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TOLERANCE OF THE LESIONED SKIN TO DERMATOLOGICAL FORMULATIONS

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Synopsis

Dermatological formulations often are applied on lesioned skin in the course of the treatment of for example dry skin, eczema or atopic disease. Therefore we felt it important to devise a test for quantification of the tolerance of these formulations. For this purpose, the horny layer was tape-stripped until the transepidermal water loss (TEWL, as measured under standard conditions) attained 40-50 g/cm²h. This needed approximately 10-15 strips. After an interval of at least 30 min during which the TEWL decreased to values of 30-40 g/cm²h, the test products were applied to the stripped area and the reactions were assessed by the volunteer according to a 4-point scale during and immediately after the applications. Standards were used for quantification, identification and comparison purposes: NaCl (0.1-10%), Urea (0.5-20%) and Propylene Glycol (1-70%). In addition the pH of the test solution was varied between 3 and 8. Curves relating-effect to concentration allowed more precise assessment of the relative roles of the different components of formulations. For example, Urea and Propylene Glycol may lead to burning reactions on lesioned skin at the concentrations usually found in dermatological products. Similarly, antimicrobials in permitted concentrations may play a role in these reactions.

Riassunto

Le formulazioni dermatologiche vengono spesso applicate su cute lesa per eccessiva secchezza, per la presenza di eczemi o di dermatiti atopiche.
È perciò importante che venga accuratamente valutata la tolleranza di queste formulazioni. A questo scopo sono stati eliminati mediante "strappo" alcuni strati del corneo fino ad ottenere una TEWL di 40-50 g/cm² h. A tal riguardo sono necessari circa 10/15 "strappi".
Dopo circa 30 minuti durante i quali i valori TEWL raggiungevano il valore di 30-40 g/cm²/h venivano applicati i prodotti da sperimentare sulle zone impoverite di corneo.
Rilevando la reattività ottenuta mediante una scala di valori numerici prima e dopo l'applicazione dei prodotti è stato possibile controllare il grado di tolleranza di alcune materie prime di uso corrente, quali ad esempio l'Urea ed il glicol propilenico.
È stata verificata anche l'influenza svolta dal pH, e dalle diverse concentrazioni d'uso permesse.

Professor Albert Kligman wrote in 1982 (1): "In our age of skin consciousness and abundant use of cosmetics and toiletries awareness is growing that the great majority of undesired reactions represent irritancy." And further: "The biologic truth is that all substances are irritating for some persons under some conditions, although extreme exposures may be necessary to bring this to light." End of citation.

The problem of irritation is far from a theoretical one. Irritation could be tentatively defined as a very general notion for undesired reactions. These can widely vary in their intensity, in their manifestations and in the moment they will be perceived as such because people differ enormously in their susceptibility to irritating substances and because the reactivity of the skin is influenced by many factors. In addition, topical preparations, whether drugs or cosmetics, are often applied to damaged skin. Indeed, it is surprising how often creams or ointments are used as home remedies for burns, abrasions, bites, and rashes of all kinds.

Hence it is not out of order or unrealistic to assess the probability of a substance being irritative under the most extreme conditions. This permits discrimination among the test products because reactions are sufficiently intense also allowing some standardisation. For this purpose, we must make sure that the substance reach the viable tissue under the horny layer. The barrier can be eliminated by scarification, as in the scarification test of Frosch and Kligman (2) or by tape stripping. Testing products on damaged skin is also necessary if, as in our case, are primarily aimed at ill people.

Among the many reactions of the skin which could be classified under irritation we were especially interested in those called "invisible reactions" by Professor Kligman (1). Topical preparations can cause disagreeable sensations - burning, itching, stinging - immediately after application and will be rejected by the consumers, especially in pediatrics. These preparations are not irritative in the ordinary sense and usually do not damage the skin. The reactions can be very intense and unpleasant, and this is why we feel it important, for the products we are selling, to try to evaluate such immediate reactions.

For all these reasons, we performed the tests on stripped skin and recorded immediate reactions. We used the following method (Fig. 1): On the ventral side of forearms, up to 5 areas of approximately 1x2 cm were stripped. Stripping was continued until the transepidermal water

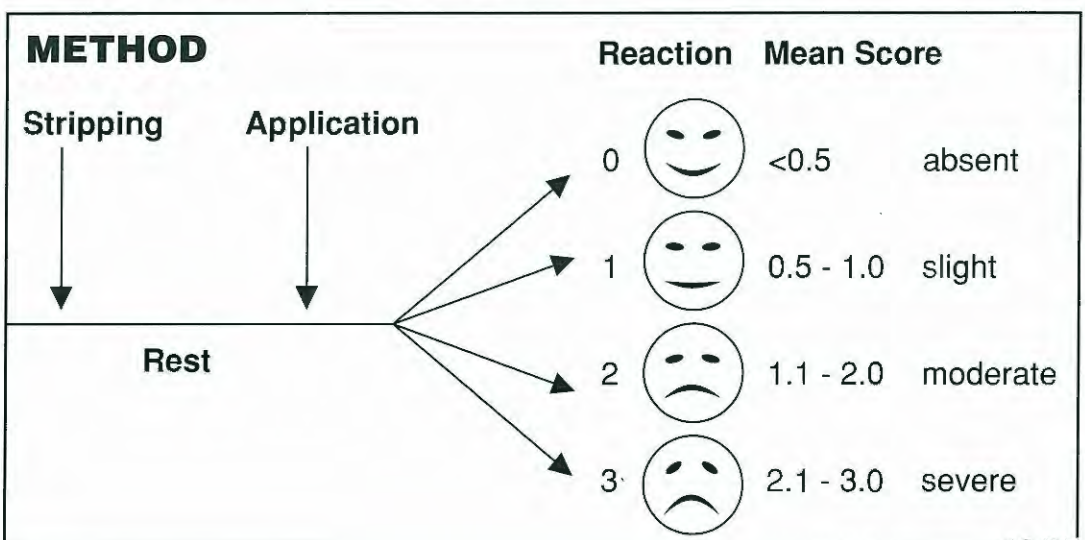

FIGURE 1

loss attained 40-50 g/m²xh which usually needed 10 to 15 strips. Thereafter, a resting period of at least 30 min was observed, during which the transepidermal water loss stabilised around values of 30-40 g/m²xh and the sensation of irritation due to the stripping vanished. The test products were applied on the stripped area taking care to avoid or minimalise any mechanical stimulation. Liquid products were dropped on the skin with a pneumatic pipette, creams and ointments were carefully pasted on the stripped surface. The test products remained on the stripped area for a maximum of 3 min. Immediate sensations, mainly burning, were graded on a four point scale from 0 (no reaction), 1 (slight), 2 (moderate) to 3 (severe). Mean scores were calculated and the results expressed as < (lower) 0.4 (no reaction), 0.5 to 1.0 (slight), 1.1 to 2.0 (moderate) and 2.1 to 3.0 (severe). This gradation has also been used by Frosch and Kligman to classify the irritation measured with the scarification test thus enabling comparisons to be made, although we are aware that the two phenomena are completely different in their nature.

First, we investigated reactions to different classes of substances used in topical formulations such as surfactants, penetration enhancers, moisturizers, all in aqueous solutions. Sodium Lauryl Sulfate (Fig. 2) is a good example to show the difference we found between the scarification test and our test. This agent is known to irritate the normal skin at the concentrations we used and scarification greatly reduces the irritation threshold. Immediate reactions were almost absent up to the concentration of 1%. On the contrary, Propylene Glycol (Fig. 3), which is incorporated in many topical formulations as a penetration enhancer, readily provoked burning sensations when applied on stripped skin but showed a low irritation potential in the scarification test.

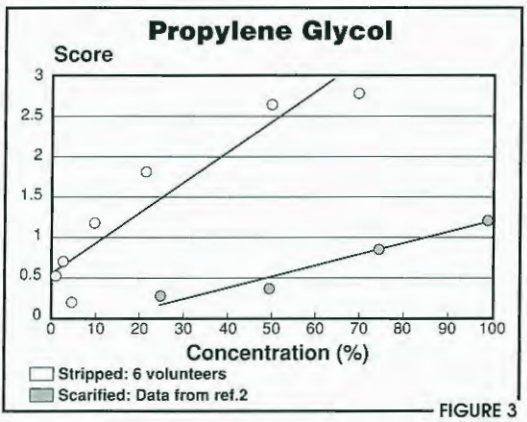

FIGURE 3

Urea (Fig. 4), as a moisturizer, led to a measurable rate of immediate reactions without great dependency on the concentration. Interestingly, higher concentrations did not much enhance this rate, which was almost comparable to the results obtained after scarification.

Among other factors which could have a major impact on the rate of immediate reactions of

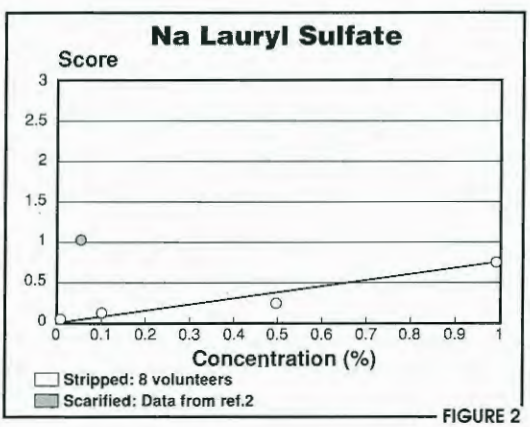

FIGURE 2

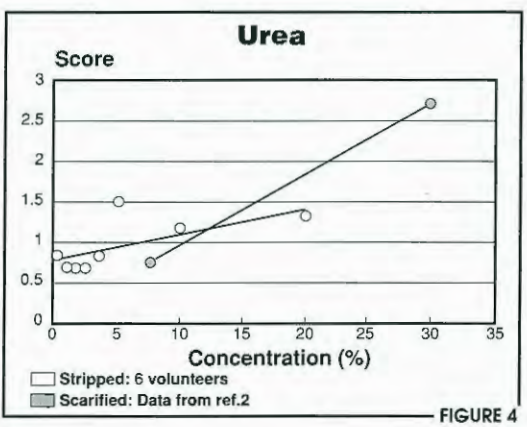

FIGURE 4

stripped skin are the osmolarity and the pH of topical formulations. Osmolarity (Fig. 5) was

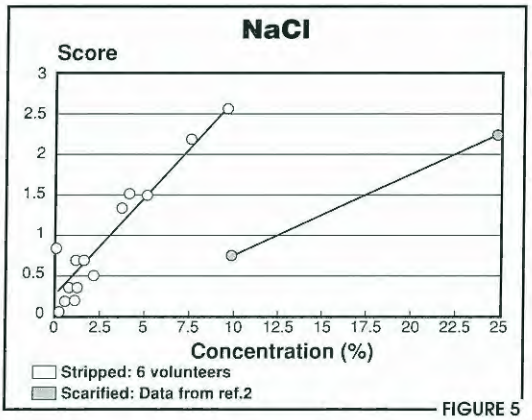

FIGURE 5

investigated with different concentrations of sodium chloride. An interesting finding was a measurable reaction score for distilled water, perhaps due to excessive hypoosmolarity. The score always diminished if a low concentration of sodium chloride was present. The dependency on osmolity was steep, again showing a

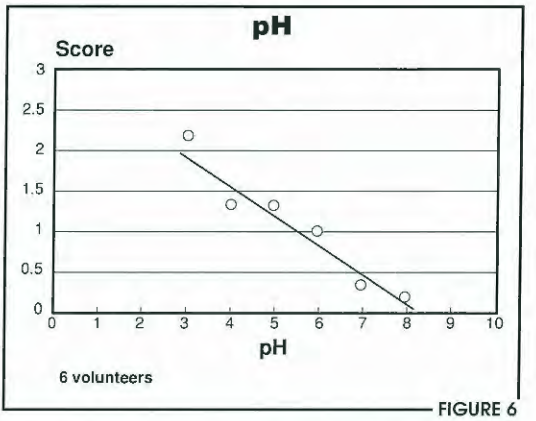

FIGURE 6

marked difference with the scarification test. Isotonic buffers of different pHs (Fig. 6) between 3 and 8 markedly influenced the score of the reactions. Most intensive responses were recorded with a pH under 4.

Table I

Antimicrobial	Concentration %	Score	Vehicle
Triclosan	0.3	0.4	O/W cream
Chlorhexidine dihydrochloride	0.3	0.5	O/Wcream
	0.3	0.8	O/W Lotio / Urea 2%
Hexamidine isethionate	0.5	0	O/Wcream
Phenylethylalcohol	0.5	0.5	O/Wcream
Phenoxyethylalcohol	2.0	2.8	O/Wcream
Trilclosan + Chlorhexidine	0.3 0.3	0.2	O/W cream
	0.3 0.3	0.6	W/O cream
Trilclosan + Phenoxyethylalcohol	0.3 0.5	1.0	W/O Lotio / Urea 2%
	0.3 1.0	2.3	W/O Lotio / Urea 2%

At this stage of the experiments, one could tentatively summarized that for our purposes, a topical product should show a neutral pH and a low osmolarity. Penetration enhancers and moisturizers should not be present or their use should be carefully considered.

It is known that other important factors which often lead to disagreeable reactions are antimicrobial agents. Some relevant data are shown in Table 1. Interestingly, we did not find high reaction scores for antimicrobials incorporated into suitable vehicles in permitted concentrations except for Phenoxyethylalcohol. Addition of urea slightly enhanced the scores as anticipated. Combination of two antimicrobials did not generally lead to higher scores, again with the exception of Phenoxyethylalcohol.

Last but not least, although we feel that immediate reactions are very important for the success of a product, the long-terms irritative potential should not be neglected. As pointed out in this communication, the test on stripped skin and the scarification test lead mostly give different results and are not predictive of each other. This was confirmed with a syndet (Table 2). That is, we do not replace the evaluation of a possible irritation as performed with the scarification test by scoring immediate reactions after application of the product on stripped skin. Rather, we consider both tests as independent but useful complements integrated in a product development scheme as an optimization of dermatological formulations before going on the market.

Table II

Washsyndet

Antimicrobials:	Triclosan 1,0 % Bronidox 0,2 %
Test	**Score**
Stripped	1 .4
Scarified	2. 3
5 volunteers	

Acknowledgement

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2. **Frosch PJ Kligman AM (1978)**: "An improved procedures for assaying irritants: The scarification test" *Current Problems in Dermatology* **7,** 69-79.