

Efficacy of stratum corneum lipid supplementation on human skin

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Recent studies suggest that supplementing intercellular lipids of the stratum corneum in ageing populations or in people with dry skin can stimulate the functioning of the skin. This work lends support to the reinforcement capacity of two different stratum corneum lipid mixtures (synthetic stratum corneum lipid mixtures, SSCL, and internal wool lipid extracts, IWL) formulated as liposomes on healthy skin of two differently aged groups of individuals. Protection of healthy skin against detergent-induced dermatitis was evaluated. Transepidermal water loss and capacitance were used to evaluate the effect of these formulations in *in vivo* long-term studies. Increase in water-holding capacity is obtained only when the formulations applied are structured as liposomes. This is slightly more pronounced for aged skin. Subsequent SLS exposure reflected the protection of healthy human skin against detergent-induced dermatitis. Slightly better results were obtained with IWL containing a mixture of natural ceramides than with SSCL with only one ceramide present in the formulation. All these results support the beneficial effects of skin lipid supplementation given their resemblance to the lipids in the stratum corneum both in composition and in the structuring of the formulation.

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Ageing skin and the increasing stress to which human skin is exposed today are some reasons for the growing interest in skin care. Supplementing the skin with compounds that are naturally found in the skin but which, as a result of age, health or environmental conditions are present in skin at suboptimal levels (1), can stimulate the functioning and conditioning of the skin (2).

The epidermal permeability barrier is known to be mediated by a series of intercellular bilayers in the stratum corneum which is rich in free fatty acids, cholesterol and ceramides (3). This particular composition allows a highly ordered arrangement of lipids known as lamellar lipid bilayers. These intercellular structures play an essential role in keeping an optimal skin barrier function (4) and in regulating the water-holding capacity (5). Modification of SC lipid organization or composition has been shown to impair these properties; patients with psoriasis (6) and atopic dermatitis (7, 8) have a diminished skin barrier function. This has been related to an overall decrease in total stratum corneum (SC) lipids (9, 10) and in particular to a decrease in ceramides (11, 12).

The skin contains at least six ceramide families which are believed to play different roles in skin properties (13). Until recently pure human skin-identical ceramides were not available, with the result that their specific functions could not be tested. In the case of Caucasians it has been shown that levels of ceramides diminish with increasing age, whereas the ratio between the ceramide subtypes remains constant (14).

Skin barrier disruption due to topical treatment with organic solvents, surfactants or removing successive layers of corneocytes by tape stripping is attributed to a selective/integral depletion or an alteration in the intercellular lipids (15, 16). It is important to emphasize that application of any one of the physiologic lipids, such as cholesterol, free fatty acids or ceramides, or a two-component mixture of them, on disturbed skin leads to a delay in barrier recovery, whereas the three component mixture in an approximately equimolar ratio allows normal recovery (15–17). Despite the controversial results of other authors when applying body lotions enriched with skin lipids on aged or sodium lauryl sulfate (SLS)-damaged skin (18),

promising results have been obtained with ceramide-containing liposomal formulations (19). The structure of liposomes, which mimics the organized lipid structures of the SC, offers a suitable strategy for achieving an accurate vehiculization of a particular compound and for incorporating an additional lipid content that modifies the water barrier function of the skin (20–22).

In this regard, an earlier study was carried out (23) topically applying two stratum corneum lipid models structured as liposomes on disturbed and intact skin. A synthetic stratum corneum lipid mixture, and a natural extract from another keratinized tissue such as wool fibre (24) have been evaluated. The ability to accelerate the repair of water barrier function was demonstrated after different disturbance methodologies, and a beneficial effect on intact skin was suggested (23).

Therefore, the main aim of this study was to lend support to the reinforcement ability of these mixtures on the healthy skin of two groups of differently aged populations, and to highlight the importance of lipid structure on bilayers in the formulation. Protection of healthy skin against detergent-induced dermatitis was evaluated after topical application of bilayer-structured formulations.

Materials and Methods

Chemicals

Palmitic acid and cholesterol were purchased from Fluka Chemicals Co. (Buchs, Switzerland). Ceramide III was obtained from Cosmoferm (Delft, the Netherlands). Ceraphyl 45 [bis(2-ethylhexyl) malate] was supplied by ISP VAN DYK Inc. (New Jersey, USA). Cholesterol sulfate was provided by Sigma (St. Louis, MO, USA). Reagent grade organic solvents, 99.0% sodium chloride (NaCl) and 99.0% SLS were supplied by Merck (Darmstadt, Germany).

Sample preparation

Two kinds of lipid samples were studied mimicking the stratum corneum lipid composition; synthetic stratum corneum lipid (SSCL) mixture and internal wool lipid (IWL) extract, which has been shown to have a similar composition to that of the stratum corneum (24). The SSCL was prepared with the following mixture (% weight): 25.0 palmitic acid, 25.0 cholesterol, 40.0 ceramide III and 10.0 cholesteryl sulfate. IWL was extracted from raw industrially scoured Merino wool following the procedure described in an earlier work (25). The composition of the main components is: 22.3% free fatty acids, 24.5% cholesterol, 21.9% ceramides and 9.8% cholesteryl sulfate (24).

These two lipid mixtures were dissolved in an organic compound widely used in cosmetics as an emollient (Ceraphyl 45, dioctyl malate) (CP) or in aqueous medium structured as liposomes, always at the concentration of 25 mg/mL. To perform the liposome samples, lipids were dissolved in chloroform/methanol 2:1 (v/v), evaporated to dryness, and hydrated with 0.9% NaCl solution. Multilamellar vesicle liposomes were formed by sonication of the suspension at 65°C as described elsewhere (23).

Subjects

Group 1 was made up of six Hispanic volunteers (1 male, 5 females) phototype II–III with a mean age of 31 ± 6 years old (range 24–42 years) with healthy but dry skin selected by a dermatologist also taking into account the evaluation of their skin capacitance values (26). Group 2 was formed by five elderly Hispanic volunteers (2 males, 3 females) phototype II–III with a mean age of 79 ± 6 years old (range 73–85 years) with healthy but ageing skin. All subjects were advised to avoid the use of topical drugs or moisturizers on the volar forearm for 24 h prior to the experiments. To obtain reliable measurements, the volunteers were acclimatized for 15 min in a conditioned room (20°C, 60% HR) before the experiments.

Biophysical measurements

Transepidermal water loss (TEWL) is a sensitive index of skin barrier integrity. This parameter evaluates the water loss in $\text{g/m}^2\text{h}$, measured using a Tewameter TM 210 (Courage & Khazaka, Cologne, Germany). Moreover, skin hydration was determined by the Corneometer CM 820, which measures skin capacitance in arbitrary units (AU). Both parameters were recorded in accordance with established guidelines (27, 28).

Efficacy on healthy human skin

Two long-term studies were performed to test the effect of the different samples on undisturbed skin of the two different populations (group 1 of subjects with healthy but dry skin and group 2 of old people with healthy but ageing skin). Baseline measurements of TEWL and skin capacitance were taken on seven marked zones of both forearms before topical application: six zones for topical treatment [Ceraphyl (CP) as placebo, IWL in Ceraphyl, SSCL in ceraphyl, NaCl solution as placebo, IWL liposomes and SSCL liposomes] and one untreated zone (control). Placebos and solutions were randomly applied (10 μL) onto marked areas of 4 cm²

Table 1. TEWL and skin capacitance (mean values $\pm$ SD) obtained for untreated skin of the different group of people evaluated

Group	Mean age (years)	Age range (years)	Number of people	TEWL (g/m ² h)	Skin capacitance (AU)
1	31 $\pm$ 6	24–42	6	9.4 $\pm$ 1.6	36 $\pm$ 7
2	79 $\pm$ 6	73–85	5	7.7 $\pm$ 2.3	43 $\pm$ 10

using an Exmire microsyringe (ITO Corp., Fuji, Japan). After 2 h and 24 h (day 1) both parameters, TEWL and skin capacitance, were evaluated and then 10 μ L of solutions were applied again. The application of the solutions was repeated on 3 more days and the parameters were measured on days 2, 3 and 7 (3 days after the last application).

Protection of healthy human skin against detergent-induced contact dermatitis

Another long-term study was performed to test the protective effect of liposomal formulated samples applied to undisturbed skin followed by SLS exposure in group 1 (subjects with healthy but dry skin). Baseline measurements of TEWL and skin capacitance were taken in four marked zones of the volar forearm before topical application: three zones for topical treatment (NaCl solution as placebo, IWL liposomes and SSCL liposomes) and one untreated zone (control). Placebo and solutions were randomly applied (5 μ L) onto the marked areas of 2 cm² using an Exmire microsyringe (ITO Corp., Fuji, Japan). After 24 h (day 1) both parameters, TEWL and skin capacitance, were evaluated and then 5 μ L of solutions were applied again. In 10 days this procedure was repeated 8 times and TEWL and skin capacitance were evaluated always after 24 h of application. Next, the four zones were exposed to 0.5% SLS aqueous solution for 24 h (see below) and the resultant irritant reaction was assessed 24 h after SLS exposure by TEWL and skin capacitance.

SLS exposure

50 μ L of an aqueous solution of 0.5% SLS was pipetted onto a layer of filter paper placed in each of several aluminium chambers (d = 12 mm, Large Finn Chambers, Epitest Oy, Finland). The chambers were fixed to the skin for 24 h with adhesive tape. Upon removal of the patch, the skin was gently rinsed with water and allowed to dry.

Data treatment

The mean values and standard deviations (SD) were calculated. Grubb's test was used for de-

tecting outliers, which were excluded from the data. One-way analysis of variance was used to determine significant differences between values obtained from different treatments (significance level accepted * P < 0.10 and ** P < 0.05).

Results

We tested two kinds of lipid samples trying to mimic the stratum corneum lipid composition; a synthetic stratum corneum lipid (SSCL) and an internal wool lipid (IWL) extract, in two different formulations: in an oily solution, Ceraphyl 45 (CP), and in aqueous medium structured as lipid bilayers in liposomes (see Materials and Methods). To evaluate the efficacy of these four formulations on healthy human skin two long-term studies were performed with the two different groups of people already described. Mean values of TEWL and skin capacitance of these two groups of people were evaluated and are listed in Table 1. Lower values of TEWL and higher values of skin capacitance in group 2 with respect to group 1 indicate better skin conditions in the group of old people than in the group of young people (which were chosen for their dry skin).

Evaluation of TEWL and skin capacitance was performed 2 h and 24 h after the first application (day 1), 24 h after a daily application (days 2, 3 and 4) and three days after the last application (day 7). Even though TEWL values were measured and statistically treated like capacitance values, no significant changes in TEWL were obtained for the different formulation applied in the two groups. This means that for healthy skin in the absence of any disturbing treatment, variations in transepidermal water loss are too small to be taken into consideration. However, significant differences were obtained in skin capacitance. The results obtained are shown in Figs 1–4.

The occlusive behaviour of the emollient (Ceraphyl) may be the reason for the increase in skin capacitance of all formulations in this medium evaluated at 2 h (Figs 1 and 3). A diminution in skin capacitance on all subsequent days (about 10%) after the application of SSCL and IWL in Ceraphyl and the placebo was obtained for the group of young people with dry skin (Fig. 1). When the same test was performed in the group

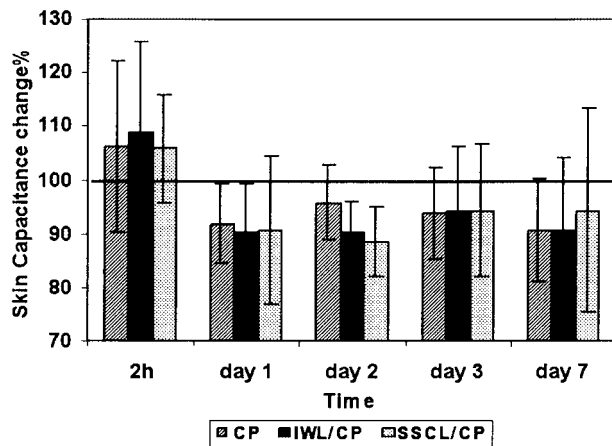


Fig. 1. Variation of skin capacitance values after non-structured lipid application in Ceraphyl (CP) in group 1 during the treatment period. Changes were doubly evaluated *versus* basal and control values.

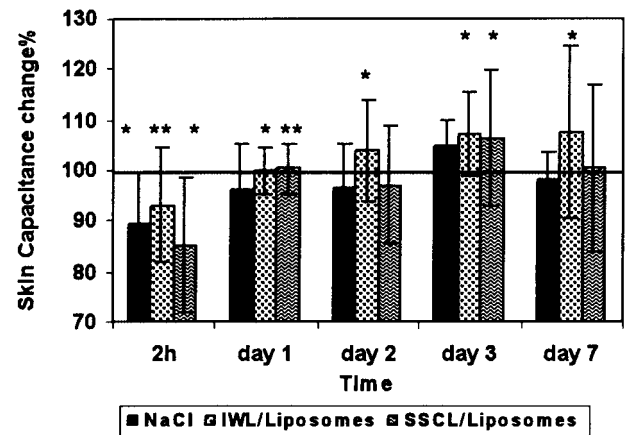


Fig. 2. Variation of skin capacitance values after bilayer-structured lipid application as liposomes in group 1 during the treatment period. Changes were doubly evaluated *versus* basal and control values (* $P < 0.10$ and ** $P < 0.05$).

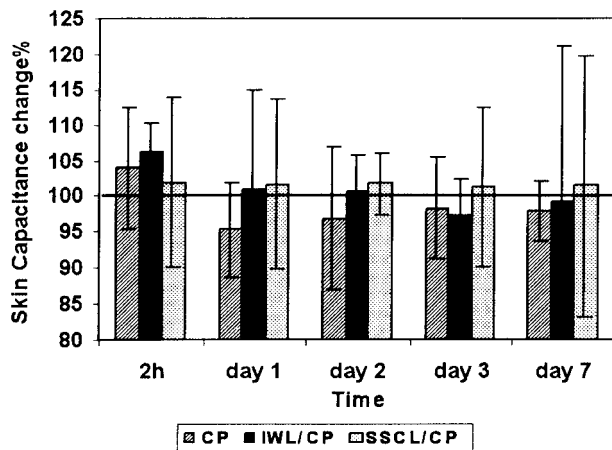


Fig. 3. Variation of skin capacitance values after non-structured lipid application in Ceraphyl (CP) in group 2 during the treatment period. Changes were doubly evaluated *versus* basal and control values.

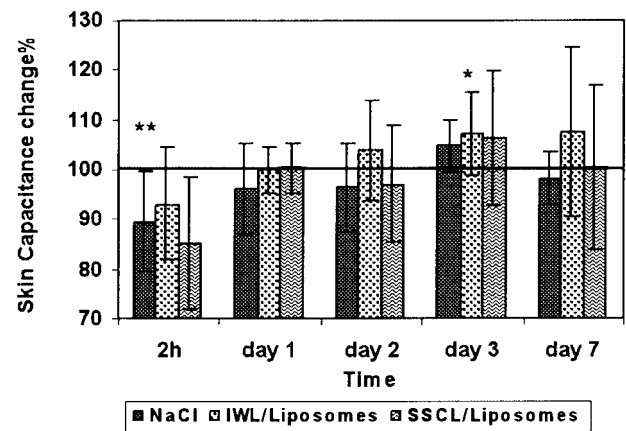


Fig. 4. Variation of skin capacitance values after bilayer-structured lipid application as liposomes in group 2 during the treatment period. Changes were doubly evaluated *versus* basal and control values (* $P < 0.10$ and ** $P < 0.05$).

of aged people, there was not much effect of any formulation on skin capacitance (Fig. 3). However, the variations obtained when SSCL and IWL structured as liposomes were applied in saline solution should be noted (Figs 2 and 4). An increase in the water-holding capacity of the skin zones took place after the third application for group 1 (Fig. 2) and already after the first application for group 2 (Fig. 4), these effects being always maintained after three days of the last application. We should also point out the greater increase in skin capacitance obtained in both cases with formulations made with IWL compared with SSCL lipids.

Statistically significant differences are indicated in Figs 2 and 4 of every formulation in liposome compared with the same formulation in Ceraphyl of Figs 1 and 3 (*90%, **95%). Skin capacitance

values in the whole treatment period of IWL/liposomes always show a statistically significant difference from the values of IWL/CP for group 1 (Fig. 2) and only after the third application for group 2 (Fig. 4).

Briefly, the two formulations of IWL and SSCL structured as liposomes in saline solution improve properties of dry and aged healthy human skin; a non-significant modification of transepidermal water loss and an increase in waterholding capacity were observed for the two groups. The latter effect appeared promptly in the elderly subjects but was more significant (compared with Ceraphyl formulations) in the young subjects with dry skin.

Therefore, to evaluate the protection of healthy human skin against detergent-induced contact dermatitis, these two formulations and the saline solu-

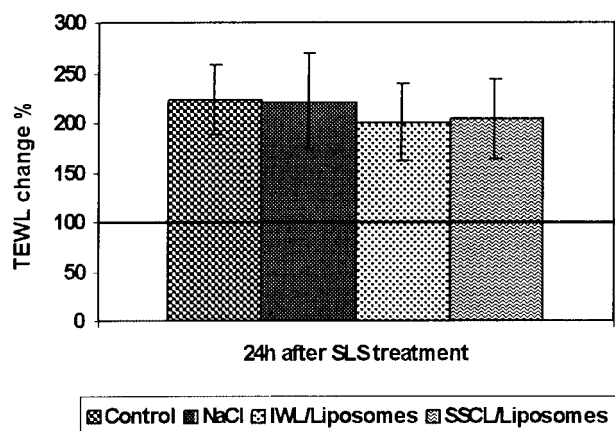


Fig. 5. Variation of TEWL values after 24 h SLS exposure of skin zones of group 1, having been previously treated with bi-layer-structured lipid samples as liposomes for 10 days (TEWL of day 10 taken as initial value). Changes were evaluated *versus* basal values.

tion as placebo were applied in a longer-term study with eight sample applications to group 1 with dry but healthy skin (Table 1). Subsequently, an irritant reaction was caused by SLS exposure, and TEWL and skin capacitance provided data on the possible reinforcement and protection of the lipid barrier due to exogenous lipid application.

As discussed above, variations in TEWL results after long-term application were not significant. Only a small diminution of about 2% was obtained for SSCL/liposome and IWL/liposome skin-applied zones at the end of the protective treatment. The skin capacitance variations during the application period again demonstrated an increase in water-holding capacity of approximately 5% for SSCL/liposome and a more marked increase for the IWL/liposome formulation of approximately 12% at the end of the application period.

24 h after SLS exposure of the control zone and the three previously treated zones, TEWL and skin capacitance were measured and values were treated taking as initial values the ones measured just before SLS exposure. The skin capacitance of all skin zones evaluated decreased by about 20%. However, this effect varied considerably depending on the person subjected to the treatment (high standard deviation), with the result that no reliable results were obtained. The effect of SLS exposure is much greater on transepidermal water loss (increase of about 220%), which directly reflects skin barrier function, resulting in much more reliable results (see Fig. 5).

Whereas the increase in TEWL for the control zone and for the placebo zone is 222.2% and 221.2%, respectively, it is possible to deduce a protection of the other two zones previously applied with IWL and SSCL liposomes from the lower in-

crease obtained, 200.9% and 203.1%, respectively. Therefore the protection of the skin barrier against detergent-induced dermatitis may be observed in these two formulations, the result being slightly better in the case of the internal wool lipid formulation.

Discussion

The status of the skin of the volunteers from the two groups was evaluated subjectively by a dermatologist and objectively by measuring TEWL and skin capacitance (Table 1). Conflicting data with respect to ageing skin have been reported. Aged skin displays an altered drug permeability and an increased susceptibility to primary (acute) irritation, suggesting an impaired epidermal barrier function with an increased TEWL (29, 30). However, a decrease in TEWL in relation to age has been reported by a number of authors, and this is probably due to low skin temperature, alterations in microvasculature, and reduction of epidermal cell turnover in the elderly (27, 30, 31). Lower values of TEWL in group 2 with respect to group 1 (Table 1) support these results. Besides, some authors reported a reduction in hydration parameters during ageing (32, 33), whereas others observed no differences at all (31, 34). In our case, the subjects of group 1 were chosen for having healthy but dry skin after a visual examination by a dermatologist and on the basis of their capacitance values (26). Therefore, the higher values of skin capacitance in group 2 with respect to group 1 were not surprising. Correlation between TEWL and the hydration state of the stratum corneum in healthy subjects is also a matter of debate. Our results support an inverse relationship between these two parameters, which is in agreement with other studies (31, 35).

The skin barrier and the water-holding capacity are the most important functions of the SC and these functions are related to the composition and structure of SC intercellular lipids (11, 30, 36). Accordingly the influence of the topical application of exogenous lipids with a similar composition and structure was assessed measuring *in vivo* changes in TEWL and skin capacitance.

The cutaneous permeability barrier is mediated by lamellar bilayers of the stratum corneum enriched in cholesterol, free fatty acids and ceramides (3, 4). A number of studies have been performed to test further the requirements of each of these three key lipids after acute disruption of the barrier by topical treatment using either an organic solvent, acetone, detergents, SLS or tape stripping (15–19, 23). In these studies, the barrier repair was mainly assessed by transepidermal water loss

evaluation. The main aim of the present work, however, was to evaluate the reinforcement of the permeability barrier of healthy skin due to application of exogenous stratum corneum lipids. Although the different tests performed in our work have a small effect on TEWL, the usefulness of skin capacitance in evaluating changes between formulations in both groups of people is considerable. Given the healthy status of the skin tested, an increase in skin capacitance whilst maintaining the equilibrium values of transepidermal water loss indicates an improvement in the skin properties, bearing in mind that the formulations applied were made up only of stratum corneum lipids, without moisturizing creams that could mask the results owing to an occlusive effect.

In the last test performed, protection of healthy skin against SLS-induced contact dermatitis was best determined by TEWL evaluation. Even though there was a significant decrease in skin capacitance due to SLS treatment, the homeostatic response (increase in epidermal synthesis) provokes fluctuations of skin hydration which hinder evaluation of the formulation. Similar results were obtained by other authors (37–39) who performed a more extensive test with SC lipids in moisturizing creams with low molecular weight humectants and salts (37, 38) or in emulsions (39), and they attributed the lack of validity of capacitance measurements to postinflammatory xerosis.

Our findings lend support to the usefulness of non-invasive instrumental techniques such as TEWL and capacitance for objectively assessing subtle changes in skin morphology and function not detectable by sensorial means.

Statistically significant results of skin capacitance changes which reflect skin hydration were observed after long-term evaluation of the different formulation tested on healthy skin. TEWL variations which reflect skin barrier function were obtained with SLS-insulted differently pretreated skin, even though the differences were not statistically significant.

In the light of our findings, the group of aged subjects seems to be a little more susceptible to barrier improvement (resulting in a prompt increase in the waterholding capacity) when SC lipids structured as liposomes are applied. Other authors recommend a target group of elderly volunteers to assess the hydrating and barrier protective properties of cosmetic products (38). According to De Paepe et al. (19), the importance of the bilayer structure of the formulations should be highlighted. In the present work, contrary effects were obtained with the same lipid mixtures when formulated in an oily solvent used as emollient, such as Ceraphyl 45, or in an aqueous saline solu-

tion structured in liposomes which, on long-term application, induced the highest hydration properties.

Some protection of healthy human skin against detergent-induced dermatitis due to a prior topical application of bilayer structured formulations of the two lipidic compositions followed by SLS exposure was detected. Moreover, slightly better results were obtained with IWL, containing a mixture of natural ceramides, when compared with SSCL, which had only one ceramide present in the formulation. We think that the failure of some studies to demonstrate the protective effect claimed for SC lipids in liposomes (18, 38, 40) could be due to the presence of only one kind of ceramide in the formulation. It is important to highlight the unique properties of each kind of ceramide that contributes to SC organization and cohesion and thereby provides; the SC with its barrier function (13). Higher capability for restoring the stratum corneum damaged barrier has been reported for emulsions containing several ceramides than for emulsions with only one ceramide in the formulation (39). Our findings confirm the work of other authors, namely that topical supplementation of individual barrier lipids or incomplete mixtures exert a negative rather than a positive influence on barrier recovery (16).

The reinforcement of the skin barrier by IWL liposome topical application together with its ability to accelerate repair (23) lend support to the view that the IWL extract might be suitable for designing new pharmaceutical or cosmetic products for skin care. All these results support the beneficial effects of skin lipid supplementation, given the resemblance of these lipids to those in the stratum corneum both in composition and structure of the formulation. Nevertheless, a more complete study using more volunteers should be undertaken in order to confirm the usefulness of specific lipid supplementation in reinforcement of the skin barrier.

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