A New Model for Assessing the Damaging Effects of Soaps and Surfactants on Human Stratum Corneum

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To elucidate the damage to the horny layers of human skin produced by surfactants and soaps, we evaluated the cytological alterations of corneocytes using an in vitro assay. Suction blisters, 8 mm in diameter, were raised on the forearms of young adult Caucasians. The roofs were cut off and the viable epidermis was removed. The discs of stratum corneum were then agitated for up to 6 h at 60°C in 1% solution of soap bars of differing irritancy. Additionally, individual examples of anionic, cationic and non-ionic surfactants were similarly evaluated. Measurements of corneocytes included: (1) the number released with time (disaggregation), (2) size (swelling) and (3) morphological change. The effects of the cationic and non-ionic surfactants did not differ significantly from those of distilled water. The anionic surfactant caused more release and less swelling and morphological change. The test soaps had vastly different effects on the structural integrity of the stratum corneum. The harsher ones caused greater disaggregation, more swelling and greater morphological deterioration of corneocytes, whereas the milder ones had less marked effects on these parameters. This model would be a useful screening technique for formulating milder soaps and might also provide insights into the complex modes of action of surfactants on the stratum corneum. (Accepted August 13, 1996.)

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A vast amount of literature testifies to the fact that soaps can provoke an acute inflammatory reaction by patch exposure and chronic dermatitis after repeated washing. In 1946, Saltzberger & Bar comprehensively reviewed existing medical knowledge of the harmful effects of soaps on human skin (1). Fifty years later, there was still no consensus regarding the everyday hazards of soaps. Then came a spate of papers declaring that the ability of soaps to induce dermatitis had been exaggerated (2, 3). Clinicians who washed the dermatitic skin of infants with a variety of soaps failed to demonstrate worsening (4, 5). Frosch & Kligman (6) then developed a soap chamber test, in which occlusive exposure daily for 5 days made it possible to separate soaps into classes of mild, moderate and severe irritancy potential. The irritancy potential was assessed by including non-invasive, instrumental measurements capable of detecting differences not apparent to the eye.

Disaggregation technique
The discs of stratum corneum were agitated by a magnetic stirring bar in the device shown in Fig. 1. The internal cage was fitted with a 100-µm mesh screen to allow the passage of individual corneocytes. A closely fitting, removable plastic tube was placed in the cage to prevent splashing and loss of fluid. This also allowed easy removal of the cage for sampling the corneocyte suspension at intervals. The discs were agitated at 60°C for up to 6 h in 1% solution of the test surfactant in distilled water.
One-microliter samples were removed at intervals, placed on a glass slide. The staining method was modified by McGinley et al. (11) and Höfle & Plewig (12). The specimens were stained with 0.1 μl of a solution of 0.5% rhodamine B and 0.75% methylene blue in ethanol:water (1:3). They were then smeared out and mounted in Paraplast® SP-15-500 Balsam (Fisher Scientific, Fair Lawn, New Jersey, U.S.A.). Morphologic changes in the corneocytes were evaluated with a conventional microscopy. The number of corneocytes per microliter was counted using the grid of a Fuchs-Rosenthal hemacytometer. To estimate the alteration of corneocyte size by surfactants, the longest diameter of 50 randomly selected cells was measured using the scale inserted into the eyepiece of an Olympus BH-2 light microscope. The change of this value allows one to assess the alteration of permeability of corneocyte envelope by surfactant solutions. These investigations were made with three discs of horn layer from different donors for each of the surfactants and soaps. The results were expressed as mean ± standard error of the mean (SEM) of the counts and the longest diameter of the corneocytes. Differences between the values in test solution and distilled water were statistically tested for significance using the Mann-Whitney U-test.

Before this study, we compared the counts and longest diameter of corneocytes released from fresh and 1-week stored materials in 1% Ivory® (Procter & Gamble Co., Cincinnati, Ohio, U.S.A.) soap solution at 60°C. We found that fresh specimens gave the same results as stored ones. To determine the optimum temperature of soap solution, we compared the corneocyte release and longest diameter of corneocytes in 1% Ivory® solution at 40, 50 and 60°C. At 40°C, there was considerably less release of corneocytes. Increasing the temperature to 50°C yielded more cells, but still substantially fewer than at 60°C (p < 0.05). The increase of longest diameter of corneocyte was almost same at 40, 50 and 60°C (p > 0.1). Over 60°C, it was difficult to keep a fixed concentration of soap solution by evaporation. We also compared the release and swelling of corneocytes in 0.5%, 1% and 2% Ivory® solutions, and 1% solution induced most release of corneocytes (p < 0.01). The increase of longest diameter of corneocyte was almost the same in 0.5, 1 and 2% solutions (p > 0.2). We examined the change of corneocytes in 1% solution of surfactants and soaps dissolved in distilled water at 60°C.

**RESULTS**

**Corneocyte counts**

Pure surfactants (Fig. 2). In distilled water, 140 to 160 corneocytes per microliter were released over a 6-h period. With SLS a peak count of 650 μl was obtained after 4 h of exposure (p < 0.02). The count for hyamine 3350 was not different from that of distilled water (p > 0.3), while the count for Triton X-100 was slightly higher (p < 0.05).

Bar soaps (Fig. 3). Ivory (p < 0.01) released almost 3,000 cells/μl after 1 h. The peak count with Dove (p < 0.02) at 2 h was 1,400 cells/μl, less than half of this amount. The release

![Fig. 2. The corneocyte counts released from horn layer discs in 1% surfactant solution and distilled water at 60°C. Mean and SEM.](image)

![Fig. 3. The corneocyte counts released from horn layer discs in 1% solutions of bar soaps at 60°C.](image)
Neutrogena peaked at about 45 μm after 2 h (p < 0.01). Minon produced a peak at 38 μm (p < 0.05). Dove had an effect only slightly greater than water (p > 0.1). The rank order of swelling, except Ivory, was quite different from that of the corneocyte counts.

**Morphologic changes**

**Surfactants.** The corneocytes obtained by tape-stripping (13) were polygonal with well-defined cell borders. The staining intensity of the cells was strong. The corneocytes released into distilled water showed no discernible changes in size or shape and stained well with rhodamine B. The cell outlines were regular polygons (Fig. 6). Triton X-100 induced slight swelling, vacuolization and moderate loss of staining intensity. However, the corneocytes retained their polygonal shapes after 6 h. The same changes occurred with SLS and hyamine 2500, but to a lesser degree (Table I).

**Bar soaps.** Bar soaps differed greatly with regard to the structural injury they produced in corneocytes. Ivory showed the most severe morphologic changes. After 1 h, the corneocytes became progressively larger with many small vacuoles (arrow), later often reaggregating into clumps of grossly abnormal corneocytes (Fig. 7a). With increasing exposure, the corneocytes showed total loss of staining and became progressively swollen, eventually undergoing rupture and total dissolution (Fig. 7b). By contrast, Dove (Fig. 7c) and Minon caused only mild alterations, and the cells maintained their polygonal shape. Both formulas of Neutrogena produced intermediate effects (Table I).

**DISCUSSION**

The process of corneocyte shedding is exceedingly complex and is just beginning to be understood. Furtasch (14) studied the chief events occurring in the outermost stratum dysjunction, where the corneocytes are beginning to disengage, by transmission electron microscopy. Just preceding desquamation, bilaminar membranes become disrupted, accompanied by detachment of desmosomes. We found in particular that exposure of blister roofs to the most damaging surfactants did not result in disintegration of the membrane.

To complicate matters, the horny layer also contains a
variety of water-soluble nitrogen compounds, the famous natural moisturizing factor. These result largely, but not solely, from enzymatic digestion of prollagin embedded in the keratohyaline granules of the stratum corneum (15). It is also known that intercellular lipids of the stratum corneum are important for the cohesion and barrier function of the horny layer (16). Imokawa et al. (17) reported that intercellular lipids were depleted by surfactants. There is also another structural locus where surfactants could act by disaggregating corneocytes, i.e. desmosomes etc. It is reported that the process of desquamation is accomplished by the breakage of desmosomal junction (18). However, they are retained in ichthyotic conditions where the stratum corneum becomes thicker. Lundstrom & Egelrud (19) have unequivocally demonstrated that surfactant-induced shedding of corneocytes is mediated by endogenous proteinase, which attacks desmosomal proteins, a process prevented in vitro by anti-protease. The process was not detected where the endogenous proteinases were located in the skin.

The importance of pH for skin health no longer has currency. Normal skin has a pH slightly on the acid side, which gave rise to the appealing “acid mantle” theory which argued that a shift toward alkalinity would adversely affect the skin. In our experiment, the pH of 1% Ivory soap solution was 9.7, and it may influence the shedding and barrier function of the horny layer.

Rhein et al. (9) described that many surfactants in solution induced swelling of isolated horny sheets, and that the swelling response correlated well with the irritant potential of surfactants. Our findings also verified that swelling, measured by an increase in the size of released corneocytes, may be a reliable indicator of potential irritancy. Like other authors (6, 8–10, 23–25), we could not demonstrate alterations in the horny layer with non-ionic and cationic surfactants. We evaluated a number of quaternary ammonium cationics, as well as typical nonionics (Triot) and never observed any significant changes (data not shown). If swelling is accepted as a key criterion for ranking anionics, Dove is the mildest of the soaps we tested. However, we hasten to point out that the absence of swelling does not signify “mildness” or lack of toxicity. Quaternary ammonium cationics like benzalkonium chloride are capable of inducing severe dermatitis, once they have penetrated into viable tissue (20). This emphasizes that there can never be a single model for assessing mildness.

In soap solution, corneocytes become swollen, accompanied by loss of staining intensity. It has been described that the permeability of the cornified envelope was very low (21, 22). Nonetheless, it seemed that soap solution flowed into the cytoplasm of corneocytes and caused swelling and rupture of corneocytes. It is assumed that the soap would alter the cornified envelope and the lipid membrane enclosing.

With our model we cannot account for the difference in ranking of anionic soaps when swelling and disaggregation were used separately as end-points. At first we postulated that corneocyte release was caused by removal of intercellular lipids. This is a popular notion, especially between researchers and clinicians who prefer to explain soap damage in terms of “stripping” of the lipids from the intercellular domains or from cell membranes (10–12, 23, 24). Froebe et al. (23) found that the ability of anionic detergents to extract lipids from isolated horny layers, even with high concentrations, was negligible, and thus not applicable to the problem of soap dermatitis. Kawai & Imokawa (24) offered the same explanation in their study of facial tightness induced by anionic surfactants. Finally, Fartasch et al. (25) provoked an inflammatory patch reaction to SLS on human skin but could not show any disruption of the intercellular bilaminar lipid membranes by transmission electron microscopy. The first structural changes occurred in the living tissue below the horny layer, interfering with the orderly sequestration of lipid-containing lamellar bodies into the intercellular spaces. This suggests that changes in lipids are secondary to earlier events. Certainly, extraction of lipids with solvents completely abol-

Table 1. Microscopic changes in corneocytes in 1% solutions of surfactants and soaps

<table>
<thead>
<tr>
<th>Corneocyte shape after 1 h</th>
<th>Swelling after 1 h</th>
<th>Rupture after 6 h</th>
<th>Intracellular vacuoles</th>
<th>Reaggregation</th>
<th>Staining intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>Polygon</td>
<td>±</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SLS</td>
<td>Roundish</td>
<td>+/±</td>
<td>–</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>Roundish</td>
<td>+</td>
<td>–</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Hyamine 3500</td>
<td>Roundish</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ivory®</td>
<td>Round</td>
<td>+++/+</td>
<td>+++/+</td>
<td>+/+</td>
<td>+</td>
</tr>
<tr>
<td>Neutrogena Original</td>
<td>Round or roundish</td>
<td>++/+/+</td>
<td>+++/+</td>
<td>+/++</td>
<td>+</td>
</tr>
<tr>
<td>Neutrogena®</td>
<td>Polygon</td>
<td>++/+/+</td>
<td>++</td>
<td>+/++</td>
<td>+</td>
</tr>
<tr>
<td>Dry skin Formula®</td>
<td>Roundish</td>
<td>+/±</td>
<td>+/</td>
<td>+/++</td>
<td>+</td>
</tr>
<tr>
<td>Minon®</td>
<td>Roundish</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dove®</td>
<td>Polygon</td>
<td>+</td>
<td>+/</td>
<td>+/++</td>
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</tr>
</tbody>
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+ + +: remarkable + +: moderate +: slight ±: little –: not found

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The swelling of the stratum corneum is not a primary event, as it occurs only after the formation of the horny layers. Therefore, the accumulation of water in the intercellular spaces cannot be the primary cause of the swelling. The swelling is more likely to be a consequence of the formation of the horny layers, as the accumulation of water is a result of the hydration of the intercellular lipids.

The swelling is also accompanied by an increase in the permeability of the stratum corneum, which allows more water to enter the intercellular spaces. This increase in permeability is caused by the formation of the intercellular lipids, which act as a barrier to the diffusion of water.

Our model shows that the swelling of the stratum corneum is not a primary event, but rather a consequence of the formation of the horny layers. The model also shows that the swelling is accompanied by an increase in permeability, which allows more water to enter the intercellular spaces.

REFERENCES

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