Environmental Effects on the Functions of the Stratum Corneum

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The stratum corneum (SC) is such an efficient barrier that only 2–5 g per h per cm² of transepidermal water loss (TEWL) occurs in normal skin. The SC also plays another important role at the skin surface in keeping our skin smooth and flexible by binding water. We exposed a simulation model of in vitro SC to various, excessive physical insults such as irradiation with 1 J per cm² of UVB, 50 J per cm² of UVA, or 3000 rad of X-ray, heating at 90°C for 3 min, freezing at −196°C for 60 s or repeated placement in an extremely dry or humid condition. None of them could cause any permanent change in the SC functions. Only the application of chemical agents such as lipid solvents or a detergent or the affliction of trauma resulted in a functional derangement of the SC. Because the viable skin tissues are more vulnerable to the effects of the environment than the SC, most of the abnormalities of the SC functions developing after environmental insults are secondarily caused by enhanced epidermal proliferation induced under the influence of underlying inflammation. These functional abnormalities were found to be demonstrable with biophysical measurements long after the disappearance of skin redness, the clinically observable sign of inflammation. The SC abnormalities in inflamed skin are also detectable as a change in the content of chemical mediators. For example, the ratio between proinflammatory IL-1 and its receptor antagonist (IL-1ra) whose production by epidermal keratinocytes is markedly enhanced by various proinflammatory stimuli, showed a deviation towards an excess of the latter in inflammatory skin. Facial skin that is always exposed to the environment is unique in that its SC shows such a deviation in the IL-1/IL-1ra ratio suggestive for the presence of mild inflammation even in normal individuals. Key words: barrier function/dermatitis/facial/IL-1RA/epidermal proliferation/stratum corneum/water-holding capacity.


The primary function of the epidermis is the production of the stratum corneum (SC) that protects our body from desiccation as well as from invasion by injurious agents (Scheef and Redelmeier, 1996). Despite its thin membranous structure of less than 20 μm, it is an efficient biologic barrier. It is comprised of a two compartment system of corneocytes, flattened dead cell bodies of keratinocytes covered by a tough, highly cross-linked protein cornified envelope, and extracellular lipid lamellae (Elias, 1981). The latter fill the continuous extracellular matrix consisting of roughly equimolar concentrations of ceramides, long-chain free fatty acids, and cholesterol released from lamellar granules of differentiated granular cells. It is the presence of the lamellar intercellular lipids that endows the SC with an effective barrier function (Elias, 1981).

Most of the body regions are covered with SC composed of about 15 tightly stacked layers of corneocytes, except for certain locations such as the face and genitals or palms and soles where the SC is extremely thin or thick, respectively (Zhen et al., 1999). Normal SC is such an efficient barrier that it hardly allows the passage of small molecules such as water. In normal skin there is only 2–5 g per h per cm² of transepidermal water loss (TEWL), whereas the inflamed skin covered with abnormal SC shows TEWL values that are far higher. Thus, the measurements of TEWL provide information about the barrier function of the SC in vivo (Nilsson, 1977). The facial skin, which shows a relatively rapid turnover rate of the SC (Baker and Kligman, 1967), is unique in that it is covered with relatively thin SC with a rather poor barrier function comparable with that noted in inflammatory skin lesions (Zhen et al., 1999).

The SC also plays another important role at the skin surface in keeping our skin smooth and flexible by binding water even in an extremely dry environment. Water renders these dry stacked layers of corneocytes sufficiently supple to allow free movement of the body without causing the SC to be cracked or fissured (Blank, 1952; Rawlings et al., 1994). As a matter of fact, there is always a water supply from the underlying hydrated living tissue and it is the water content at the superficial portion of the SC that keeps the skin smooth and soft, as long as its water-holding capacity is intact (Obata and Tagami, 1989). For the evaluation of such a skin surface hydration state in vivo, we can utilize electrical measurements that provide data about the hydration state of the SC instantly (Tagami et al., 1980). High frequency conductometry is more suitable for measurement of the hydration state of the skin than capacitance measurements, whereas the latter are more sensitive for dry skin conditions than the former (Hashimoto–Kumasaka et al., 1993; Fluhr et al., 1999). Again, it is the presence of the lamellar intercellular lipids that endows the SC with softness by its water-holding capacity (Imokawa et al., 1991b) together with sebum that

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covers the skin surface (O’goshi et al., 2000) and water-soluble amino acids (natural moisturizing factor; NMF) produced by enzymatic degradation of filaggrin. The NMF helps structural keratin proteins acquire their elasticity via the reduction of intermolecular forces (Okura et al., 1995).

The SC produced in pathologic skin that is deficient in its water-holding capacity is firm and brittle, developing fissures and scales even under normal ambient conditions (Takenuchi et al., 1986; Denda et al., 1992). We can at least partially compensate this deficiency by applying effective topical agents (Rawlings et al., 1994; Tagami, 1994). Moisturizers not only supply water but they increase the water-binding capacity of the SC by supplying the SC with humectants. In contrast, occlusive emollients such as petrolatum and oils retard the TEWL to form a water reservoir at the skin surface like sebum (Obata and Tagami, 1990; Tagami, 1994).

Water is uniformly distributed in isolated in vitro samples of SC; however, the water content of the in vivo SC is not uniform. There is a concentration gradient of water even within this thin membrane from the lowermost portion that faces the fully hydrated viable epidermis to the uppermost layer that is exposed to dry air (Tagami et al., 1980; Warner et al., 1988). Thus, for functional analyses of the SC in vitro, we have devised a simple simulation model of the in vivo SC in which a concentration gradient of water exists between the surface and the lowermost portion of the SC. It consists of an isolated sheet of SC that tightly occludes the underlying water-saturated filler paper placed as in a diffusion chamber (Obata and Tagami, 1989; Hashimoto-Kumasaka et al., 1993).

Harsh physical insults from the environment, such as UV or X-ray irradiation, heating, and freezing, always induce acute irritant dermatitis in the skin where the formation of blisters covered with intact SC may take place several hours to a few days later. These findings suggest that the SC is more resistant than the underlying viable epidermis to their direct damaging effects; however, we do not have any evidence whether the SC retains its functional integrity after these drastic environmental insults. It is because we cannot use the skin of human volunteers to assess the effects of harsh physical or chemical insults on the SC. For this purpose we chose a simulation model of in vivo SC. Using it, we could easily assess the effects of various harsh physicochemical stimuli that might be afforded on the skin from our environment.

MATERIALS AND METHODS

A simulation model of in vivo SC For the preparation of SC sheets, we used normal skin samples obtained from amputated limbs. Epidermal sheets that were separated by placing the skin samples in water at 60°C for 30 s were dipped into a solution of 1 × 10^(-7) trypsin in 5% aqueous sodium bicarbonate for 18 h at 37°C. After removing the epidermis mechanically by rubbing, isolated sheets of the SC were then rinsed in distilled water for 1 h with one change of water and afterwards stored in a desiccator over silica gel (Kligman and Christoffers, 1963).

The model utilizes a SC sheet placed over a pad of five sheets of filter paper saturated with phosphate-buffered saline (PBS). The filter paper is mounted on a glass slide and all free edges of the SC sheet are sealed to the glass with a biophysical frame of adhesive vinyl tape with a large hole. The surface of the SC is exposed to the ambient atmosphere only through the hole, and passage of water is allowed only through this portion of the SC. The underlying water-saturated filter paper is the water source for the overlying SC like the fluid-saturated cutaneous tissue in vivo, and is also the conducting medium that allows the formation of an adequate electric field (Obata and Tagami, 1989). Although we utilized skin samples heated at 60°C for 3 min to isolate the SC samples, the prepared SC model showed TEWL rates similar to those noted in normal skin.

All the following treatments were carried out in a room at 20°C with 70% relative humidity.

Biophysical measurements For biophysical measurements we measured TEWL with an Evaporimeter (ServoMed, Sweden) as described by Nilsson (1977) and high frequency conductance with a skin surface hygrometer (Skicon-200, IBS, Hamamatsu, Japan) (Tagami et al., 1980). Skin surface pH was measured with a Skin-PH meter PH900 (Courage + Khazaka, Cologne, Germany). Skin redness was determined with a Minolta CR-310 (Minolta, Osaka, Japan) chromameter according to the L*, a*, b* CIE 1976 system, where a* is an attribute on the redness-green scale.

Physicochemical insults on the SC Massive doses of UVB or UVA that can produce sunburn reactions in any Japanese, i.e., 0, 5, 1, and 2 J cm(-2) of UVB or 50 J cm(-2) of UVA, were irradiated on the SC sheet of the in vivo simulation model. The UV source was a bank of five fluorescent sun lump tubes (FL20E-30/DMR, Toshiba Medical Supply, Tokyo, Japan) that emitted total UV 80% (with a spectral radiation output ratio of UVA 25%, UVB 54%, and UVC 1%) with a peak of 305 nm. The UVA source was a bank of 10 black fluorescent tubes (ULTRAS BL/DMR, Toshiba Medical Supply) that emitted total UV 90% (UVA 89% and UVB 1%) with a peak 315 nm.

For ionizing radiation, we exposed the SC model to 1000 or 3000 rad of X-ray from a Softex (Softex, Tokyo, Japan).

For heating, we firmly pressed a glass containing heated water against the surface of the SC sheet for 3 min. The temperature of the water was adjusted to 70°C or 90°C. For freezing, we firmly pressed a cotton-tipped doped in liquid nitrogen (−196°C) against the surface of the SC for 10−60 s. All these treatments of the skin in vivo definitely produce a burn or cold injury.

Effects of the exposure of the SC model to extremely humid or dry ambient conditions were examined by simply placing it in environments with high or low relative humidities (RH). RH of 0%, 69%, and 97% were obtained by putting different saturated salt solutions at the bottom of desiccation chambers (Middleton, 1968).

For chemical insults on the SC an open-ended cylinder of 2 cm in diameter was pressed onto the surface of the SC sheets for different time periods with acetone/ether (1:1), chloroform/ethanol (1:1), hexane, or 10% sodium lauryl sulfate (SLS) aqueous solution for a short period of time.

Experimental production of irritant or allergic contact dermatitis on the skin of guinea pigs Because we cannot produce various types of severe inflammation in human volunteers to examine the proliferative activity of the epidermis, we used Hartley strain guinea pigs weighing 500–800 g for the following in vivo studies. We experimentally produced allergic contact dermatitis with C. albicans antigen in five guinea pigs that had been made contact-sensitive to this antigen by the production of experimental cutaneous candidiasis 1 mo before, as reported previously (Tagami et al., 1985). Briefly, cutaneous candidiasis was induced by inoculation of 10^9 yeast cells of C. albicans to a 2 × 2 cm^2 area under occlusion for 24 h. A 1:200 Candida antigen aqueous solution (Tori Pharmaceutical, Tokyo, Japan) was applied under occlusion for 24 h on the back of the animals after removing their hair with an electric hair clipper and electric shaver. At the patch test site we obtained various biophysical parameters 2, 4, 7, 9, 11, 13, and 18 d after application of the patches.

Subsequently, we tried to compare these changes with those observed in irritant dermatitis showing a similar severity of clinically observable inflammation in guinea pigs. By conducting various preliminary trials, we found that a diffusely bright erythematous reaction comparable with that noted in allergic contact dermatitis to Candida allergen at 48 h is produced by the application of 10% sodium lauryl sulfate (SLS) in petrolatum for 24 h.

Evaluation of DNA synthesis of the epidermis The proliferative response of the epidermis to dermatitic changes experimentally produced in the guinea pigs was evaluated by obtaining biopsy specimens from the skin lesions at various intervals. The epidermal proliferation was analyzed by using FACScan (Becton Dickinson Immunocytometry System, Franklin, NJ) (Bata Czorgo et al., 1993).

RESULTS AND DISCUSSION

Direct effects of drastic physicochemical insults on the SC functions It is well known that SC absorbs up to 50% of both irradiated UVB and UVA (Kaidbey et al., 1979). Moreover, solar-simulating UV radiation and the reactive oxidant pollutant ozone are reported to induce SC lipid peroxidation and protein oxidation (Thiele et al., 1997, 1999); however, in these experiments, we could not find any abnormality in the barrier function or in the water-holding capacity of the SC after irradiation with the massive doses of UVB or UVA (data not shown). It was also reported that changes of the skin surface in vivo do not occur immediately after UV irradiation. Miyauchi et al. (1992) demonstrated a significant
Figure 1. Changes in transepidermal water loss after chemical treatments. Effects of the exposure of the stratum corneum sheet to various chemicals for 2 min (●) and 4 min (○) on TEWL.

Table I. Influences of drastic physical insults on the biophysical parameters of the stratum corneum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Transepidermal water loss</th>
<th>High frequency conductance</th>
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<tbody>
<tr>
<td>Irradiation of</td>
<td></td>
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<tr>
<td>UVB (1 J per cm²)</td>
<td>temporary increase</td>
<td>temporary increase</td>
</tr>
<tr>
<td>UVA (50 J per cm²)</td>
<td>temporary decrease</td>
<td>temporary decrease</td>
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<tr>
<td>X ray (3000 rad)</td>
<td>temporary decrease</td>
<td>temporary decrease</td>
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<tr>
<td>Heating at 90°C for 3 min</td>
<td>temporary increase</td>
<td>temporary increase</td>
</tr>
<tr>
<td>Freezing at −19°C for 1 min</td>
<td>temporary decrease</td>
<td>temporary decrease</td>
</tr>
<tr>
<td>Repeated exposures to</td>
<td></td>
<td></td>
</tr>
<tr>
<td>humid air (96% RH) for 24 h</td>
<td>temporary increase</td>
<td>temporary increase</td>
</tr>
<tr>
<td>dry air (0% RH) for 24 hr</td>
<td>temporary decrease</td>
<td>temporary decrease</td>
</tr>
</tbody>
</table>

*No change.

Acetone, chloroform, and methanol readily induced a slight decrease in the hydration levels of the skin surface 24 h after UVB irradiation of guinea pig skin. Thus, we can state that the functional disturbances of the SC observed in UV irradiated skin are not due to the direct effect of UV on the SC, but only reflect secondary changes possibly caused by the UV damage of the viable skin tissues that induces an epidermal proliferative response and thymocyte-mediated events (Hiratake et al., 1998). It was also the case with ionizing radiation, heat, or cold treatment, because they did not leave any permanent change in the TEWL or high frequency conductance levels (Table I). Exposure of the SC model to 1000 or 3000 rad of X-ray produces no particular change. When we applied high temperatures on the SC sheets, water vapor permeation of the SC exhibited a temperature-dependent increase just after the heat application, but it was always reversible after cooling of the SC sheet. Thus, the initial temperature-dependent elevation in TEWL quickly decreased in the following 5 min, being followed by a later gradual decrease that returned to the preheating levels after up to 30 min. Although the temporary increase in TEWL indicates that such a heat application definitely changes the state of the intercellular lipids of the SC into a fluid phase (Pilgram et al., 1999), this change seemed to be totally reversible. In contrast, high frequency conductance showed consistent values even after the heat application, reflecting only a mild change occurring in the hydration state of the SC.

Applications of liquid nitrogen definitely caused freezing of the water in the SC as well as that in the underlying filter paper. In contrast to heating, TEWL showed an initial decrease. The prominent initial changes reflected an air flow taking place in the inside of the cylinder of the applied probe of the Evaporimeter that was induced by the cooling of the SC surface. In this case the direction of the airflow seemed to be opposite to that induced by the heating. It was followed by an increase in TEWL that was again reversible, returning to pretreatment levels depending upon the length of the freezing period (data not shown). Conductance values were again not influenced by the treatments.

From the reversible changes in TEWL observed after freezing, we think that the architecture of the SC is resilient enough to withstand the formation of space-occupying solid ice that is produced from the free water present in the deep portion of the SC (Takenouchi et al., 1986; Warner et al., 1988).

All of these present data correspond well with our clinical experience that even in the scalded skin or skin with cold injury, there at first takes place the formation of blisters that reflect the functional integrity of the covering SC.

It is difficult to expose the skin to extreme changes of relative humidity for a long period of time. Thus, we placed the simulation model of SC in vivo in environments with high or low relative humidities (RH) for 24 h. Agner and Serup (1993) found that occluding the upper arm skin with a Finn chamber containing 60 μl of sterile water led to a significant increase in TEWL. Immersion of the SC in water for a prolonged period of time always induces disintegration of the SC, which is observed as maceration, due to excessive hydration in the SC (Kligman, 1994; Tagami et al., 1998). In our experiments, however, alternative exposures to an extremely humid or dry environment for 24 h each did not induce any substantial change in the barrier function of the SC (data not shown). Thus, as compared with the direct exposure to water, the exposure to humid air is not harsh enough to induce barrier damage. Its effect seems to be similar to that noted after the application of an empty Finn chamber for 24 h as utilized for control patch testing.

Recently, Denda et al. (1998) reported that exposure of murine skin to a dry condition with a relative humidity of less than 10% produced an increase in the weight and thickness of the SC with a commensurate increase in the water barrier function. They further found that exposure to low humidity increased epidermal DNA synthesis, which was probably a homeostatic response to protect the animal from excessive TEWL in a dry environment, and that such skin epidermal hyperplasia and mast cell hypertrophy were easily induced after barrier disruption. (Denda et al., 1998). All these changes may be induced by a signal of dryness to the viable epidermal tissues rather than to the SC, because the functional integrity of the SC was found to be unimpaired after its exposure to a dry environment in this study.

In contrast to the innocuous nature of the so far mentioned physical stimuli, which can induce only a mild or reversible functional derangement of the SC functions at most, the effects of lipid solvents that can extract the intercellular lipids were remarkable. A significant increase in TEWL together with a conspicuous decrease in high frequency conductance occurred after the treatment with chloroform/methanol for 2 min. Its treatment for 4 min induced degradation of the integrity of the SC sheet leading to the development of fissures. In vivo experiments, acetone treatment even for only a few minutes selectively removed glycerolipids and sterols from the SC. In this study acetone/ether treatment induced a moderate increase in TEWL as well as a decrease in high frequency conductance after 2 or 4 min. Among the studied lipid solvents, the effects of hexane seemed to be mild on the SC; there occurred just a minor decrease in high frequency conductance after treatment with hexane. In contrast with the other solvents, we did not find any increase in TEWL but rather a slight decrease (Figs 1, 2).

As with these lipid solvents, there occurred an increase in TEWL after treatment with 10% sodium lauryl sulfate (SLS) for 4 min
Corresponding to the increased DNA synthesis in the epidermis, TEWL showed a continuous increase until day 4. Such a delayed increase in the barrier damage of the SC seemed to reflect the hyperproliferative state of the epidermis as noted in allergic contact dermatitis and psoriasis, where it is difficult for keratinocytes to attain sufficient differentiation. Thereafter, the elevated TEWL values began to decrease but took more than 15 d to return to the control levels (Fig 3c). A similar pattern was also observed in the changes of the skin surface pH (Fig 3d). It gradually increased until day 4, keeping significantly higher levels for 3 d and began to return to the normal levels by day 11. Thus, the elevated TEWL as well as the skin surface pH values reflected the prolonged course of the inflammation in allergic contact dermatitis that could not be detected only by measurements of the erythematous changes. Unfortunately, we could not measure the hydration state of the SC in such animals because of the presence of remaining hair stubs; however, in our preliminary study, in an experimental M. furfur-induced allergic contact dermatitis in hairless guinea pigs, the conductance levels suddenly dropped to zero when the erythematous lesions that developed on day 2 began to show diffuse scaling on day 5 (Yoshimura and Tagami, unpublished data).

In this study we further compared these changes with those observed in irritant dermatitis induced by SLS showing a similar severity of clinically observable inflammation in guinea pigs. A diffusely bright erythematous reaction was produced by the application of 10% SLS in petrolatum for 24 h on the back skin of guinea pigs. The grade of erythema at 24 h was slightly stronger than that of the allergic contact dermatitis caused by Candida allergen at 48 h, and it returned to normal levels by day 5 (Fig 4a).

An analysis of the increase in epidermal proliferation in such skin by using FACSscan revealed that the peak increase in DNA synthesizing epidermal cells occurred on day 1, and then the level quickly returned to normal by day 4 (Fig 4b). These data indicate a somewhat shorter course of epidermal proliferation after SLS irritation than in allergic contact dermatitis to Candida; however, an increase in TEWL that reflects the SC barrier dysfunction due to epidermal proliferation was noted even until day 5. Thereafter, TEWL showed a gradual decrease with a return to normal levels around day 10 (Fig 4c). Thus, the damage of the SC barrier function seemed to be restored much quicker after SLS irritation than in allergic contact dermatitis. Likewise, the elevated skin surface pH returned to normal around day 10 (Fig 4d). Again, the total course was much shorter than that in allergic contact dermatitis that initially showed similar erythematous reactions.

These results indicate that the barrier function of the SC is a more sensitive indicator of the hyperproliferative epidermis present in dermatitic skin than the erythematous changes that have been regarded as the crucial sign of inflammation. Furthermore, we think that the skin surface pH can be used as a more practical biophysical parameter to quantitatively assess the inflammatory skin than the redness of the skin.

Functional abnormalities of the stratum corneum in atopic xerosis

Dry scaly skin, xerosis, occurs in the skin whose water-holding capacity of the SC is deficient. In this study we found that clinically observable erythematous changes seem to be demonstrable only in an acute phase of the inflammatory processes of the skin; however, microscopically observable inflammation persists much longer than the visible redness, provoking the proliferative activity of the epidermis that is associated with an insufficient keratinization process that is observed clinically as xerosis. Similarly, SC that has a mild functional deficiency is always observed as clinically noninflamed xerotic skin in patients with atopic dermatitis (Watanabe et al., 1991), like that noted in the skin more than 1 wk after tape-
stripping (Tagami and Yoshikuni, 1985; Denda et al., 1992). In such skin, there are mild residual inflammation and increased proliferative activity of the epidermis accompanied by rapid turnover of the SC.

The skin of atopic xerosis shows higher TEWL and lower hydration of the skin surface than those of normal individuals because of the reduced content of ceramides (Imokawa et al., 1991b). This reduction in the ceramide content in the SC of atopic xerosis is no longer observable when the xerotic change disappears (Tanaka et al., 1997; Matsumoto et al., 1999). The SC of atopic xerosis also contains substantially lower amounts of water-soluble amino acids, which play a role in the water-retaining capacity of the SC, than do those of controls. Although the number of SC cell layers in atopic xerosis (21 ± 4) was substantially larger than that in controls (15 ± 1), its turnover time (7 ± 2 d) was appreciably shorter than that for controls (14 ± 2 d). As noted in skin showing increased epidermal proliferation, the size of superficial corneocytes in patients with atopic xerosis was substantially smaller than that in controls. All these changes seem to reflect increased epidermal proliferation due to an ongoing low-level dermatitis. (Watanabe et al., 1991).

Patch testing with 1% SLS in petrolatum for 24 h on atopic xerosis induces much more severe damage in the SC barrier function, which lasted over 1 wk, than that in normal individuals (Tabata et al., 1998). This is in sharp contrast to the effects of tape-stripping in atopic xerosis in which the barrier recovery is not impaired as compared with normal individuals (Gfesset et al., 1997; Tanaka et al., 1997). Possibly, the damaging effects of chemical insults are more far reaching in the living tissue than those of the physical trauma of tape-stripping, which are mainly confined to the superficial epidermis.

Although clinically resembling each other in terms of their dry pruritic skin changes, the functional characteristics of the SC in senile xerosis are distinct from those of atopic xerosis. Senile xerosis develops on the background of a normal aging process of the skin without any involvement of skin inflammation (Hara et al., 1993). It only produces inflammation secondary to the development of fissuring of the SC and repeated scratching. The SC samples collected from both atopic xerosis and senile xerosis contain a substantially lower amount of water-soluble amino acids that help structural keratin proteins acquire their elasticity via the reduction of intermolecular forces in the presence of water (Jokura et al., 1991).
A reduction in the content of amino acids in the SC is a consistent feature observable in any kind of xerotic skin (Takenouchi et al., 1985; Horii et al., 1989; Denda et al., 1992; Tanaka et al., 1997). The intercellular lipids as well as skin surface lipids are also greatly decreased in senile xerosis, which occurs as a physiologic skin change from aging (Hara et al., 1993); however, the barrier function of the SC is not impaired but rather better than normal in senile xerosis. It seems to be compensated by the presence of a thickened SC caused by the retention of extremely enlarged and flattened corneocytes on the skin surface, probably due to a slower SC turnover rate that reflects decreased epidermal proliferation in the elderly skin (Table II).

It is of note that the facial skin of normal individuals shows high TEWL values almost comparable with those noted in scaly chronic dermatitic skin, which far exceed those of atopic xerosis (Zhen et al., 1999). A simple barrier dysfunction of the SC at a level noted in atopic xerosis cannot alone explain the development of dermatitis. Fissures and cracks of the SC induced by xerosis stimulate the free nerve endings of C fibers extending into the epidermis to elicit pruritus. Furthermore, scratching due to pruritus opens a new pathway in the SC for aeroallergens consisting of large molecular proteins. As in scratch patch testing, it allows even the permeation of large molecular proteins, facilitating the development of eczematous dermatitis in the presence of contact sensitivity to these allergens (Tanaka et al., 1994). In fact, nummular eczema tends to occur in those with senile xerosis who also retain cellular immune functions that allow the development of contact hypersensitivity to penetrating aeroallergens as in the patients with atopic dermatitis (Aoyama et al., 1999).

**Biologically active substances in the stratum corneum** Like the earth that contains numerous historic remains from the past, the SC of inflammatory skin also contains various biologic mediators that have played an important role in the preceding processes of inflammation in the skin. For example, psoriatic scales are a rich source of various chemical mediators and cytokines involved in the pathogenesis (Tagami and Aiba, 1998). Epidermal keratinocytes have the capability of producing a variety of cytokines and even complement components, particularly under the stimulation of proinflammatory cytokines (Terui et al., 1997).
Among them, IL-1 is constitutively produced by the keratinocytes, so that their end product, SC, is a major reservoir for active IL-1 (Gahring et al., 1985; Takematsu et al., 1986). The release of IL-1 from the epidermis after stimulation is a primary event that leads to skin inflammation through the induction of various cytokines, other proinflammatory mediators, and adhesion molecules (Kupper, 1990).

IL-1 receptor antagonist (IL-1ra) that is also produced by keratinocytes functions as a specific and competitive inhibitor of IL-1, because IL-1ra binds to the same receptors as IL-1α and β without eliciting any biologic response. Thus, the balance between IL-1α and IL-1β is important for maintaining homeostasis in the skin. An excess of 10–100 times more IL-1ra than IL-1 is required to abrogate the IL-1 induced biologic response by 50%. Acute and chronic disruption of the cutaneous permeability barrier increases mRNA levels for TNF, GM-CSF, IL-1α, and IL-1β in the epidermis. The cutaneous response to barrier disruption includes mechanisms that increase IL-1 and IL-1ra mRNA levels in a coordinated manner. The net result provides a regulatory mechanism for controlling the biologic effects of increased IL-1 production (Wood et al., 1994). Irradiation of the skin with UVB was also noted to result in striking elevation of IL-1ra in the desquamating scales (Hirao et al., 1996). Furthermore, an increase in the ratio of IL-1ra to IL-1α was consistently observed in SC samples obtained from inflammatory dermatoses such as psoriasis and atopic dermatitis (Terui et al., 1997). Therefore, this increase in the ratio is a nonspecific phenomenon that can occur in any kind of inflammatory skin disease regardless of the characteristics of the inflammation.

From this viewpoint it is interesting that the SC of the exposed normal facial skin contains markedly higher amounts of IL-1ra than that of unexposed areas (Hirao et al., 1996). As compared with that of other areas, the SC cell layer of the facial skin is much thinner and the barrier function is extremely poor. Its SC shows a rapid turnover of around 7 d as also noted in atopic xerosis (Baker and Kligman, 1967; Watanabe et al., 1991). These findings suggest that the facial skin is unique in that it is always in a mildly inflamed state, probably due to exposure to the environment, because of the rather compromised state of the barrier function.

### Influence of traumatic injuries from the environment on the SC functions

The skin is always exposed to various mechanical traumas, including scratching and cutting. When the viable epidermis is directly exposed to the air by total removal of the SC by experimental tape-stripping, its barrier function is quickly restored in the initial few days as assessed by TELW measurements (Tagami and Yoshikuni, 1985). There occurs a rapid increase in the synthesis and release of the intercellular lipids in the epidermis that shows a profound increase in proliferation (Menon et al., 1992). This recovery process is delayed in elderly skin (Ghahdally et al., 1996); however, it takes more than 10 d for the complete recovery of the residual mild increase in TELW as well as the decrease in the water-holding capacity of the SC (Tagami and Yoshikuni, 1985).

Interestingly, it was shown that the initial recovery process of the SC barrier function was not delayed (Tanaka et al., 1997) or was rather hastened in atopic dry skin (Glessner et al., 1997). By contrast, the restoration of its barrier dysfunction after a chemical injury induced by the application of 1% SLS in petrolatum for 24 h was more severe and prolonged than in normal individuals (Tabata et al., 1998). In this case, the damage is not confined only to the SC and upper epidermis but involves the epidermis and dermis to exacerbate the mild, clinically unobservable inflammation that persists in the skin of atopic xerosis.

Because suction blisters are produced by separation at the lamina lucida of the basement membrane, we can produce erosive lesions lacking total epidermis by breaking the top of suction blisters. In this case the restoration of the normal SC barrier function takes three times longer (45 d) than that required for the normalization of the SC after total SC removal by tape-stripping (15 d) (Suetake et al., 1996). No scar formation occurs, however, after the healing of the erosive lesions because there is little damage to the dermal tissues. In contrast, injuries involving the dermal tissues always induce scar formation, including hypertrophic scars or keloids depending on their depth and accompanying inflammation. To study the restoration of the SC function in mid dermal wounds, we can use the donor sites for split-thickness skin grafts because a reproducible depth of the wound is attainable with a dermatome. Although re-epithelization of such wounds was completed within 2 wk, abnormal SC functions expressed by elevated TELW values persisted beyond expectations. It ranged from about 150 to 500 d depending on the depth of the wound. There was also a location-dependent difference. When the thigh and the lower abdominal rare were compared, the thigh skin appeared to take much longer to normalize. Interestingly, the SC functional changes measured in hypertrophic skin and keloids were highly abnormal, corresponding to those of the split-thickness skin donor sites approximately 50 d after graft harvesting (Suetake et al., 1996).

### CONCLUSION

Some of the chemical and traumatic injuries can induce disintegration of the SC architecture; however, the viable skin tissues are more vulnerable to the effects of the environment than the SC. Thus, most of the functional abnormalities of the SC developing after environmental insults are secondarily caused by enhanced epidermal proliferation induced under the influence of underlying inflammation. Biophysical parameters and content of bioactive substances that are easily obtainable in a noninvasive fashion in the SC of the skin exposed to various environmental insults, can be utilized as a sensitive indicator for the skin damage.

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### REFERENCES

Agner T, Serup J: Time course of occlusive effects on skin evaluated by measurement of transepidermal water loss (TEWL). Including patch test with sodium lauryl sulphate and water. *Contact Dermatitis* 26:6–9, 1993


### Table II. Comparison of abnormalities of the stratum corneum between atopic xerosis and senile xerosis

<table>
<thead>
<tr>
<th>Functional analyses</th>
<th>Atopic xerosis</th>
<th>Senile xerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barrier function</td>
<td>Impaired</td>
<td>Normal or slightly better</td>
</tr>
<tr>
<td>Hydration state</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Turnover time</td>
<td>Quick</td>
<td>Slow</td>
</tr>
<tr>
<td>Number of cell layers</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Chemical analyses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin surface lipids</td>
<td>Not decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Inter cellular lipids</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Water-soluble amino acids</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
</tbody>
</table>

This table compares the abnormalities of the stratum corneum between atopic and senile xerosis.


Zhen Y-X, Suetake T, Tagami H: Number of cell layers of the stratum corneum in normal skin relationship to the anatomical location on the body, age, sex, and physical parameters. *Arch Dermatol Res* 291:555–559, 1999