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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 Caucasoids. 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 five soap bars of differing irritancy. Additionally, individual examples of anionic, cationic and nonionic surfactants were similarly evaluated. Measurements of corneocytes included: (1) the number released with time (disaggregation), (2) size (swelling) and (3) morphologic degradation. 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 morphologic 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.

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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, Sultzberger & Baer 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, i.e. Doppler velocimetry, electrical conductance, degree of erythema assessed by colorimetry, attenuated total reflectance, image analysis of Silflo replicas, and transepidermal water loss (TEWL) (7). Nonetheless, the variables assessed by these in vivo tests are affected by season, dew point, humidity, age, body region, race and other influences.

By contrast, in vitro tests can be precisely standardized and rigorously monitored. Pape & Hoppe (8) measured the capacity of surfactants to cause hemolysis of bovine red blood cells, as a measurement of membrane damage. Swelling and extension of plane of sheets of the horny layer is another parameter that seems to correlate well with irritancy potential (9, 10). However, few studies have investigated the cytological alterations in isolated human corneocytes produced by surfactants.

On the other hand, there is a need to select a laboratory model that can assess and predict the safety profile of cleansing agents. This is a topical issue, since the market is preoccupied with claims of enhanced "mildness." Consumers have become increasingly concerned with the safety of household products that are used daily for decades. A bevy of new liquid and bar soaps are briskly competing with each other, centering chiefly around "mildness" claims.

While cleansers may injure skin in different ways, a key feature pertinent to mildness is interaction with the stratum corneum, the tissue at the first point of contact. Structural damage affecting the integrity and physico-chemical state of the horny layer barrier has figured prominently in the development of in vitro models.

We present a new in vitro model for measuring the effects of surfactants on discs of human stratum corneum.

MATERIALS AND METHODS

Stratum corneum specimens

Eight-millimeter suction blisters were raised on the volar forearms of young adult Caucasoids using a hand-held vacuum pump (Meward Enterprise Co., Cucamonga, California, U.S.A.) adjusted manually to a negative pressure of two atmospheres.

The time taken to raise a fluid-filled blister ranged from 90 to 120 min. Histologic examination showed that the plane of separation was precisely at a dermo-epidermal interface. Three blisters were conveniently obtained from each forearm. These heal without scarring and are not painful, though hyperpigmentation is a frequent sequela.

The blister roofs were cut off and their undersurface gently rubbed with a saline-moistened cotton swab to remove the viable epidermis. Histologic examination showed that the cleaned roof comprised the entire stratum corneum. The number of horny cell layers in the blister ranged from 14 to 17.

The specimens were placed a surface side up on a glass slide and dried at room temperature for storage until use within 1 week.

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.

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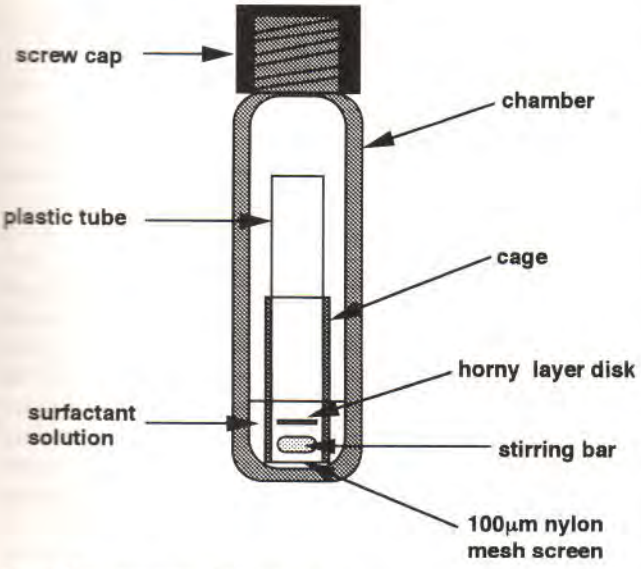

Fig. 1. The structure of the chamber.

One-microliter samples were removed at intervals, placed on a glass slide. The staining method was modified by McGinley et al. (11) and Hölze & 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 Paramount® 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 horny layers 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 indueed 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.

Test surfactants

Bar soaps. We arbitrarily selected four brands covering the range from mild to strong, as ranked by the soap chamber assay on human skin (6):

(1) Ivory (Procter & Gamble Co., Cincinnati, Ohio, U.S.A.)
(2) Neutrogena, Original formula (Neutrogena Corp., Los Angeles, California, U.S.A.)
(3) Neutrogena, Dry skin formula (Neutrogena Corp.)
(4) Minon (Yamanouchi Pharmaceutical Corp., Tokyo, Japan).
(5) Dove (Lever Brothers, New York City, New York, U.S.A.)

Pure surfactants. Anionic; Sodium lauryl sulfate (SLS; Sigma Chemical Co., St. Louis, Missouri, U.S.A.) Cationic; Benzalkonium chloride (Hyamine 3500, Rohm & Haas Co., Philadelphia, Pennsylvania, U.S.A.) Non-Ionic; T-octylphenoxy-polyethoxy ethanol (Triton X-100, Sigma Chemical Co.)

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 3500 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

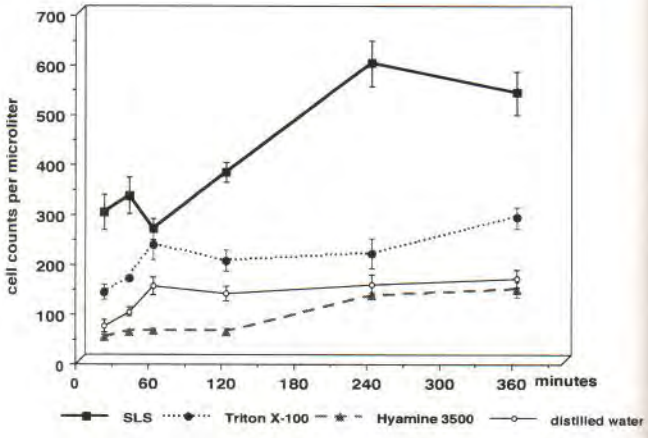

Fig. 2. The corneocyte counts released from horny layer discs in 1% surfactant solution and distilled water at 60°C. Mean and SEM.

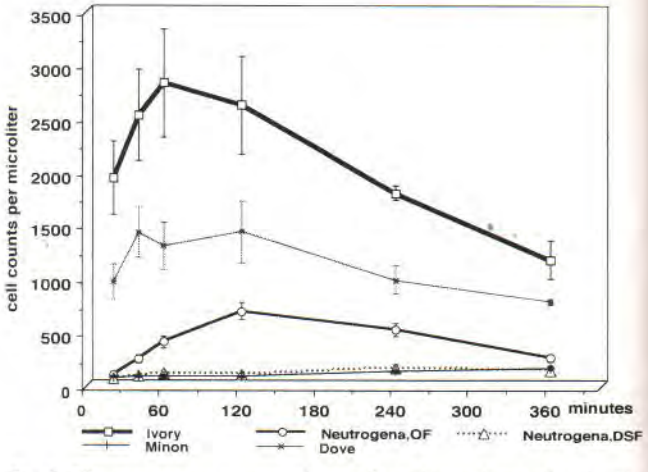

Fig. 3. The corneocyte counts released from horny layer discs in 1% solutions of bar soaps at 60°C.

by original formula Neutrogena ($p<0.05$) was 700 cells/μl, one quarter of that for Ivory. The results for Minon and Neutrogena dry skin formula were less than 170 cells/μl and not significantly different from that of distilled water ($p>0.1$). It should be noted that the corneocyte counts generally peaked after 1 to 2 h. With Ivory, the count fell from 3,000 to 1,200 cells/μl after 6 h. Microscopy revealed that this was due to progressive swelling of corneocytes with loss of their cell contents and eventual cell dissolution.

Corneocyte size (longest diameter)

Surfactants (Fig. 4). Distilled water and hyamine 3,500 caused a negligible increase in corneocyte size, from 28 to 34 μm ($p>0.1$). Triton X-100 ($p<0.05$) gave the greatest increase, but only to 38 μm. Surprisingly, swelling with SLS was only slightly greater than that with distilled water ($p>0.3$).

Bar soaps (Fig. 5). There were striking differences among the soaps. The greatest swelling was observed with Ivory, reaching a mean diameter of 49 μm in less than 1 h ($p<0.01$). The swelling produced by dry skin formula Neutrogena peaked at 48 μm after 6 h ($p<0.01$). That produced by original formula 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.

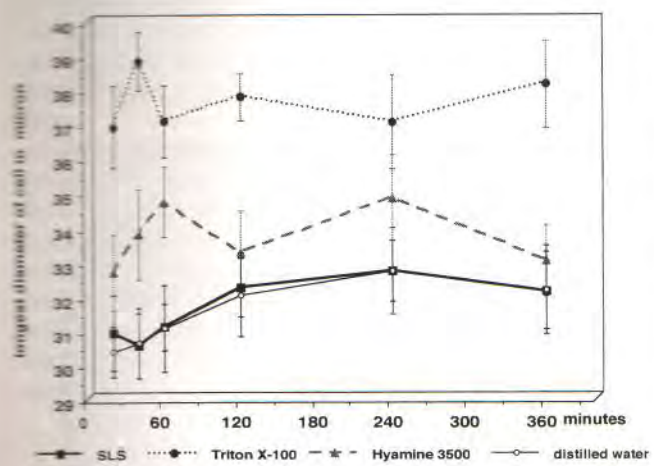

Fig. 4. The longest diameter of corneocytes released from horny layer discs in 1% surfactant solutions and distilled water at 60°C.

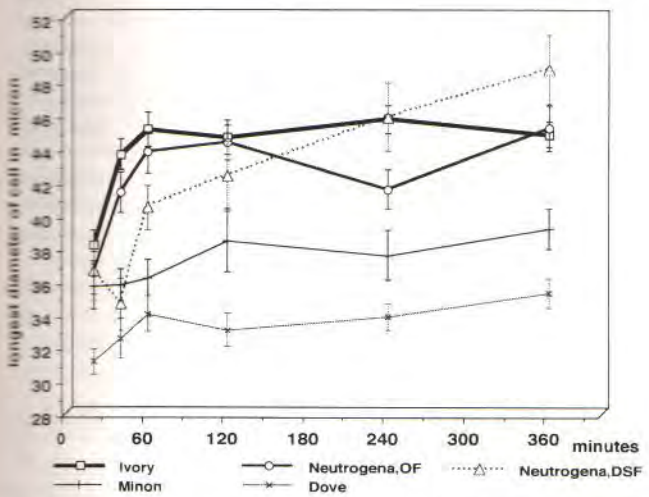

Fig. 5. The longest diameter of corneocytes released from horny layer discs in 1% solution of bar soaps at 60°C.

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 3500, 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. Fartasch (14) studied the chief events occurring in the outermost stratum dysjunctum, 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

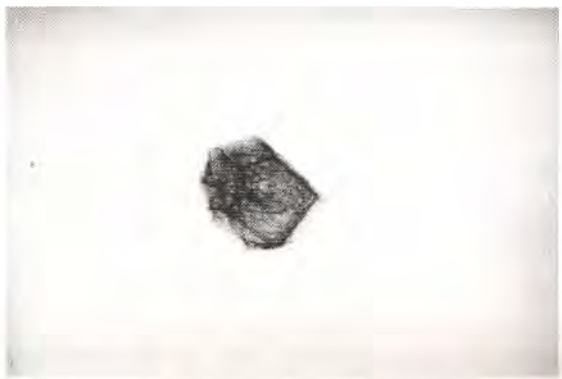

Fig. 6. Corneocytes after 6 h of exposure in distilled water. These are polygonal with well-defined cell borders. The corneocytes show no discernible changes in morphology. Rhodamine-B and methylene blue staining, ×400.

variety of water-soluble nitrogen compounds, the famous natural moisturizing factor. These result largely, but not solely, from enzymatic digestion of profillagrin 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 accompanied by the breakage of desmosomal junction (18). However, they are retained in ichthyotic conditions where the stratum corneum becomes thicker. Lundström & 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-proteinases. The process was not detected where the endogeneous 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 irritancy potential of surfactants. Our findings also verify 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 (Tritons) 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 became 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*

	Corneocyte shape after 1 h	Swelling after 1 h	Rupture after 6 h	Intracellular vacuoles	Reaggregation	Staining intensity
Distilled water	Polygon	±	−	−	−	Strong
SLS	Polygon	+/±	+	±	+	Slightly strong
Triton X-100	Roundish Polygon	++	+	±	+	Moderate
Hyamine 3500	Roundish Polygon	+	±	−	−	Slightly strong
Ivory®	Round	+++/++	+++/++	+++	+++	Very faint
Neutrogena Original Formula®	Round or roundish Polygon	+++/++	++	++/+	+	Faint
Neutrogena Dry skin Formula®	Round or roundish Polygon	+++/++	++	++/+	+	Faint
Minon®	Roundish Polygon	++/+	+	±	±	Moderate
Dove®	Roundish Polygon	+	+	−	±	Slightly strong

+++: remarkable ++: moderate +: slight ±: little −: not found

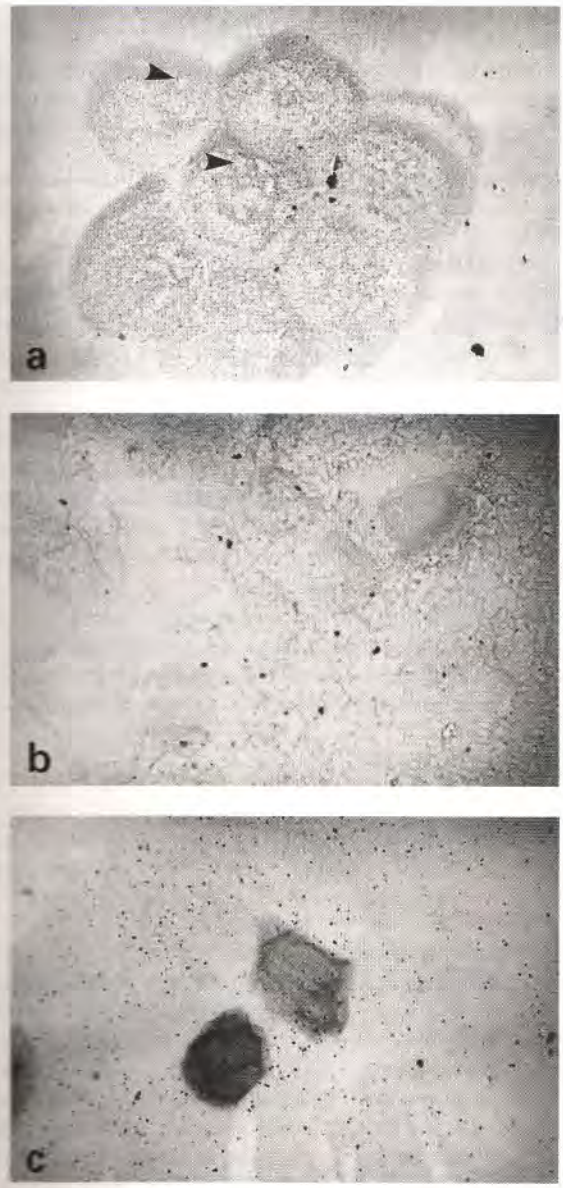

Fig. 7. Cytology of corneocytes in soap solutions. Rhodamine-B and methylene blue staining, ×400. (*a*) Corneocytes after 1 h in 1% Ivory solution. The corneocytes have become greatly swollen with many vacuoles (*arrow*) and fine granules. The cells are reaggregating into clumps. (*b*) Corneocytes after 360 min in 1% Ivory. The swollen cells have disintegrated and do not have defined borders or shapes. The cells are swollen, eventually undergoing rupture and total dissolution. (*c*) Corneocytes after 360 min in 1% Dove. The cells are less swollen and their staining intensity has slightly decreased. Rhodamine-B and methylene blue staining, ×400.

ishes the barrier and is accompanied by total loss of intercellular lipids ultrastructurally (26).

Our model examined the effects of surfactants exclusively on the horny layer, and we focused on applying it for analysis of cleansing products that can meet a claim of mildness. The model might also prove useful in basic studies of the properties of the horny layer in relation to age, race, disease and experimentally induced alterations. We have also found that suction blisters from blacks are considerably more resistant to surfactants than those from whites (unpublished data). The superior barrier properties of black skin are usually attributed to a greater number of corneocyte cell layers, but our findings suggest that other factors may also be operative.

In conclusion, in vitro models cannot address important clinical problems associated with subjective, sensory responses to soaps. We agree with Jackwerth & Krachter (27) that these discomforts will drive consumers away, no matter how "mild" a cleaning product has been shown to be by objective demonstration.

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