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Pyranine, a fluorescent dye, detects subclinical injury to sodium lauryl sulfate

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Synopsis

Anionic surfactants may damage the horny layer barrier in the absence of clinical signs of irritation. Increased permeability increases susceptibility to exogenous chemical insults.

We describe herein a rapid method for detecting invisible disruption of the barrier by sodium lauryl sulfate (SLS). Low concentrations of SLS, insufficient to induce visible changes, were applied occlusively in Hill Top chambers to the volar forearms of normal women for 24 hours. One hour after removal of the chambers, pyranine, a water-soluble fluorescent dye, was applied with a cotton-tip applicator and allowed to dry. The dye was gently washed off 30 minutes later. The intensity of the fluorescence was visualized under Wood's light immediately and 24 hours later. Ultraviolet photos were obtained for photographic documentation. Measurements of transepidermal water loss were made at the same time.

Concentrations of 0.025% and 0.1% SLS caused, in most of the subjects, no visible reactions. However, in comparison to a control water patch, both sites fluoresced strongly under Wood's light, more so with 0.1% SLS. Transepidermal water loss also increased, proportionally to concentration, validating barrier disruption. 0.005% SLS was below the threshold of injury by this methodology.

Pyranine fluorescence appears to be a useful technique for evaluating subclinical barrier damage from detergents.

INTRODUCTION

Irritant reactions are common and important in clinical and occupational dermatology. There has recently been a great interest in the mildness of skin care products, especially surfactants and soaps. In a competitive market place, manufacturers vie with each other to prove claims of superior mildness.

Clinical methods for grading visible signs of irritation are giving way to bioengineering techniques, which provide more accurate, more discriminating, and more objective data in comparative studies. A noteworthy advantage is the ability to measure subclinical, nonvisible changes. Many sophisticated instruments are available for measuring various features of the irritation reaction, including transepidermal water loss (TEWL), scanning

laser-doppler velocimetry, ultrasound imaging, and conductance (1–3). No chemical irritant has been more intensively and comprehensively studied than sodium lauryl sulfate (SLS), an anionic surfactant. A huge literature has been built up dealing with multifarious ways of characterizing the response of human skin to SLS. SLS is now the classic prototype of an irritant whose major impact is on the horny layer barrier.

The most complete description and review of the numerous methods available to study SLS reactions, entitled "Guidelines on sodium lauryl sulfate exposure tests," has recently been published by the European Society of Contact Dermatitis (4). The consensus is that TEWL is the most sensitive procedure for detecting tissue damage by SLS. Invisible damage to the horny layer can be reliably, quickly, and easily estimated by the evaporimeter (2,5), which measures vapor tension at two fixed positions above the surface. International standards for proper use of the evaporimeter have also been published (6).

Interest is growing in methods that will detect early, subclinical damage by anionic surfactants. Dansyl chloride is a fluorescent dye that stains the entire thickness of the horny layer. Disappearance of the dye under Wood's light is a measure of the turnover of the stratum corneum (7). More rapid extinction of fluorescence has been shown to correlate with subclinical injury to SLS (8). However, there are technical limitations to the technique, which is also time-consuming (9,10).

Dermatologists, including us, frequently mark patch test sites with yellow fluorescent pens. The indicator dye in these pens is pyranine. We now present a method using pyranine dye to detect early, invisible damage to the horny layer barrier by SLS.

MATERIALS AND METHODS

The subjects were 11 healthy Caucasian women, ages 30 to 50. One hundred fifty microliters of the test solutions was pipetted into the cotton pad of 11-mm Hill Top chambers (Hill Top Research Inc., Cincinnati, OH). These were fixed to the volar forearm skin. On one forearm, chambers containing distilled water and 1%, 5%, and 10% SLS (Sigma Chemical Co.) were applied for one hour. On the opposite forearm, chambers containing distilled water and 0.005%, 0.025%, and 0.1% SLS and one empty chamber were applied for 24 hours. The sites were briefly washed with water after removal and left open.

The sites were evaluated clinically one and 24 hours later and scored as follows: 0 = no reaction; 1 = patchy mild erythema; 2 = uniform mild erythema; 3 = moderate erythema, slight infiltration, few vescicles; 4 = strong erythema, infiltration, vescicles; 5 = strong erythema, infiltration, bullae with exudation.

The source of pyranine (solvent green no. 7) was a yellow Hi-Liter® pen (Avery-Dennison, Diamond Bar, CA). At first, the dye was squeezed out of the pen into a jar. One hour after removing the chambers, each patched site was evenly covered with 0.15 ml of the dye. The dye was applied with a cotton-tipped applicator to each area of 6.5 cm^2 that included both patched and surrounding untreated skin. The solution was left to air dry for 30 minutes, and the site was briefly washed with Dove® soap and patted dry.

The sites were visualized under Wood's light immediately and 24 hours later. Fluorescence was scored as follows: 0 = no fluorescence or less than surrounding area; 1 = mild fluorescence comparable to surrounding area; 2 = moderate fluorescence; 3 = strong

fluorescence, with punctuate accentuation; 4 = strong, bright fluorescence in large patches.

Fluorescent photographs were also taken under two oppositely placed Wood's lamps (Spectroline®, MB-100, 365 nm, Spectronics Corp., Westbury, NY) positioned 15 cm from the surface at a 45° angle. A Haze 2A Tiffen filter was placed in front of the camera lens. The film was 1600 ASA Kodak Ektachrome, processed at P2.

TRANSEPIDERMAL WATER LOSS (TEWL)

TEWL was measured 30 minutes after removal of the chambers, with the evaporimeter (EP2, Servo Med, Stockholm, Sweden) connected to a dedicated software. Measurements were conducted inside an environmental chamber at 21°C degrees and 41% relative humidity, after equilibration, following international guidelines for the use of this instrument (6).

STATISTICS

Significance was estimated by the Wilcoxon signed rank two-tailed test.

RESULTS

CORRELATION OF VISIBLE IRRITATION WITH FLUORESCENCE

None of the one-hour patch tests, including 10% SLS, induced any visible reaction. However, with 10% SLS there was a non-significant increase in fluorescence when compared to water.

With 24-hour exposures, only four of the 11 subjects showed a visible reaction to 0.025% SLS (grade 2) and 0.1% SLS (grades 2 and 3) one hour after removal. At 24 hours after removal of the chambers, two subjects with 0.025% SLS and three with 0.1% SLS still had a mild erythema (Table I). The irritation was non-significant at any time compared with the water chamber. By contrast, strong fluorescence was observed (grades 3 and 4) in 100% of the sites exposed to 0.1% SLS ($p < 0.005$, compared with all the other test sites), and in 63% of those exposed to 0.025% SLS ($p < 0.005$, compared with water), one hour after removal. Fluorescence was still strong 24 hours after removing the chambers, although reaching significance only for 0.1% SLS ($p = 0.001$), in comparison with water (Table I). No increase in fluorescence was observed with 0.005% SLS (Figure 1).

TEWL

After the one-hour exposures, the TEWL values did not increase significantly. By contrast, with 24-hour patches TEWL values became significantly elevated ($p < 0.1$), with all concentrations at one and 24 hours post removal (Figure 2). These increases were significant compared to water for 0.025% and 0.1% SLS ($p < 0.1$). It should be noted that water also caused barrier damage compared to the empty chamber.

Table I
24-Hour Patch Tests: Grading 24 Hours After Removal of Patches

	Dry		Water		0.005% SLS		0.025% SLS		0.1% SLS	
Subject	Clin	Fluo	Clin	Fluo	Clin	Fluo	Clin	Fluo	Clin	Fluo
P.B.	0	0	0	2	0	2	0	2	1	4
M.S.	0	0	0	0	0	1	1	0	3	2
D.R.	0	0	0	2	0	2	0	3	1	4
M.H.	0	0	0	1	0	1	0	3	0	4
D.F.	0	0	0	0	0	2	0	4	1	3
N.L.	0	1	0	2	0	3	0	3	0	4
T.B.	0	2	0	0	0	0	0	0	0	3
L.C.	0	0	0	1	0	2	0	3	1	3
E.L.	0	0	0	0	0	1	3	1	3	4
B.C.	0	0	0	0	0	0	2	2	2	3
L.P.	0	0	0	0	0	0	0	0	0	3

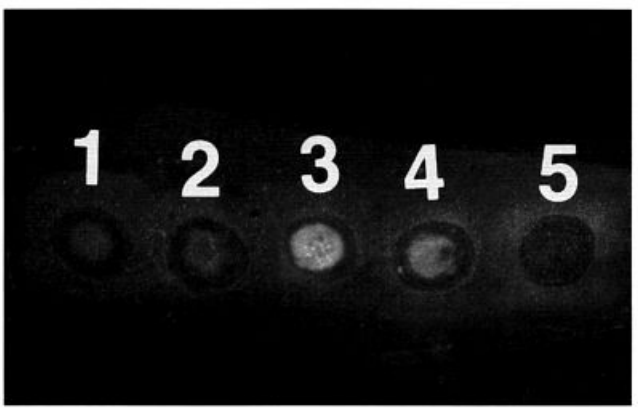

Figure 1. 24 hours post removal of 24-hour SLS applications. Fluorescent evaluation: 0.1% SLS (#3) shows large patches of bright fluorescence (score = 4). 0.025% SLS (#4) is scored 3. 0.005% SLS (#2) and water (#1) are scored 1. The empty chamber (#5) is scored 0. Note the halo of fluorescence at the sites in contact with the tape, suggesting that a slight skin damage has occurred.

Fluorescence grades and TEWL values of 24-hour exposure chambers correlated very well at one and 24 hours after removal ($r = 0.86$ and $r = 0.94$). Clearly, even the slightest damage is detected by both methods.

DISCUSSION

Until now, identification of dubious, subclinical reactions to anionic surfactants required the use of complex, expensive, bioengineering instruments such as 20 MHz ultrasound, conductance, and evaporimetry (2,3,5). By contrast, the pyranine dye technique is the ultimate in simplicity, requiring only a Wood's lamp to visualize fluorescence.

This method has also great sensitivity since we regularly observed strong fluorescence (grades 3 and 4) at 24-hour exposure sites that were clinically negative. TEWL mea-

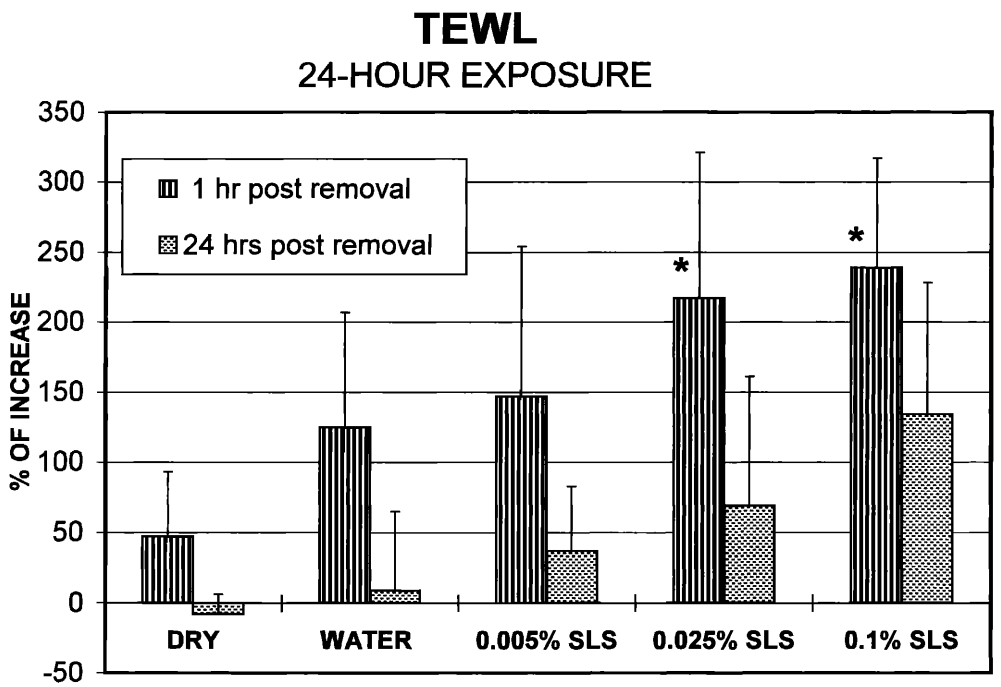

Figure 2. At 24-hour exposure sites TEWL for 0.025% and 0.1% SLS increased significantly (*$p < 0.1$) compared to water.

surements correlated very well with the intensity of fluorescence, validating the reliability of the method. Fluorescence remained positive for days, enabling serial follow-up.

Interestingly, strong inflammatory reactions provoked by higher concentrations of SLS (for example, 0.5% SLS) do not fluoresce after application of the pyranine dye (unpublished results). At first glance this seems paradoxical, but it is explainable by the knowledge that higher concentrations result in total loss of the severely damaged horny layer.

Preliminary studies showed that as little as a 30-minute application of the dye to normal volar skin enabled most of the stratum corneum to become fluorescent. It took 30 cellophane tape strippings to completely remove the dye, short of the strippings necessary to reach the glistening layer. Moreover, overnight dye exposure on normal skin stained corneocytes, vellus hairs, and the follicular infundibula for over one week (unpublished observations). The intensity of fluorescence and the time for extinction of fluorescence was less than after a 24-hour exposure to 10% dansyl chloride. We speculate that pyranine binds to keratin filaments in corneocytes, but this has not been properly studied.

It is important to realize that 24-hour exposure to water alone increased TEWL and resulted in a slightly enhanced fluorescence. Therefore, a water control must be included as a reference control in all tests of water-soluble materials. It is well known that water alone can overhydrate the horny layer and make it more permeable (11).

We have chosen SLS as a paradigm for this study because its primary effect is to disrupt the barrier. We predict that the pyranine test will be helpful in evaluating the mildness

of cleansers by identifying products that might induce stratum corneum damage. Also, pyranine fluorescence might find useful applications in chronic dermatologic diseases with an impaired skin barrier, such as in atopic dermatitis, especially in following the response to therapy.

Finally, comedones on the face of acne patients also stain brightly, and the benefit of comedolytic agents could be assessed by their steady disappearance during treatment (unpublished observations).

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