

The effects of prolonged use of surfactants on the skin of normal and photo-exposed hairless mice*

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Laboratory tests to assess the irritant potential of materials, such as skin cleansers, which are normally used over a long period by humans, fail to mimic actual use. Most washing tests last a few days or at most a few weeks. Skin sites and techniques are often not standardized. The more standardized patch test involves occlusion and results in exaggerated reactions, since even water and blank patches produce visible and pathophysiologic changes. All of these tests rely on visual assessment despite strong evidence that similarly appearing skin can be very different histologically. The primary objective of this study was to use a well-defined animal model to evaluate the cumulative effects of repeated skin exposure to low levels of surfactants of varying skin irritation potential. A secondary aim was to examine whether or not surfactant-induced skin changes were exacerbated by suberythral UV radiation. Test materials were applied topically, 2× daily to the dorsal areas of normal and low-dose solar simulator exposed mice for 15 weeks. Our results show that, with conditions mimicking typical normal use, these surfactants and skin cleansers produce little or very mild histological changes in the skin. UV irradiation alone produced the greatest change in all histological parameters examined, with no synergistic or additive effects with the topical treatments.

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Abbreviations:

SLS: sodium lauryl sulfate
syndet: synthetic detergent
UVR: ultraviolet radiation
SSR: solar simulating radiation
TEWL: transepidermal water loss
GAGs: glycosaminoglycans

Assessment of the irritant potential of detergents and surfactants on human skin is fraught with inconsistencies. In the absence of a standard protocol, results depend largely on how and with what subjects the studies are performed. In practice, skin sites may be face, hands, legs, inner or outer arm, sun-exposed or not. Ages of subjects vary widely. Application or washings may be with gloved hands, cloths or other abrasive materials, once to multiple times per day for a few days or a few weeks. Water hardness adds another variable

as does the concentration of materials. Environmental factors such as temperature and humidity can also play a rôle.

The more standardized patch test eliminates some of these problems but adds yet another, that of occlusion. Water under occlusive conditions or even empty chambers produce visible and pathophysiologic changes that range from redness to a damaged horny layer with increased permeability (1). Despite this, an occasional report on skin irritation will fail to include either a water or blank control patch (2). Although a rare patch test study will include biopsy of the sites (3, 4), for the most part, assessment is visual (5) or by somewhat more sensitive non-invasive bioengineering methods (6–8). Visual assessment can be misleading since Kligman has clearly demonstrated in humans that similarly appearing skin can be vastly different histologically (9).

The aim of this study was to explore, in a well-defined animal model, the histological effects resulting from exposure to surfactants at doses and frequencies which closely mimic human ex-

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perience. We chose the hairless mouse for a number of reasons. Extensive studies have shown that its skin responds to ultraviolet radiation (UVR) in a manner similar to that of humans with regard to erythema, connective tissue damage and tumorigenesis (10–13). Furthermore, recent studies (14) show that hairless mouse skin responds to chronic application of topical chemicals such as lactic acid, benzalkonium chloride and isopropyl myristate with a varied panoply of histologic changes that appear to be similar to those seen with UVR. Furthermore, since cleansing products may be used for decades, the model is amenable to chronic application of these products. Additionally, ample amounts of tissue are available for biopsy, thus avoiding the sampling error inherent in small punch biopsies.

Since surfactants and skin cleansers are used on sun-protected as well as sun-exposed skin, in this study 1/2 of the mice were also exposed to low-level solar simulating radiation (SSR) corresponding to a modest daily exposure to sunlight. Surfactants were used for 15 weeks. The widely-studied sodium lauryl sulfate (SLS, 5%w/w) was used as a positive control (15). SLS concentrations of approximately 5% under occlusion, produce significant changes in skin color, transepidermal water loss (TEWL), epidermal morphology, inflammation and mitotic activity (3, 4, 7, 8, 16). Our studies indicated that with normal use, rather than with occlusive techniques, surfactants and skin cleansers produce little or very mild changes in the skin.

Materials and Methods

Topical treatments

Groups of 8 female albino Skh-hairless-1 mice (Charles River Laboratories, Wilmington, MA), age 8–10 weeks, were treated on the dorsal trunk 2× daily 5× a week for 15 weeks with 100 μ l of 1 of the following 5% aqueous solutions: sodium lauryl sulfate (SLS: Sigma Chemical Co., St. Louis, MO); soap bar (sodium tallowate and sodium cocoate); synthetic detergent bar (syndet: sodium cocoyl isethionate, stearic acid and sodium cocoate/tallowate); liquid soap (sodium laureth 2 EO, cocoamido propyl betaine and decyl polyglucose). Deionized water alone was used as a control. Materials were gently rubbed on the skin for 30 s using a finger cot and were gently rinsed away with 1–2 ml of deionized water. Skin was allowed to air dry. The study was carried out during Jan–May 1996 at the University of Pennsylvania. Animal handling was performed in accordance with the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Solar simulating radiation (SSR)

Another cohort of mice (8/group) was first exposed to 1 MED (minimal erythema dose) of SSR (solar simulated radiation). 2 to 3 h later, the mice were treated with the test material as described above. The solar simulator was a forced air cooled compact 5700 watt xenon arc (Kratos Analytical Instruments, Ramsey, NJ). The collimated beam was passed through a water filter before impinging on a 45° UV-reflecting dichroic mirror and finally through a 1 mm Schott WG 320 filter. Mice (8 at a time) were placed on a turntable in a circular cage that prevented them from shielding one another. Their dorsal surface was 16 in below the UV source. To deliver 1 MED dose ($\sim 0.054 \text{ J/cm}^2$ UVB), the animals were exposed for ~ 5 min with irradiances of 0.16 and 4.78 mW/cm^2 , respectively for UVB and UVA, as measured with an IL-700A Research Radiometer (International Light, Inc., Newburyport, MA). The UVB sensor had a peak sensitivity at ~ 290 nm, whereas that of the UVA was at ~ 360 nm.

Transepidermal water loss (TEWL)

TEWL was evaluated at the beginning and end of the study with a Servo Med Evaporimeter (Stockholm, Sweden). The probe was placed on the lower dorsum.

Histologic evaluation

On Monday following the final Friday treatments, animals were sacrificed (CO_2 asphyxiation) and biopsies (2×0.5 cm) were taken from the central dorsal skin, perpendicular to the long axis of the trunk. The biopsies were fixed in 10% buffered formalin and prepared for light microscopy. Skin sections were stained as follows: hematoxylin and eosin (H&E) for general morphology and assessment of inflammation, Luna's aldehyde fuchsin (17) for elastic fibers, Masson's trichrome for collagen and Mowry's colloidal iron for glycosaminoglycans (GAGs).

Global assessment of histological changes were performed on all stained specimens. The observer (LHK) was blinded as to the nature of the treatment.

Quantitative analysis of histology sections was carried out using 2 contiguous histological sections, each photographed at the midpoint of the section (H & E stain: $\times 100$ magnification). Quantitative measurements, including the epidermal thickness (hyperplasia), dermal thickness and number of inflammatory cells, were performed at 3 different points on the photographs. These

points were the same for all the photographs and were measured equidistantly from the edges of the photographic field. 5 measurements were made for each parameter. Statistical analysis of the differences among the means for all the treatment groups was performed using the analysis of variance method, ANOVA.

Results

Clinical and biophysical assessment

During the course of and at the completion of the study, clinical examination of the skin surface and color revealed little or no changes in any of the topically-treated, unirradiated mice (Fig. 1a). SSR produced a modest wrinkling of the skin and the topical treatments had no consistent additive effect (Fig. 1b).

TEWL values were unchanged by any of the topical treatments in unirradiated and irradiated

mice. Measurements ranged from 5.35–6.54 g/m²/h (data not shown).

Histologic assessment

Many aspects of skin morphologic changes and inflammatory sequelae were examined in the 10 treatment groups. To conserve space, no attempt has been made to present all changes in all treatment groups. The most typical findings are illustrated below:

Epidermal changes and inflammation (H & E stain)

The normal hairless mouse. The epidermis of normal untreated hairless mouse is thin and composed of 3–5 layers of cells including 1–2 granular layers (Fig. 2a). The dermis consists of 2 histologically distinct layers of approximately equal size. The upper 1/2 contains mainly collagen bundles in



Fig. 1. Clinical appearance. (A) Right: untreated normal mouse. Dorsal trunk is yellowish pink with fine wrinkles that appear and disappear with body movement. Left: liquid soap treatment: the skin is the same as in the untreated animal. (B) Right: SSR only: skin appears slightly thickened and shallow transverse wrinkles have appeared. Left: SSR+liquid soap: the skin is similar to the SSR control.

loose random array. The lower 1/2 is comprised of a mixture of variable amounts of lipocytes, large keratinizing cysts scattered in 1–2 rows and a focal, mild granulomatous reaction resulting from occasional rupture of the cysts (11).

Topically-treated and unirradiated. In all topically-treated unirradiated groups, parameters such as total skin thickness, epidermal cell layers, keratinocyte staining characteristics, epidermal

lymphocytic infiltrate (exocytosis), number of dermal cysts and extent of the granulomatous reaction appeared similar to those seen in untreated controls. The only notable change was in the degree of upper dermal cellularity, which was within the normal range in SLS-treated skin (cf. Fig. 2a, b), but focal and slightly increased in most specimens treated with the soap bar, liquid soap, syndet, and with water alone (Fig. 2c–f). In general, the

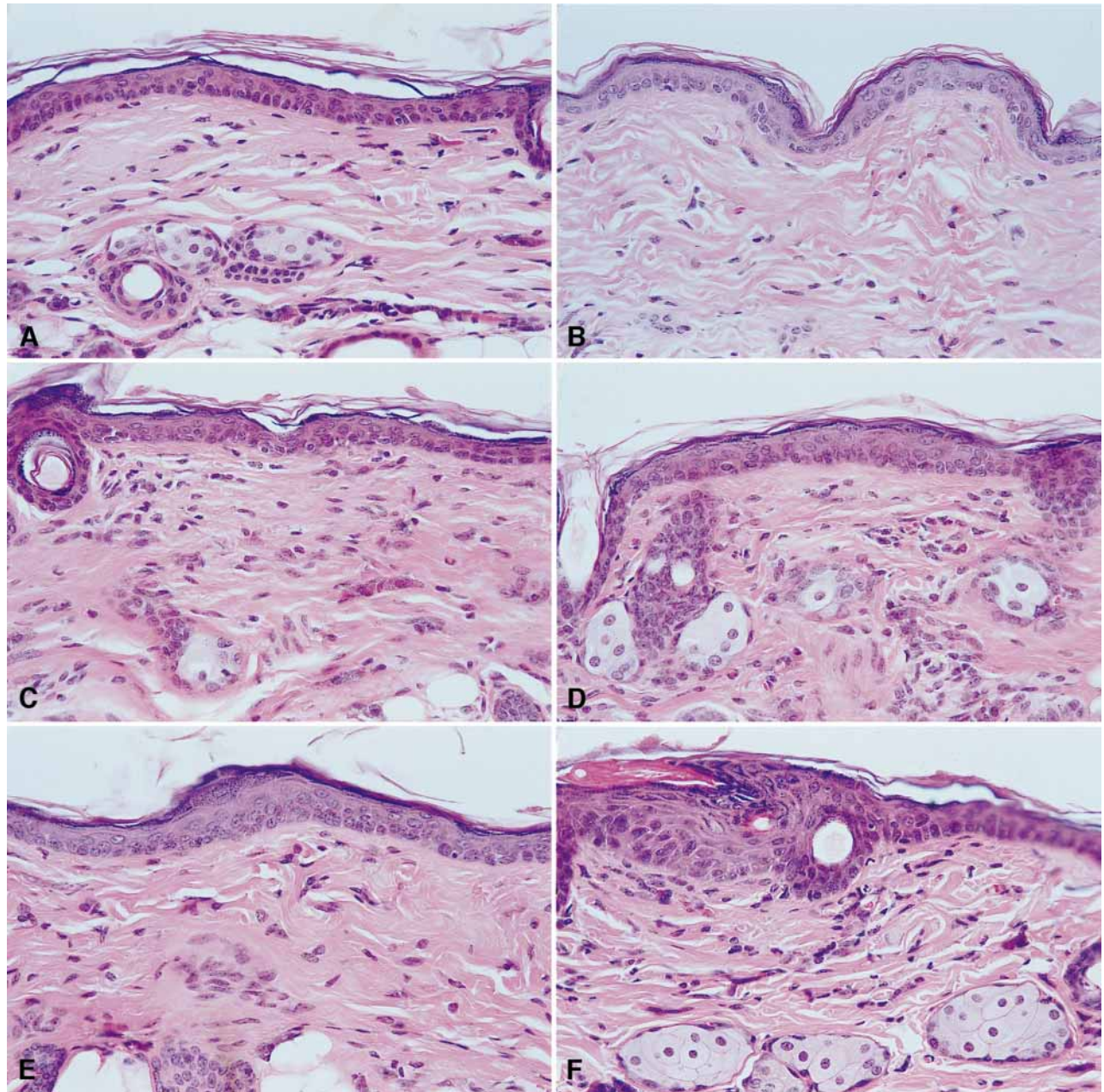


Fig. 2. Hematoxylin & Eosin (H&E) stain of unirradiated skin ($\times 100$ magnification) (A) Untreated skin: the normal epidermis consists of 3–5 cell layers including 1–2 granular layers. The upper dermal cellularity consists of some scattered lymphocytes and fibroblast-like cells. (B) SLS treatment: the skin and its cellularity is comparable to untreated control. (C, D, E) Topically treated with soap bar, liquid soap and syndet, respectively. Dermal cellularity was mildly and focally increased by all treatments. The increase was due, mainly, to fibroblasts. (F) Water treatment. The increase was more of an inflammatory nature and appears primarily due to increased lymphocytes. The thickened area of the epidermis is a sectioning artifact.

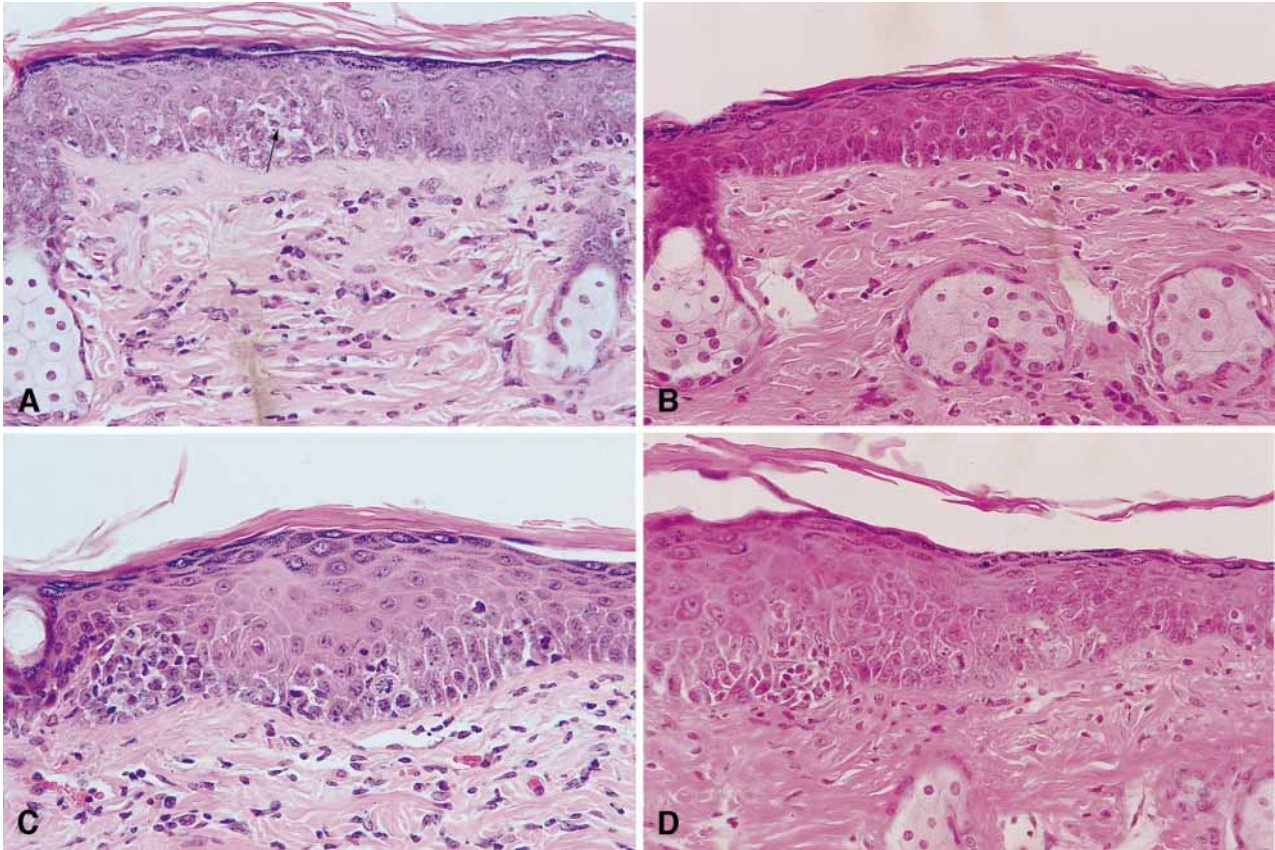


Fig. 3. H & E stain of SSR irradiated skin ($\times 100$ magnification) (A) SSR only. The epidermis is hyperplastic with cellular atypia, loss of polarity and exocytosis ($\rightarrow$). Dermal cellularity is greatly increased by a number of lymphocytes. (B) SSR + SLS treatment: the topical treatment did not enhance the SSR effect. (C) SSR + soap bar. The inflammatory infiltrate is more severe than with SLS but is within the range seen with SSR alone. (D) SSR + Syndet. One of the few specimens in the group with a more severe inflammatory infiltrate that, however, was still within the range of SSR alone.

detergent-induced increase in dermal cellularity was due mainly to increased fibroblasts whereas with water it was more of an inflammatory nature.

Topically-treated and irradiated. Changes in the

specimens from topically-treated and irradiated mice tended to be somewhat variable. For example, downgrowths of the epidermis and atypia varied from animal to animal in all groups. Similarly,

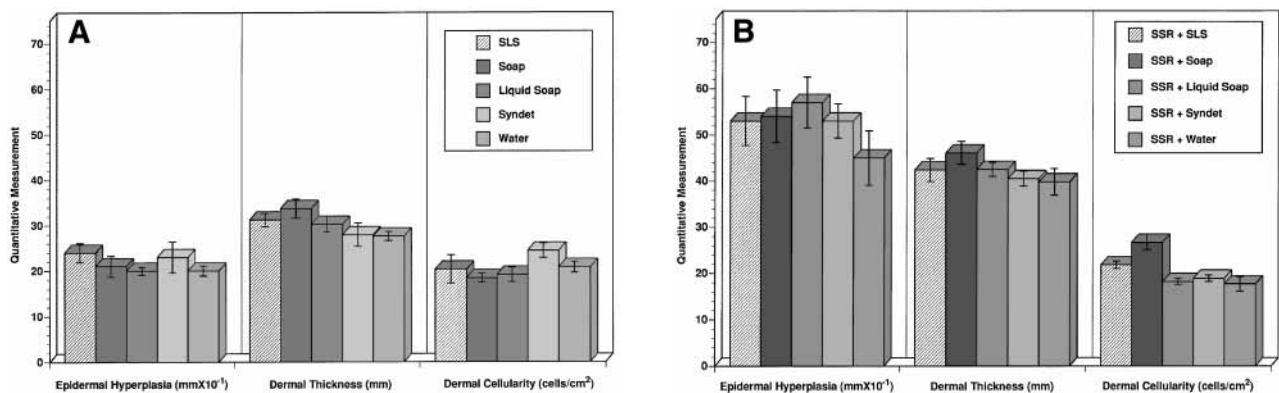


Fig. 4. Quantitative analysis of histological sections (H & E $\times 100$ magnification). The histograms are used to compare the quantitative measures of the 5 treatment groups ($n=7$). They show an interval representing ± 1 S.E. but are not intended to demonstrate statistically significant differences among the treatments. (A) Unirradiated mouse skin after 15 weeks of topical treatment. (B) Irradiated mouse skin after 15 weeks of topical treatment.

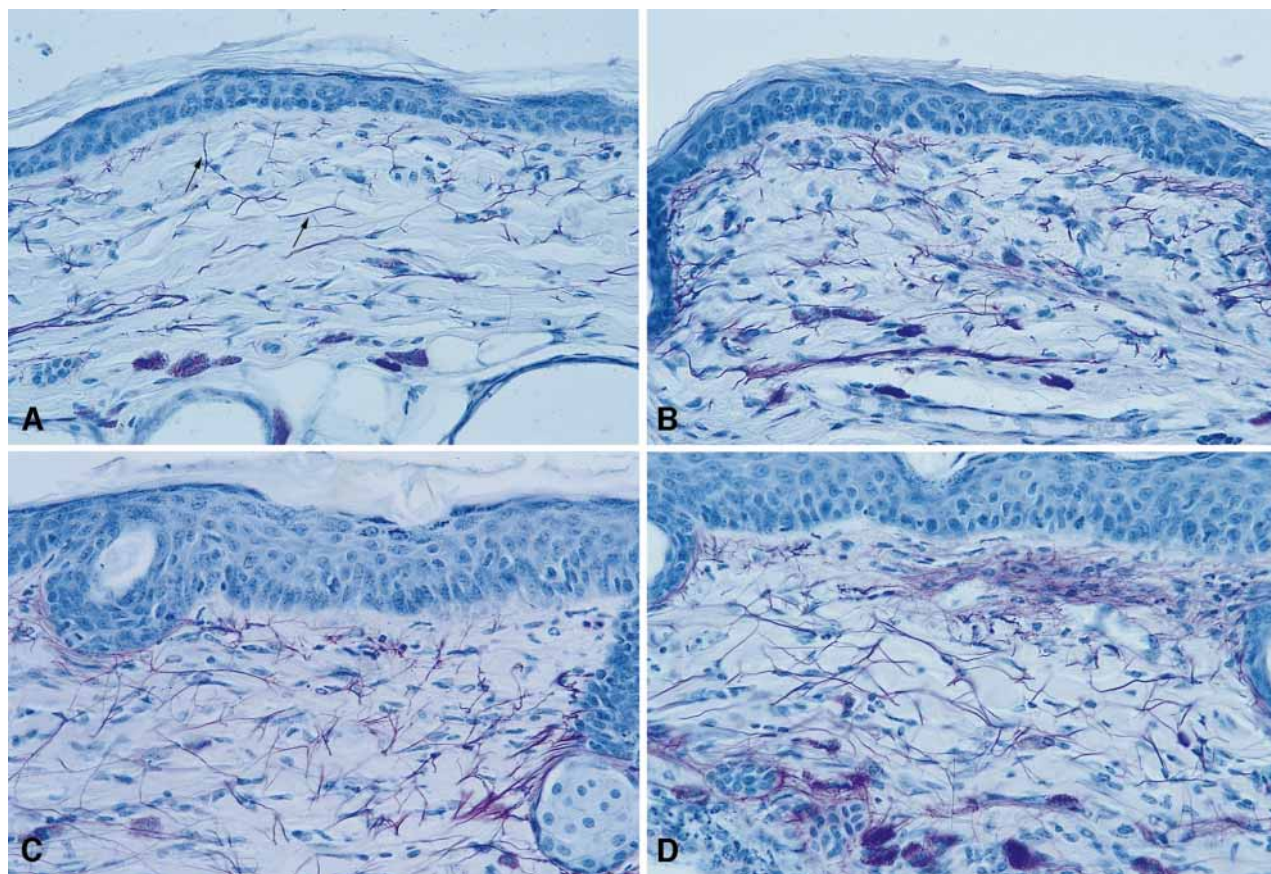


Fig. 5. Luna's stain for elastic fibers ($\times 100$ magnification). (A) Untreated skin: The thread-like elastic fibers are thin and sparse ($\rightarrow$). The ovoid purple structures are mast cells. (B) Syndet: elastic fiber hyperplasia is mild and focal. (C) SSR only: elastic fiber hyperplasia is less focal than in unirradiated skin and it varies from mild to moderate. (D) SSR+water: most specimens had a moderate degree of elastic fiber hyperplasia.

overall skin thickness, as judged microscopically, was increased in only 1/2 the SLS and soap bar-treated animals. In general, only a few specimens (1–2) from each topically-treated group showed a greater degree of epidermal hyperplasia than SSR control. However, most specimens in SLS and water-treated groups were similar to the SSR control (Fig. 3a, b). Exocytosis was mild and focal in all groups.

Upper dermal cellularity was similar to SSR controls in most specimens (Fig. 3a, b), although a few specimens from the soap and syndet groups had a more severe infiltrate (Fig. 3c, d). In all groups, including SSR controls, the increased cell population consisted of fibroblasts, lymphocytes and a few eosinophils. Changes in the lower dermis were similar in all groups and involved a slight enlargement of the cysts and a variably exacerbated granulomatous reaction. Thus, the varied morphology of the topically-treated SSR irradiated groups, as revealed by H & E stain, was judged to be due, largely, to the usual range of response of these animals to SSR.

An attempt was made to quantify some of the described histological changes (Fig. 4a, b). Similar to the subjective assessment, differences between treatment groups were mainly small and not statistically significant. These differences were sometimes not in complete agreement with the observer scores. For example, in Fig. 4b, in SSR+soap-treated skin, *only* dermal cellularity was significantly higher than other treatments. The well-known focal nature of histological changes in the skin contributed to these discrepancies.

Elastic fibers: Luna's stain

Normal hairless mouse skin contains sparse amounts of thin elastic fibers (Fig. 5a). In some animals, a few foci of very fine fibers appear with increasing age. The only change observed in the topically-treated unirradiated animals was with syndet treatment (Fig. 5b) and water controls which produced a mild to moderate focal elastic fiber hyperplasia in approximately 1/2 of the mice.

SSR controls had mild to moderate elastic fiber

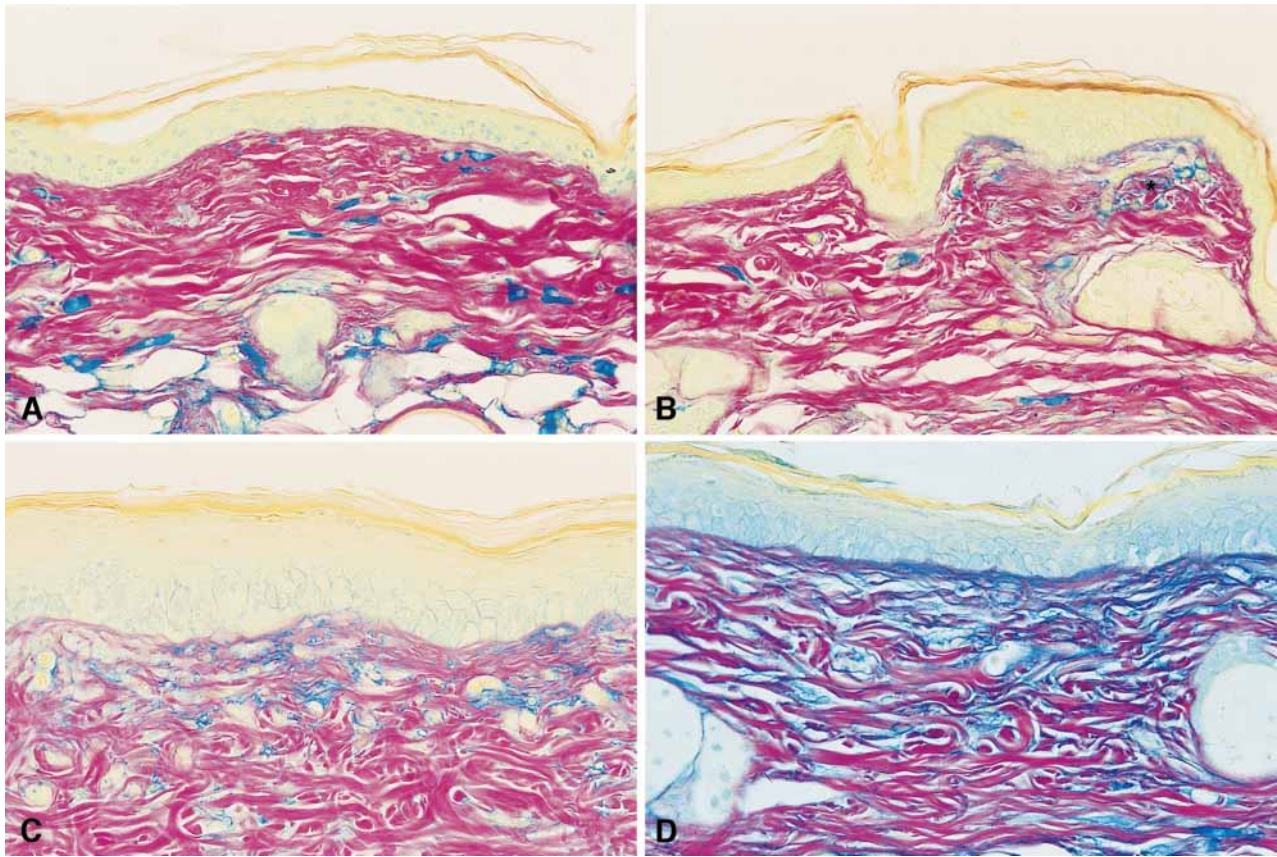


Fig. 6. Mowry's stain for glycosaminoglycans ($\times 100$ magnification). (A) Untreated: blue staining GAGs can rarely be discerned in normal skin. The ovoid blue structures are mast cells. (B) SLS treatment: this tiny focus of blue staining GAGs (*) is similar to what can occasionally be seen in untreated skin. (C) SSR only: a generalized mild scattering of blue staining GAGs is present subepidermally across large area of the specimen. Note mild increase in intercellular epidermal GAGs. (D) SSR+water: water treatment and syndet (not shown) produced the greatest increase in GAGs. Intercellular epidermal GAGs are increased.

hyperplasia (Fig. 5c). There were some foci of fine fibers whose density exceeded that found in age-matched unirradiated controls. There were, however, no thickened elastotic accretions. As in SSR controls, all topically-treated, irradiated mice had a mild to moderate elastic fiber hyperplasia. Most specimens from the soap bar, syndet and liquid soap groups were in the mild category whereas SLS (Fig. 5d) and water-treated specimens were in the moderate category.

Glycosaminoglycans: Mowry's stain

Normal age-matched control skin contains very sparse quantities of blue staining GAGs in the upper dermis. This ranges from none to scattered faint traces between collagen bundles (Fig. 6a) and an occasional tiny diffuse focal deposition. In the case of the treated, unirradiated animals, all groups showed a very mild focal increase in GAGs. The water-treated group had the greatest number of specimens (6/7) with increased GAG content in this category (Fig. 6b).

Similar to SSR control animals, most of the topically-treated, irradiated animals exhibited a mild, generalized increase in dermal GAGs (Fig. 6c). In addition, the greatest increase in dermal GAGs was seen in a few specimens from the syndet group and the water-treated group (Fig. 6d). Focal intercellular epidermal GAGs were present in all specimens, including the SSR controls.

Collagen: Masson's trichrome

All specimens from topically-treated unirradiated animals stained a brilliant blue, as did the collagen of normal age-matched controls (Fig. 7a).

In irradiated control animals, collagen damage, as judged by loss of affinity for the stain, was virtually nonexistent. However, in some areas of the skin and with some treatments there were foci of thin collagen bundles that did not stain as intensely as the thicker bundles. Fibroblasts were abundant in these areas, suggesting new collagen synthesis (Fig. 7b). These minor variations were mainly due to the focal and random nature of

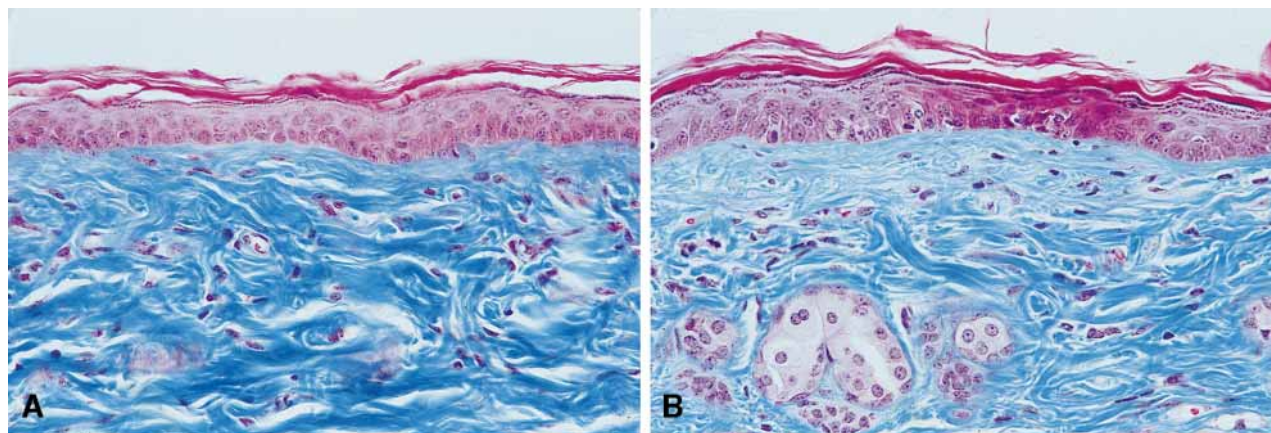


Fig. 7. Masson's trichrome stain for collagen ($\times 100$ magnifications). (A) Untreated skin: collagen in the entire dermis stains with the same intensity. All topically treated skin had the identical staining property. (B) SSR+SLS: typical of SSR controls and all other topical treatments, this specimen shows a small area of thin subepidermal collagen bundles which stain more lightly than the collagen below.

changes in mouse skin. None of the topical treatments had any additive or synergistic effects.

Discussion

Under experimental conditions intended to mimic normal human use, surfactants and skin cleansers appeared to cause little visible or histologic damage to the unirradiated skin of hairless mice. In contrast, exposure to low level SSR, for approximately $\sim 15\%$ of the animal's lifetime, was more damaging than repeated applications of these surfactants on unirradiated skin. Furthermore, the use of the cleansers did not significantly or consistently add to the SSR-induced damage.

In unirradiated, topically-treated skin all parameters examined with H & E stain were within the normal range with the exception of upper dermal cellularity. The 3 cleansing solutions (soap, syndet and liquid soap) evoked a mild focal increase that was due mainly to fibroblasts. However, water alone induced the greatest increase in cellularity with a mixture of fibroblasts and lymphocytes thus suggesting an irritant response. The stronger reaction with water may be related to greater frictional effects (i.e., "lack of slip") relative to the surfactant solutions. In irradiated skin, in general, the increase in dermal cellularity for both control and topically treated, was due to fibroblasts, lymphocytes and a few eosinophils. Other changes in the general morphology were also mainly attributable to the SSR alone. In the case of the few specimens that exhibited more marked changes, these were well within the range of variability of the response of these mice to UV irradiation.

The low-grade histological changes induced by the surfactants treatments were unexpected. This could suggest that the skin of hairless mouse was unresponsive to such challenges. However, the unirradiated hairless mouse is shown to be capable of displaying a wide range of responses to topical chemicals. In a previous study (14), an open application of 1.5% benzalkonium chloride applied for only 8 weeks induced an intense inflammatory infiltrate and increased numbers of fine elastic fibers in the dermis. Concurrent studies in this laboratory with mice exposed to SSR for 12 weeks showed that topically-applied benzalkonium chloride (2.5%) produced dermal inflammation, increased mast cells, elevated glycosaminoglycans and elastic fiber hyperplasia to an extent greater than that found in SSR controls (L. Kligman unpublished data).

The modest changes observed in this study, including the insignificant SLS effects, contrast sharply with the more severe skin reactions seen in occlusive patch testing in humans. Pirot et al. (8) examined an SLS dose response in a 24 h occlusive patch test on the mildly sun exposed volar forearm and reported a significant increase in redness and TEWL with 5% SLS compared to water patch tested sites. Significant changes in TEWL values were seen with SLS doses as low as 0.3% indicating barrier damage. Similarly, in a 24 h occlusive patch test study on human subjects of the 3 cleansers used in this study, the soap bar and syndet solutions produced significant redness compared to water filled or empty chambers (data not shown).

In a 48 h occlusive patch test using 5% aqueous SLS on the volar forearm, Willis et al. (3, 4)

demonstrated even more drastic alterations. The epidermis was parakeratotic with spongiosis, exocytosis and an increase in proliferating keratinocytes (3, 4). Ultrastructural studies revealed keratinocytes with numerous lipid droplets and membrane bound vesicles (18). In addition, SLS produced a large and significant increase in leukocyte infiltration into the dermis (225 cells/mm epidermis versus 75 cells/mm epidermis in the water control) (4). By contrast, SLS-treated non-occluded mouse skin, whether irradiated or not, appeared similar to the respective controls.

Histologic examination of connective tissue also revealed little changes as a result of washings with the surfactants and skin cleansers. Elastic fiber content in the majority of unirradiated specimens was in the normal range. Only water and the syndet induced a mild elastic fiber hyperplasia in a few of the specimens. SSR alone induced a greater change with half the specimens developing a moderate increase in elastic fibers whereas water treatment of irradiated skin shifted the majority of specimens into the moderate category. Glycosaminoglycans in most unirradiated specimens and in all treatment groups were very mildly and focally increased. Only the SSR controls and SSR plus water groups had a few specimens in the moderate category. None of the treatments induced histochemically detectable losses in collagen. Indeed, all irradiated groups showed a few foci of increased fibroblasts and thin collagen bundles suggesting areas of collagen synthesis.

Taken together, these results indicate that under normal use conditions, as opposed to occlusive patch testing or exaggerated washing conditions, these surfactants and skin cleansers are relatively benign. Many of the mild changes seen here may be the result of rubbing during application. Interestingly, the result of this study is in agreement with previous clinical home-use studies (up to 2 week duration) confirming that the same soap and syndet bars do not induce skin irritation and erythema in humans (personal communications, P. Rizvi, unpublished data). UV irradiation alone produced the greatest change in all histological parameters examined. Intriguingly, we found that low level SSR was more damaging to the skin than repeated contacts with surfactants (e.g., 5% SLS) and skin cleansers (e.g., soap). The use of topically-applied surfactant-based products on mildly photodamaged skin resulted in no additional changes, indicating little interactions between surfactants and UV exposure.

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