low) temperature when working with detergent solutions to reduce the risk of developing irritant contact reactions. *Key words: Skin penetration; Detergents; SLS; Irritant contact dermatitis.*

During the past two decades an increasing interest has been focused on irritant contact dermatitis. One type of these contact reactions is the chronic irritant dermatitis (the so-called traumiterative contact dermatitis) (1). This involves contact reactions due to repeated exposures to an irritant stimulus or the exposure to a combination of several different stimuli. One major group of irritants that might produce this type of contact reactions is detergents (2). It is known that detergents interact on several functional levels in epidermis. They have a direct effect on lipids and proteins in stratum corneum, the major diffusion barrier in the skin (3, 4). Detergents also influence the keratinocytes, the epidermal Langerhans' cells and might induce inflammation (2, 5–9). In addition, it is thought that the exposure to detergents causes deterioration of the epidermal barrier, subsequently increasing the susceptibility to other noxious, external stimuli. The effect on the skin of applied detergents will depend on several factors such as how frequently they are applied, the area of contact, the concentration of the detergent and the initial condition of the skin.

In experimental studies on irritant contact dermatitis, sodium lauryl sulfate (SLS) has often been used as a model substance (cf. 8). It has been demonstrated in vivo that the irritant response to SLS in humans depends on the dose and the frequency of application (10). Concerning the irritant capacity of substances applied to the skin, one factor that has been discussed only to a minor extent is the influence of the temperature of the skin surface. Detergents are often used at temperatures above 30°C. It is known that the absorption of substances through the skin depends on the activating energy (11). However, little is known about the relation between the temperature and irritant contact dermatitis. In human subjects it has been shown that e.g. the irritancy of lemon perfume is temperature-dependent in the interval of 23°C to 43°C (12).

Research over the last ten years has provided an increasing understanding of the structure and function of the epidermal barrier. It has been shown that the major diffusion barrier of the skin is located in stratum corneum (13). The structure of stratum corneum has been described in terms of a two-compartment model, "the brick and mortar model" (14), which visualizes the corneocytes as the bricks and intercellular lipids as the mortar. The intercellular lipids in stratum corneum have a specific composition, giving stratum corneum its unique capacities (15, 16). The structure and function of the stratum corneum lipids are, among other things, dependent on the temperature. Several studies have shown that the molecular packing of the lipids varies in the temperature interval from 22°C to 60°C (17–19). It is thus possible that the temperature of the water in which detergents are dissolved might influence the barrier function in several ways and thus determine how the detergents affect the skin. In the present study, we used an in vitro model to evaluate how the penetration of SLS through the skin (and subsequently the effect on epidermis) varies with the temperature in the range of 22°C to 60°C. To our knowledge, this has not previously been studied in human skin.

MATERIALS AND METHODS

Chemicals

For the diffusion studies, SLS labelled with 35-S, specific activity 0.2 mCi/ml, and $NiCl_2$ labelled with 63-Ni, specific activity 1 mCi/ml, were obtained from Amersham International, Stockholm, Sweden. Unlabelled SLS, purity grade 99.9%, was obtained from KEBO AB, Stockholm, and unlabelled $NiCl_2$ from E. Merck, Darmstadt.

Skin specimens

Full thickness human skin was obtained from plastic reconstruction surgery of female breast. The skin was either used at once or kept frozen at −28°C for various periods of time before experiments were started. The skin was completely covered with gauze and packed in plastic bottles when frozen. When thawing the skin, the procedure was to let the skin be in its packaging, either at, ambient temperature (22°C) for 3–4 h or 7–8 h in a refrigerator (temperature 4°C).

Table I. *The penetration of 2% SLS through human skin at 22°C, 40°C and 60°C*

The results from all samples. The scintillation CPM values are given. (CPM = counts per minute.)

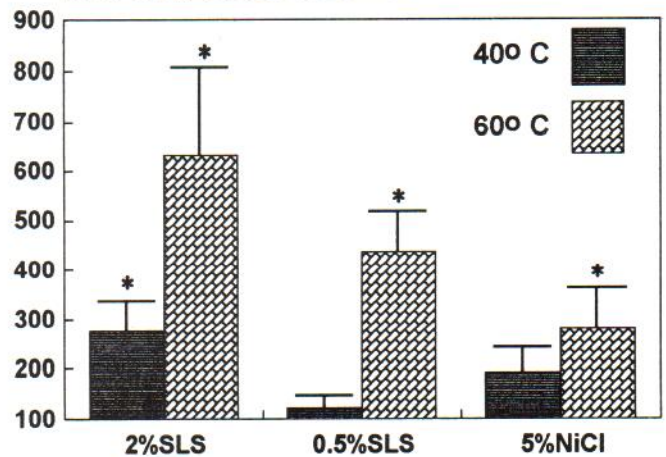| Temperature	22°C	40°C	60°C
Mean	308	602	1250
Median	96	389	526
Minimum	45	57	120
Maximum	1447	2416	5547
Number of samples	16	16	16
Statistical analysis compared to 22°C		$p<0.05$	$p<0.05$

Fig. 1. The increase in penetration at 40°C and 60°C is given in the figure. Penetration at 22°C is set to 100. *CPM* = counts per minute at scintillation. * = $p <0.05$ compared to the penetration att 22°C. Mean and standard error of the mean are given.

Diffusion cell model

A horizontal static diffusion cell model was used for the penetration studies. Distilled water was used as vehicle to avoid the protein-extracting capacity of physiological salt solutions.

Diffusion cell studies

The whole skin was mechanically freed from subcutanous fat tissue, using a razor blade, and cut into three pieces, approximately 1.5 cm × 1.5 cm. The skin samples were then mounted in three diffusion cells with the epidermal side of the skin towards the donor chamber. The area of skin exposed to solutes was 1.33 cm². The volume of each chamber was 60 ml. All three diffusion cells were prepared and run at the same time at ambient temperature (22°C) and with thermostatic control at 40°C and 60°C respectively, for a period of 18 h. The choice of penetration time was decided upon after a pre-study of different penetration times: 3, 6, 12, 18 and 30 h. It was found that for penetration periods shorter than 18 h the detectable amount of 35-S-SLS was close to background level. The following different donor solutions were used in the study:

1) 2% SLS with 35-S-SLS added. A 2% SLS solution (w/v) with 0.5 ml 35-S-SLS added, i.e. total activity 0.03 mCi in each donor chamber. Total number of triplet sets (*n*) = 16, frozen <1 month *n* = 10, frozen ≥1 month *n* = 6.
2) 0.5% SLS with 35-S-SLS added. A 0.5% SLS solution (w/v) with 0.5 ml 35-S-SLS added, i.e. total activity 0.03 mCi in each donor chamber. *n* = 15, all frozen <1month.
3) 5% NiCl with 63-Ni added. A 5% NiCl solution (w/v) with 0.5 ml 63-Ni added, i.e. total activity 0.17 mCi in each donor chamber. *n* = 14, all frozen <1 month.

Scintillation

The radioactivity in donor and recipient chamber was determined by liquid scintillation counting (Packard liquid spectrometer system, model 2425, Packard Inc.) using Biofluor (NEN Research) scintillation fluid. Determination of background level was performed on sample of pure scintillation fluid (*n* = 20) and was established for both the 35-S and 63-Ni experiments. In addition, dilution series were performed with 35-S-SLS in a 2% SLS solution (w/v) and 63-Ni in 5% NiCl solution to establish that there was a linear correlation between the amount of radioactivity and measured counts per minutes (CPM values). At the end of the experiment (t = 18 h), a 2 ml sample of the donor solution was taken and 3 samples, each 2 ml, were taken from the recipient chamber of each temperature set. The sample solution was dried in heat chambers overnight. Four ml Biofluor scintillation fluid was then added. Samples were mechanically shaken by a Heidolph DSG 304 machine, and the radioactivity was determined by scintillation 48 h after addition of the scintillation fluid. An average CPM value was calculated for each recipient chamber, and the data of the 22°C experiment was set to 100%, to which the measured data at 40°C and 60°C were related. If the radioactivity in the donor solution was reduced with more than 10%, the experiment was discarded due to an assumed barrier damage.

Processing of measured data

The penetration at 40°C and 60°C was compared with the penetration at 22°C in each group, using the non-parametric Wilcoxon's rank sum test. A *p* value lower than 0.05 was considered significant.

RESULTS

The penetration of 35-S-SLS (2% applied) at 40°C and 60°C

Table II. *The penetration of 0.5% SLS through human skin at 22°C, 40°C and 60°C*

The scintillation CPM values are given. (CPM = counts per minute.) N.S. = not significant.

Temperature	22°C	40°C	60°C
Mean	247	252	579
Median	78	87	319
Minimum	55	62	148
Maximum	2263	2265	3134
Number of samples	15	15	15
Statistical analysis compared to 22°C		N.S.	$p<0.05$

Table III. *The penetration of 5% NiCl through human skin at 22°C, 40°C and 60°C*

The scintillation CPM values are given. (CPM = counts per minute.) N.S. = not significant.

Temperature	22°C	40°C	60°C
Mean	179	386	463
Median	150	159	269
Minimum	95	108	122
Maximum	520	1799	1982
Number of samples	14	14	14
Statistical analysis compared to 22°C		N.S.	$p < 0.05$

was significantly increased (Table I, Fig. 1) as compared to "baseline" penetration at ambient temperature (22°C). When considering a possible difference between non-frozen and frozen samples, we found that the increase in penetration related to temperature was not as high in the samples which had been frozen ≥1 month. The penetration of 35-S-SLS (0.5% applied) was significantly increased at 60°C but not at 40°C (Table II, Fig 1). The same result was found for NiCl, where the penetration of 63-Ni was significantly increased at 60°C but not at 40°C (Table III, Fig. 1).

DISCUSSION

In the present study, it is demonstrated that in the in vitro modell with diffusion chambers, the penetration of SLS increases when the temperature is raised from 22°C to 40° or 60°C. The increase in penetration was higher when a higher concentration of SLS was applied.

In vitro models with diffusion chambers have been used for several years in studies of percutaneous absorption (cf. 20, 21). It must be remembered that this technique has limitations but the methodology can provide accurate predictions on in vivo absorption (22). Performing experiments on skin specimens stored at low temperatures is a possible source of error. The use of human skin stored at a low temperature has been reported previously. Major effects on the barrier function were not detected when 3H water was used to evaluate the penetration through skin stored for various time intervals up to 1 year (20). There are other data indicating that storage at a low temperature of hydrated skin specimens might alter the penetration of drugs (23). Our results show that, for SLS, there was a minor difference in the skin penetration when the skin samples were stored for longer periods than 1 month. There are three other steps in the handling of skin specimens that might influence the barrier function of the skin, that is the mounting in the diffusion chambers with the hydration of the skin, the possibility of a mechanical injury at separation of the sub-cutaneous fat from dermis and the performance of experiments at 60°C, as this temperature can be used to separate dermis from epidermis. Bond et al. (24) have reported that hydration of human stratum corneum does not significantly affect the penetration barrier over a period of 8 days (192 h). Thus, in this study, it seems unlikely that the hydration of stratum corneum has affected the penetration barrier in a

crucial manner, due to the relatively short penetration time (18 h). Temperature separation of epidermis from dermis is possible by immersing the skin sample in 60°C water or bringing it in contact with a polished metal surface holding 60°C. However, the separation rift generally occurs between the viable epidermis and the dermis (25). This means that the gross morphology of the barrier part of the epidermis is little affected. Looking at our primary scintillation data, there is a variation in the obtained data. It is known that in vitro measurements with diffusion chambers produce data with a marked variation even for control materials (26). The use of full thickness (epidermis + dermis) skin in the diffusion chamber might introduce a more pronounced inter-individual variation than that seen in experiments with separated epidermis (20). We believe that by using three adjacent skin specimens from the same patient in each experiment (22°, 40° and 60°C), we have virtually eliminated experimental procedures as a cause of the registered increased penetration.

Penetration of detergents through human skin was first demonstrated by Blank & Gould in 1959 (27). It has been shown that the penetration of SLS is a function of the concentration applied (28). However, the relation is not linear, and the concentration of free SLS is related to the critical micellar concentration (CMC) which is 0.24% for SLS at 22°C (29). The penetration of SLS increased more when going a) from a concentration lower than CMC (0.1%) to a concentration higher than CMC (1.0%), than going b) from a concentration higher than CMC (1.0%) to an even higher concentration (10%) (28). The increase in penetration was in the latter case linear. In our study we used two concentrations of SLS, 0.5% and 2.0%, which are concentrations commonly used in experiments of irritancy (cf. 8). In the temperature interval 22°–55°C the CMC for SLS will vary from 0.24% to approximately 0.27% (29). Thus the penetration of 35-S-SLS in our study is unlikely to have been affected by a shift in penetration capacity due to changes in CMC in the temperature interval used.

The fact that the penetration of the 2.0% SLS solution was higher than that of the 0.5% solution and of NiCl indicates that the increase is not only dependent on a pure temperature effect on SLS. One possible explanation is that SLS extracts both lipids and proteins from stratum corneum and that the application of a higher concentration of SLS could have a more pronounced effect on the barrier function of stratum corneum. Blank et al. demonstrated (30) that by removing the lipids from stratum corneum, they decreased the activating energy for water and both polar- and non-polar substances, thus increasing the absorption. In addition, detergents are known to interact with biological membranes and the effect may be an increased permeability (31). In stratum corneum this might lead to an interaction with the intercellular lipid bilayers, inducing increasing disorder in the packing of the lipids (17), and in addition causing a decrease of the transition temperature for the layer as a whole (31). Another tentative explanation is that the molecular arrangement of lipids in stratum corneum does depend on the temperature. Available data suggest that the thermal transition of the stratum corneum lipids from the close packed gel state to a more fluid crystalline state occurs within the temperature range of 38°C to

40°C (19), i.e. at temperatures well above normal skin temperature.

The conspicuous increase in SLS (2% solution) permeability at 40°C compared to that at ambient temperature is in harmony with the concept that in the fluid crystalline state, at temperatures above the transition temperature, the lipid bilayer units are less densely packed. This will result in a higher permeability for water and other compounds. At 60°C the lipid bilayers show a still higher degree of disorder, which may even approach a chaotic state. Hence the barrier function will drop towards a minimum.

The penetration of NiCl did not increase as much as did SLS. In 1965 Wahlberg (32) determined the penetration of metal salts (sodium chromate, cobaltous and mercuric chlorides) through human and guinea-pig skin using diffusion chambers at different temperatures. He found that the absorption was higher at 34°C than at 24°C. When comparing the results from the two different SLS solutions with the results from the NiCl solution, we believe that the difference demonstrates that SLS interacts with the intercellular lipid bilayers in a rather complex manner. The SLS interaction with the lipid bilayer obviously affects the penetration more than is the case for NiCl. It is in this context of interest to note the enhancement of Ni penetration caused by SLS when Ni and SLS were applied together on guinea-pig skin (33).

In conclusion, in the present study we have shown that the penetration of SLS and Ni through the epidermal barrier increases as the temperature of the solution is increased. This is probably in part dependent on the increase in chemical activity brought about by increasing the temperature. However, the increase in SLS penetration seems to depend also on other factors, such as the noxious effect on the barrier itself and possibly an alteration in the molecular structure of the lipid phase of stratum corneum due to the increased temperature. This implies that when considering possible mechanisms for irritant contact dermatitis caused by detergents, the temperature at which the products are used should be taken into account.

ACKNOWLEDGEMENTS

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