Acta Derm Venereol (Stockh) 1998; 78: 279–283

Cumulative Irritation in Older and Younger Skin: a Comparison

DORIS A. SCHWINDT[1], KLAUS -P. WILHELM[2], DAVID L. MILLER[3] and HOWARD I. MAIBACH[1]

[1]*Department of Dermatology, University of California, School of Medicine, San Francisco, CA, USA,* [2]*proDERM, Institute for Applied Dermatological Research, Schenefeld, and Department of Dermatology, Medical University of Lübeck, Germany and* [3]*bioNET, Inc., Dallas, TX, USA*

Changes in stratum corneum properties due to sodium lauryl sulfate cumulative irritation were determined as a function of age. We irritated the backs of 7 younger (27.7 ± 4.6 years, mean ± standard deviation) and 10 older (69.8 ± 5.5 years) volunteers on 5 consecutive days with open application of a 7.5% aqueous sodium lauryl sulfate solution. Water- and untreated skin served as controls. Transepidermal water loss, stratum corneum capacitance, Desquamation Index, and skin roughness parameters were evaluated on 5 days of the 1st week, and on 3 days of the 2nd week. All parameters revealed a delayed and decreased reaction of older compared to younger skin and recovery appeared to be prolonged. We conclude that under these test conditions irritancy to repetitive sodium lauryl sulfate exposure of older versus younger skin resembled that previously observed after single occlusive exposure. In neither age group was the recovery effect reduced by repeated irritation, demonstrating sufficient skin barrier and recovery function. ***Key words: barrier function; desquamation; roughness; stratum corneum hydration; transepidermal water loss.***

(Accepted February 23, 1998.)

Acta Derm Venereol (Stockh) 1998; 78: 279–283.

Howard I. Maibach, MD, Department of Dermatology, School of Medicine, Box 0989, Surge 110, University of California, San Francisco, CA 94143-0989, USA.

Since many changes occurring in aging skin lead to a decline in structural and functional properties it has been speculated that aging skin is more prone to develop irritant contact dermatitis (1), and special attention has been addressed to the care of aging skin, to preventing irritation and dryness. In several studies comparing acute irritancy in aging versus young skin (2, 3), results depended on the agent, but sodium lauryl sulfate (SLS)-induced acute irritation was less pronounced and developed more slowly in older subjects.

Chronic contact dermatitis, due to repeated exposure to low doses of an irritant, i.e. a detergent, is a major clinical problem. Predominant features are skin dryness, scaling and roughness. Wilhelm et al. (4) followed the course of cumulative contact dermatitis due to SLS in subjects between 24 and 46 years of age. Barrier impairment and skin dryness reached greatest or lowest values, respectively, when treatment was discontinued, and did not reach baseline until 2 weeks after the previous exposure. Widmer et al. found an increased transepidermal water loss (TEWL) 9 weeks after cumulative irritant contact dermatitis, stressing the importance of complete healing after irritation (5).

The course of cumulative contact dermatitis is determined by a superposition of stimulus and recovery. The observation that older skin reacts more slowly to irritation could imply a prolonged recovery time. It has not yet been investigated how older skin reacts to cumulative irritation. This study investigates whether cumulative irritation of older skin results in a condition in which recovery would be reduced by repeated irritation, leading to severe skin damage.

We utilized short-term repetitive SLS exposure to model cumulative irritant dermatitis, focusing on stratum corneum properties, i.e. water content, TEWL, desquamation and relief in groups of younger and older subjects using bioengineering- and image analysis techniques. The intensity of reaction and the time course are compared, and possible correlations between the measured parameters discussed.

MATERIALS AND METHODS

Study population

Seventeen healthy Caucasian volunteers participated after providing informed consent. The study population was divided into a group of 7 younger (27.7 ± 4.6 years, mean ± standard deviation) and another of 10 older (69.8 ± 5.5 years) subjects. The study was approved by the University of California Committee on Human Research.

Induction of irritation

Each volunteer's back was treated on 5 consecutive days (days 0 – 4) according to the following scheme: To avoid anatomic selection bias the back was divided into 8 areas, 1 for each day of measurement. Within each area 3 sites for different treatments were selected: 1 for application of 7.5% aqueous SLS solution (SLS, Sigma Chemical Company, St. Louis, MO, 99% purity), and 1 for application of demineralized water; 350 µl of SLS solution or water, respectively, was pipetted on cotton pads, 19 mm in diameter. A 3rd site within each area was left untreated. The treatment sites within each area and the day of measurement for the 8 areas were randomized. Untreated and water-treated sites served as controls. Cotton pads were removed after 35 min, and the skin was allowed to dry. Measurements were taken every day of the 1st week before starting treatments (days 0 – 4) and on 3 days of the 2nd week (days 7, 9, and 11, no treatments) at each of the 3 treatment sites within the respective area. To minimize the impact of previous consecutive measurements the following order of measurements was chosen: capacitance, TEWL, D-Squame®, and replica.

Measurements

TEWL was measured with an evaporation meter (Tewameter TM 210, Courage Khazaka, Cologne, Germany, and Acaderm, Menlo Park, CA) (6). The device assesses the water vapor pressure gradient above the skin by means of 2 hygrosensors located in an open probehead at different heights (7). TEWL was recorded continuously and expressed in g/(m^{2}h). Mean readings were taken when values had stabilized.

Water content of the stratum corneum was evaluated in triplicate with a capacitance meter (Corneometer CM 820 PC, Courage & Khazaka, Cologne, Germany, and Acaderm, Menlo Park, CA). The measured capacitance of the stratum corneum increases with its water content (6, 8). Through probe design a constant application pressure of 3.56 N during the measurements is ensured.

Skin scaliness was quantified using digital image analysis of D-Squames® (Cu-Derm Corporation, Dallas, Texas) (9). D-Squames® are clear, adhesive-coated disks that sample loose corneocyte clusters and scales from the superficial stratum corneum (10). The disks were handled, and image-analyzed to provide the DI as described by Schatz et al. (9).

Skin roughness was assessed using the Skin Visiometer 400 (Courage & Khazaka, Cologne, Germany, and Acaderm, Menlo Park, CA) (11), a photometric device that analyzes dyed translucent silicon (negative) replicas of the skin, based on Bouger-Lambert's law: $I_{ex} = I_{in} e^{-kd}$, where I_{in} is the unattenuated light intensity, I_{ex} is the transmitted light intensity, k is the napirian absorption coefficient of the medium, d is the thickness of the medium. Thus, light transmission through a replica containing a constant concentration of blue dye (k = const.) depends only on its thickness. Providing a constant overall thickness of 0.5 mm light transmission through the replica varies with the skin relief.

Replicas were prepared and analyzed using the Visiometer as described by Articus et al. (11). Vertical resolution of the image varies from 0.6 up to 4 μm per grey level; horizontal resolution is approximately 20 μm. The thickness of the replica at each pixel was used to calculate surface parameters (12). The definitions of the surface parameters are standardized as given in ISO and DIN specifications (ISO 4287, DIN 4762–4768). For the description of the skin surface the amplitude parameters R are most commonly used. R_a is the arithmetic mean determined from the absolute deviations from the profile mean along a defined sampling line (arithmetic mean roughness value). For the calculation of R_z the sampling line is divided into five consecutive sections. The mean of the maximum peak to valley heights of every section is R_z (mean roughness depth). One hundred and eighty radial sampling lines were defined to account for the anisotropy of the replica. The mean of R_z and R_a from all 180 lines were taken as the roughness parameter for a given replica (12). During the measurements, room temperature was 20°C and humidity 65-70%.

Statistics

Statistical analysis was performed using general linear model repeated measures design. Thus each person served as their own control for the treatment sites; a parallel design compared the old and the young. The independent, "between-subject" variable age (two levels, younger and older), and the "within-subject" (repeated) variables treatment (three levels, baseline, SLS and Vehicle, Vehicle), and time after initial patch application (8 levels) were used as coded indicator variables. Multiple comparison of independent variables was done *a priori* using orthogonal contrasts (13). The chosen level of significance was $p < 0.05$; p-values are given for the comparison between untreated and SLS-treated skin.

RESULTS

Transepidermal water loss (TEWL)

TEWL time courses differed significantly between the age groups (Fig. 1). In younger subjects increased TEWL values were observed after 1 day of SLS treatment (p_1=0.001) and reached a maximum after 4 days of treatment, but were still significantly increased on day 11 (p_{11}=0.001). Older subjects showed a 1-day delayed onset of increasing TEWL, with the maximum on day 2. Generally, older untreated skin showed significantly lower values than younger untreated skin ($p < 0.001$).

Capacitance

Changes in stratum corneum hydration differed significantly in the age groups (Fig. 2). In younger subjects, capacitance decreased continuously, reaching lowest values on day 9. In contrast, the onset of dehydration was again 1 day delayed in older subjects, and lowest values were reached on day 2, although baseline values had not been reached at the end of study. After 3 days of SLS treatment, mean capacitance of each group was at the same level. In general, untreated stratum corneum of older subjects was less hydrated than of younger subjects ($p < 0.001$).

Desquamation index (DI)

The DI for SLS versus vehicle-, and untreated skin differed insignificantly on any day. Younger subjects tended to have an increased DI during the 2nd week, whereas older subjects showed greatest DI during the 1st week, with a maximum on day 3.

Roughness parameters

SLS-treated sites of younger subjects reached significantly increased roughness on days 3, 4 and 7 (R_z: p_3=0.004 p_4=0.007 (Table I), and R_a: p_3=0.028, $p_4 < 0.001$, p_7=0.045), and tended to stay rougher till the end of study. Older skin was significantly rougher only on days 3 and 4 (R_z: p_3=0.04, and R_a: p_4=0.003). R_z and R_a during the 2nd week were at the same level as before any SLS treatment. In general, older untreated skin was significantly rougher than younger skin ($p < 0.001$).

DISCUSSION

TEWL is a valuable parameter for assessing the barrier integrity and barrier function of stratum corneum. Although

Table I. *Observed means and standard errors of the means of R_z (μm) for water-treated, untreated, and SLS-treated skin during 2 weeks. Measures of older and younger women are given*

Day	Old			Young		
	Water	Untreated	SLS	Water	Untreated	SLS
0	96.0±7.5	92.7±4.6	104.0±7.8	78.7±3.3	88.4±5.3	82.0±3.1
1	102.0±5.9	108.5±6.5	93.3±3.9	87.7±3.3	75.4±3.8	82.0±3.1
2	98.9±7.3	97.5±3.5	100.7±6.0	86.1±6.8	75.7±4.6	79.1±3.7
3	98.0±6.8	95.9±5.3	116.1±8.1[a]	74.7±3.0	76.3±3.6	79.9±5.5[a]
4	105.0±6.8	97.9±5.5	110.3±6.3	75.0±4.5	72.4±3.1	85.1±5.3[a]
7	102.1±6.1	104.5±5.5	107.1±9.0	81.3±7.1	69.7±3.6	85.7±7.8
9	112.4±9.7	105.6±6.0	104.5±8.6	84.6±4.2	80.1±3.7	82.0±6.7
11	101.4±4.0	105.1±4.0	95.9±3.4	83.7±1.7	81.9±7.1	89.4±10.0

[a]Indicates significance at $p < 0.05$ compared to untreated skin.

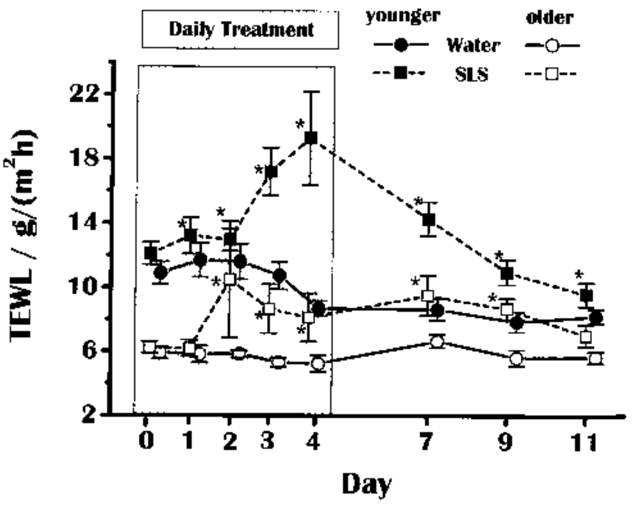

Fig. 1. Time course of the observed transepidermal water loss for younger and older subjects. The skin of the back was treated daily with 350 μl of 7.5% aqueous SLS solution; with water in open application for 35 min on days 0–4; or was left untreated. Data of untreated skin were very similar to those of water-treated skin, and are not shown for clarity. The error bars are 1 standard error of the mean.

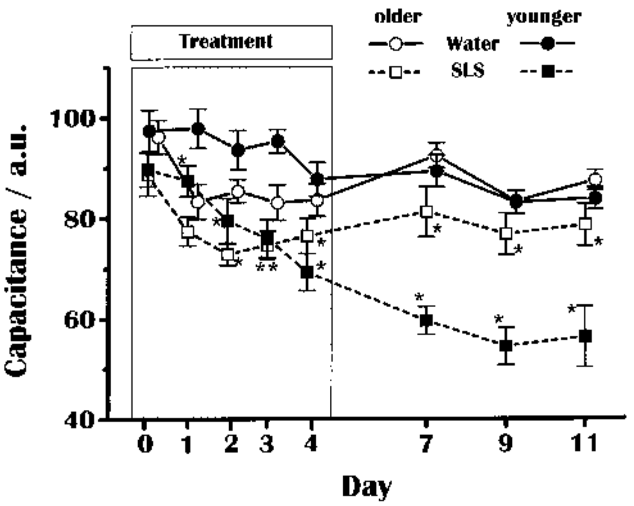

Fig. 2. Time course of the observed skin capacitances for younger and older subjects. The skin of the back was treated daily with 350 μl of 7.5% aqueous SLS solution; with water in open application for 35 min on days 0–4; or was left untreated. Data of untreated skin are not shown for clarity (s.a.). The error bars are 1 standard error of the mean.

Roskos et al. did not observe age-dependent TEWL differences (14), several studies reveal a decreased TEWL in aging compared to young skin (3, 15, 16). We confirmed the latter findings, with the mean TEWL of younger subjects being (9.0 ± 0.3) g/(m^{2}h) (mean ± SEM), and of older subjects (5.5 ± 0.1) g/(m^{2}h).

TEWL is a water flux driven by passive diffusion and depends on the diffusion constant, D, which is a material property of the stratum corneum, and on the water concentration gradient across the stratum corneum ($\partial c/\partial x$). It is likely that D and ($\partial c/\partial x$) are not independent quantities. Structural changes of aging stratum corneum could therefore directly change D; in addition, the altered stratum corneum hydration might change D indirectly, although not necessarily in the same direction.

Ghadially et al. found a global reduction of lipids with reduced numbers of extracellular bilayers in aging mice (17). Rogers et al. confirmed these findings for human stratum corneum (18). As the intercellular space is the primary domain in which water diffusion occurs (19), the effective diffusion constant of the stratum corneum is reduced by the decreased intercellular space (20, 21).

The measurement of stratum corneum hydration which should reflect the water concentration gradient ($\partial c/\partial x$) revealed decreased values for older [(86.6 ± 1.1) a.u.] compared to younger [(92.7 ± 1.1) a.u.] subjects, confirming the finding of Potts et al. (22). Thus, both the decreased hydration ($\partial c/\partial x$) and/or altered intercellular space (D) may account for the decreased TEWL in older skin. Consequently, the latter is not merely an indication of improved barrier function.

Structural changes of the stratum corneum due to chemical insult in most cases affect water diffusion. Thus, TEWL is used to assess the extent of barrier defects and to document recovery (4, 23, 24). When irritating younger subjects repeatedly with SLS, we found a sharp increase in TEWL reaching its maximum on the last (5th) day of treatment (see Fig. 1). It is tempting to attribute this observation to changes in the intercellular space, i.e. the intercellular lipids. The lipid bilayers of the upper stratum corneum, however, seem to stay intact at least in the short term (25, 26), so that the apparent barrier disruption has been attributed to changes in the amino acids and/or to protein denaturation within the corneocytes. Based on these findings one might speculate that disrupture of intracellular keratin provides an alternative pathway for water diffusion. Repeatedly irritated stratum corneum rearranged and gradually dried, indicated by decreasing capacitance values (Fig. 2), as altered stratum corneum has reduced waterholding capacity. The dried stratum corneum prevented further water loss, as suggested by a TEWL that at this stage reached almost baseline values.

In aging skin the impact of repeated SLS irritation was less pronounced. This may be the result of a decreased percutaneous absorption of the irritant governed by (i) larger corneocytes and smaller intercellular space, (ii) a flattened dermoepidermal interface [see (27) and references therein], and (iii) a reduced partitioning of the irritant into the stratum corneum due to decreased sebaceous gland activity (28). Moreover, a compromised immune response reduces inflammation. Thus, the same stimulus that led to subacute but clinically visible irritation in younger subjects caused only slightly increased TEWL and decreased capacitance in older subjects. The supposedly milder damage yielded nearly symmetrical time courses of TEWL and capacitance (Figs. 1 and 2). The onset of reaction was delayed 1 day compared to younger subjects. But despite the comparatively smaller and in most older people invisible damage, capacitance did not reach baseline level at the end of study.

Assuming that SLS irritation results in a linear increase and recovery in a linear decrease of TEWL with time (Fig. 1), we estimated the SLS insult and recovery effects to be 3.27(45) and 1.43(16) g/(m^{2}hday) for younger and 1.80(84) and 0.26(20) g/(m^{2}h day) for older skin, respectively. The numbers in parentheses are 1 standard deviation in units of the last digit given. Thus, TEWL recovery and insult effects remained constant during the investigated period and the net effect (insult minus recovery) was similar for young and old skin [1.84(48) and 1.54(86) g/(m^2h day), respectively]. This demonstrates sufficient skin barrier and recovery function in both age groups, although the SLS insult and recovery effect for older skin was decreased. Indeed, Ghadially et al. found that lipid synthesis after acute barrier damage was decreased in aged epidermis (29).

The surface of human skin shows a pattern determined by intersecting primary and secondary lines. These lines are present throughout the stratum corneum and are due to elastic fibers that reach the dermal papillae (30). The term "rough skin" is often used to describe different phenomena. Rough in a physical sense means a surface with lines of certain width and depth and can well be quantified by roughness parameters. Thus, aging skin with fewer and deepened primary and secondary lines mainly due to dermal changes will be defined rougher, although it is not necessarily rough to touch. Rough skin in the common sense means scaly and fissured skin, mainly reflecting changes of the (superficial) stratum corneum. Imokawa thus used a scaliness score to grade detergent-induced rough skin (31). However, irritation affects the entire stratum corneum. When applying detergents for 24 h Kawai et al. observed deepened furrows and membranous desquamation (32). Agner & Serup found 4 days after irritation with SLS (for 24 h, under occlusion) a rough pattern of non-parallel deep grooves (33).

R_z and R_a are sensitive measures for assessing depth changes of the microrelief. In our study the mean difference of R_z baseline values between aging and younger skin was 26.5 μm with a standard error difference of 2.6 μm (R_a: 7.0 ± 0.75 μm). Changes due to treatment were apparent after 4 days of repetitive irritation (Table I). In younger subjects R_z and R_a were still at a higher level at the end of the study, although not significantly so. Aging skin again showed a delayed onset of increasing roughness, but had reached low levels by the end of study.

Scaling is a phenomenon caused by corneocytes not shedding off singly, but rather in clusters. It appears that proteolytic degradation of corneosomes is required to regulate normal desquamation (34). Suzuki et al. found an elevated activity of proteases in SLS-induced hyperproliferation (35, 36) and suggested their essential role in SLS-induced scaling. The role of stratum corneum hydration in this process is unclear, but the observed decreased water content might well be a consequence of the proteolytic activity. Despite this fact, normal desquamation might require a sufficiently hydrated stratum corneum. Possibly both factors are indirectly correlated rather than being directly dependent on each other.

In our study results of DI were not significant, due to considerable variation. In tendency, DIs were greater towards the end of the study in younger subjects, but were increased during the 1st week in older subjects, thus roughly mirroring capacitance curves.

In conclusion, the results of this study using repeated open SLS irritation show a similar pattern of reactivity as single, occlusive irritation: older skin showed a delayed onset and less intense reaction. Although we could estimate a longer recovery time for TEWL after SLS irritation for older skin, this did not result in more severe cumulative reactions, since the SLS insult effect is less, too. The net effect was constant for both age groups. It appears that a more massive exposure over a longer time period is required to challenge older skin. Predisposition could be all forms of predamaged skin that facilitate penetration.

ACKNOWLEDGEMENT

We thank AcaDerm, Inc., Menlo Park, CA., for kindly providing the skin visiometer and materials for the skin replicas.

REFERENCES

1. Gilchrest BA. Skin aging and photoaging: an overview. J Am Acad Dermatol 1989; 21: 610 – 613.
2. Cua A, Wilhelm K-P, Maibach HI. Cutaneous sodium lauryl sulphate irritation potential: age and regional variability. Br J Dermatol 1990; 123: 607 – 613.
3. Elsner P, Wilhelm D, Maibach HI. Sodium lauryl sulfate-induced irritant contact dermatitis in vulvar and forearm skin of premenopausal and post-menopausal women. J Am Acad Dermatol 1990; 23: 648 – 652.
4. Wilhelm K-P, Freitag G, Wolff HH. Surfactant-induced skin irritation and skin repair: evaluation of a cumulative human irritation model by noninvasive techniques. J Am Acad Dermatol 1994; 31: 981 – 987.
5. Widmer J, Elsner P, Burg G. Skin irritant reactivity following experimental cumulative irritant contact dermatitis. Contact Dermatitis 1994; 30: 35 – 39.
6. Distante F, Berardesca E, Hydration. In: Berardesca E, Elsner P, Wilhelm K-P, Maibach HI, eds. Bioengineering of the skin: methods and instrumentation. Boca Raton: CRC Press, 1995: 5 – 12.
7. Nilsson GE. Measurement of water exchange through skin. Med Biol Eng Comput 1977; 15: 209 – 218.
8. Hashimoto-Kumasaka K, Takahashi K, Tagami H. Electrical measurement of the water content of the stratum corneum in vivo and in vitro under various conditions: comparison between skin surface hygrometer and corneometer in evaluation of the skin surface hydration state. Acta Derm Venereol (Stockh) 1993; 73: 335.
9. Schatz H, Kligman AM, Manning S, Stoudemayer T. Quantification of dry (xerotic) skin by image analysis of scales removed by adhesive discs (D-Squames). J Soc Cosmet Chem 1993; 44: 53 – 63.
10. Miller DL. Sticky slides and tape techniques to harvest stratum corneum material. In: Serup J, Jemec GBE, eds. Handbook of non-invasive methods and the skin. Boca Raton: CRC Press, 1995: 149 – 151.
11. Articus K, Khazaka G, Wilhelm K-P. The skin visiometer – a photometric device for the measurement of skin roughness. In: Wilhelm K-P, Berardesca E, Elsner P, Maibach HI, eds. Bioengineering of the skin: skin surface imaging and analysis. Boca Raton: CRC Press, 1997: 59 – 72.
12. Hoppe U. Topologie der Hautoberfläche. J Soc Cosmet Chem 1979; 30: 213.
13. SPSS. In: Advanced Statistics, 7.5 Chicago: SPSS Inc, 1997: 89 – 96.
14. Roskos KV, Maibach HI, Guy RH. The effect of aging on percutaneous absorption in man. J Pharm Biopharm 1989; 17: 617 – 630.
15. Wilhelm K-P, Cua AB, Maibach HI. Skin aging effect on transepidermal water loss, stratum corneum hydration, skin surface pH, and casual sebum content. Arch Dermatol 1991; 127: 1806 – 1809.
16. Leveque JL, Corcuff P, De Rigal J, Agache P. In vivo studies of the evolution of physical properties of the human skin with age. Int J Dermatol 1984; 23: 322 – 329.
17. Ghadially R, Brown BE, Sequeira-Martin SM, Feingold KR, Elias PM. The aged epidermal permeability barrier: structural, functional, and lipid biochemical abnormalities in humans and a senescent murine model. J Clin Invest 1994; 95: 2281 – 2290.
18. Rogers J, Harding C, Mayo A, Banks J, Rawlings A. Stratum cor-

neum lipids: the effects of ageing and the seasons. Arch Dermatol Res 1996; 288: 765 – 770.
19. Elias PM, Cooper ER, Korc A, Brown BE. Percutaneous transport in relation to stratum corneum structure and lipid composition. J Invest Dermatol 1981; 76: 291 – 301.
20. Marks R. Measurement of biological aging in human epidermis. Br J Dermatol 1981; 104: 627 – 633.
21. Rougier A, Lotte C, Corcuff P, Maibach HI. Relationship between skin permeability and corneocyte size according to anatomic site, age, and sex in man. J Soc Cosmet Chem 1988; 39: 15 – 26.
22. Potts RO, Buras EM, Chrisman DA. Changes with age in the moisture content of human skin. J Invest Dermatol 1984; 82: 97 – 100.
23. Agner T, Serup J. Individual and instrumental variations in irritant patch-test reactions – clinical evaluation and quantification by bioengineering methods. Clin Exp Dermatol 1990; 15: 29 – 33.
24. Lee CH, Effendy I, Maibach HI. Acute irritant contact dermatitis: recovery time in man. Contact Dermatitis 1997; 36: 285 – 290.
25. Fartasch M, Diepgen TL, Hornstein OP. Morphological changes of epidermal lipid layers of stratum corneum in sodium lauryl sulfate induced dry skin: a functional and ultrastructural study. J Invest Dermatol 1991; 96: 617.
26. Froebe CL, Simion FA, Rhein LD, Cagan RH, Kligman A. Stratum corneum lipid removal by surfactants: relation to in vivo irritation. Dermatologica 1990; 181: 277 – 283.
27. Harvell JD, Maibach HI. Percutaneous absorption and inflammation in aged skin: a review. J Am Acad Dermatol 1994; 31: 1015 – 1021.
28. Pochi PE, Strauss JS, Downing DT. Age-related changes in sebaceous gland activity. J Invest Dermatol 1979; 73: 108 – 111.
29. Ghadially R, Brown BE, Hanley K, Reed JT, Feingold KR, Elias PM. Decreased epidermal lipid synthesis accounts for altered barrier function in aged mice. J Invest Dermatol 1996; 106: 1064 – 1096.
30. Montagna W, Carlisle K. Structural changes in aging human skin. J Invest Dermatol 1979; 73: 47 – 53.
31. Imokawa G. Comparative study on the mechanism of irritation by sulfate and phosphate type of anionic surfactants. J Soc Cosmet Chem 1980; 31: 45 – 66.
32. Kawai K, Nakagawa M, Kawai J, Kawai K. Evaluation of skin irritancy of sodium lauryl sulphate: a comparative study between the replica method and visual evaluation. Contact Dermatitis 1992; 27: 174 – 181.
33. Agner T, Serup J. Skin reactions to irritants assessed by polysulfide rubber replica. Contact Dermatitis 1987; 17: 205 – 211.
34. Lundström A, Egelrud T. Stratum corneum chymotryptic enzyme: a proteinase which may be generally present in the stratum corneum and with a possible involvement in desquamation. Arch Dermatol Res 1991; 71: 471 – 474.
35. Suzuki Y, Nomura J, Koyama J, Horii I. The role of proteases in stratum corneum: involvement in stratum corneum desquamation. Arch Dermatol Res 1994; 286: 249 – 253.
36. Suzuki Y, Nomura J, Hori J, Koyama J, Takahashi M, Horii I. Detection and characterization of endogenous protease associated with desquamation of stratum corneum. Arch Dermatol Res 1993; 285: 372 – 377.