J. Soc. Cosmet. Chem., 47 1–11 (January/February 1996)

An improved procedure for conducting lactic acid stinging tests on facial skin

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Received November 15, 1995.

Synopsis

The lactic acid stinging test was proposed as a means of identifying women with hyperirritable "sensitive" skin of the face. These are normal persons who, in the absence of clinical signs of irritation, experience various neurosensory discomforts after using certain cosmetics and skin care products unaccompanied by signs of irritation. The original test has come under sharp criticism for unreliability, because of inconsistent results among laboratories.

The present studies were undertaken to improve the accuracy, reliability, and reproducibility of the test. It consists of a ten-minute exposure of the cheek to 10% racemic lactic acid in a Hilltop chamber. Two values are obtained: (1) the time required for stinging to be perceived and (2) the intensity of peak stinging on a 0 to 3 interval scale.

The salient findings were: Identical results were obtained when the test was repeated on the same group after a period of one year. Increasing the concentration to 20% and greater may induce visible irritation without appreciably increasing the peak intensity scores. Deliberately damaging the skin by repeated washing with a highly detersive soap does not convert "non-stingers" to "stingers." Substances cannot be simultaneously tested on both cheeks. Strong stinging on one side enhances the perception of stinging on the opposite side.

The revised test helps producers of skin care products to ascertain before marketing whether persons with "sensitive" skin will experience adverse subjective discomforts after daily use. A typical protocol involves twice-daily applications to the face for two weeks. Intensified stinging is a warning that consumers with sensitive skin may experience itching, burning, or stinging.

In 1977 Frosch and Kligman published a method for appraising the capacity of topically applied substances to induce stinging on facial skin (1). An unusual experience stimulated their investigation. A sunscreen that contained amyl-dimethyl-p-aminobenzoic acid had passed all the conventional tests for topical safety. Nonetheless, after marketing, the manufacturer received numerous complaints of stinging, necessitating recall. Intense stinging occurred in the complete absence of visible signs of irritation. This is sometimes called "subjective" irritation, to differentiate it from objective signs such as erythema and scaling (2).

Producers of facial products have become increasingly aware that adverse neurosensory reactions such as itching, burning, and stinging are fairly common and are usually not

predicted by routine toxicologic procedures. Surveys have shown that many women who have no skin disorders describe themselves on questionnaires as having "sensitive skin" (3). Fragrances, sunscreens, and moisturizers are often singled out as causative. Green and Bluth have comprehensively reviewed the psychophysical methods for measuring adverse sensory reactions to chemicals (4). They suggest the term "chemosensory irritability," rather than subjective irritation. Since a substantial number of women report that they have "sensitive" skin (3), it is a daunting challenge to identify these women prior to marketing.

By definition, "sensitive" skin refers to women without preexisting skin disease. Dermatologists are well aware that patients with rosacea, atopic dermatitis, ichthyotic disorders, and other dermatoses experience subjective irritation with much greater frequency (5) than other women. These patients can be identified by history and examination. Identifying persons with sensitive skin has been the object of serious investigation in our laboratory for many years.

It is important to define stinging explicitly to avoid confusion with other subjective responses. A prototype for burning is the chloroform:methanol (20:80) model described by Soschin and Kligman (6). When applied to the cheek in a glass cup, intense burning develops in a minute or less. The end point is the time to experience intolerable burning. An example of itching is the sensation provoked by histamine, prompting an urge to scratch.

Lactic acid stinging develops in a crescendo-type pattern. It begins to be perceived after a few minutes, gradually intensifying to peak intensity in about 3–5 minutes. It then disappears after 15 to 20 minutes. There are usually no clinical signs of irritation. A mild, transient erythema may appear in unusually susceptible persons.

Stinging must be differentiated from pain, as exemplified in the model used by Armstrong *et al.* (7). They applied irritating chemicals to the bases of unroofed cantharidin blisters. Pain was rapidly induced. Green and Bluth have emphasized that individuals differ greatly in their responses to chemical stimuli (4): A response to one stimulus does not predict subjective irritation to others. Different sensations may occur concomitantly; for example, stinging may be accompanied by itching.

There is some evidence that strong stingers are more readily damaged by known irritants. Dimethylsulfoxide induces larger wheals in stingers (1). Lammintausta *et al.* found that stingers were more vulnerable to irritating chemicals (8). Thus, the lactic acid test might provide a way to identify persons with hyperirritable or delicate skin.

In the original model, stinging was most consistently provoked when the subjects were brought to a state of profuse sweating in an environmental chamber at 120°F. A 5% solution of lactic acid on a soaked cotton swab was then rubbed briskly over the nasolabial fold and cheek. Stinging was scored at 2.5, 5.0, and 8.0 minutes on a 0 to 3 interval scale. The mean of these three readings comprised the final score. Frosch and Kligman suggested a facial sauna as a convenient substitute for inducing sweating. Finally, they suggested that increasing the concentration to 10% would provoke equivalent stinging in the absence of sweating. Grove *et al.* subsequently proposed guidelines to standardize performance of the test (9).

The lactic acid stinging model has come under sharp criticism. Mayne *et al.* could not obtain reproducible results when the procedure was repeated at different times on the

same panelists (10). Furthermore, discrepancies occurred when the right and left cheeks were simultaneously compared. Many contract laboratories now perform lactic acid stinging tests on facial products. Unfortunately, the results of inter-laboratory reports are disturbingly great (personal communication). In this report, we shall summarize the investigations that led to a rigorous refinement of the original method.

MATERIALS AND METHODS

Ten percent racemic D-L lactic acid is prepared in distilled water from 85% syrup (Sigma Chemical). Fifty microliters is then pipetted onto the absorbent pad contained in a 2-cm-diameter Hilltop chamber. This volume saturates the pad without leakage. The adhesive around the chamber is trimmed to leave two short 2-mm tabs on opposite sides, sufficient to seal the chamber to the skin (Figures 1, 2).

The exposure time is ten minutes, after which we remove the chamber and briefly hand-wash the face with a mild liquid soap. During the exposure, the subject records stinging each minute on a 0 to 3 interval scale: 0 = none, 1 = slight, 2 = moderate, 3 = severe.

Skin care products are discontinued for two weeks prior to the test, except for lipstick

Figure 1. A Hilltop chamber with its backing trimmed to minimize adhesive in contact with the skin. Lactic acid solution is applied to the absorbent pad that fills.

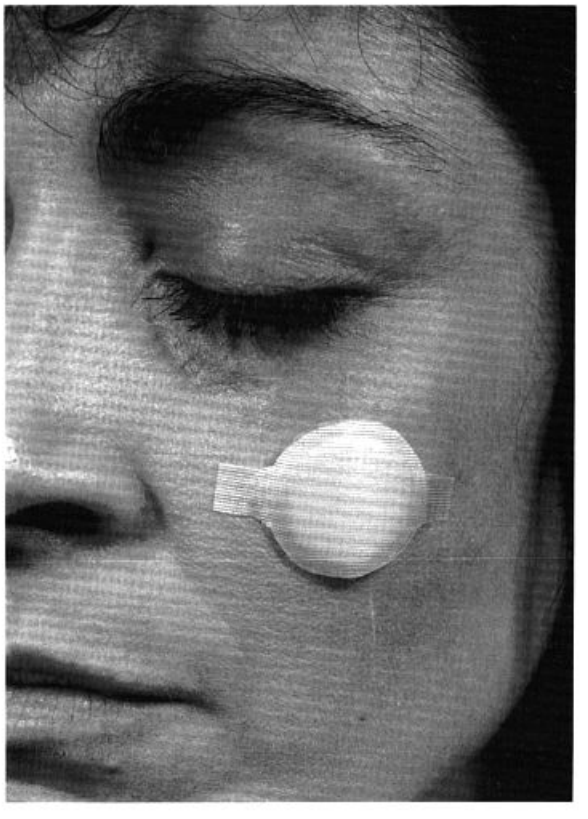

Figure 2. A Hilltop chamber in place on the skin.

and eye-area make-up. The panelists are also given a mild soap bar or liquid wash to be used only once daily. Coffee, tea, alcohol, and smoking is prohibited on the day of the test. The panelists report to the laboratory one hour before testing and remain in a quiescent state in a noiseless, pleasant room. The subjects are comfortably seated during the exposure.

Two values are recorded: (1) The time in minutes when stinging is first unequivocally perceived (short times usually presage intense stinging). (2) The peak intensity reached during the ten-minute exposure, on a 0 to 3 scale. Occasionally, stinging declines before the end of ten minutes. An alternative system is to sum the ten scores and to calculate the mean.

SUBJECT SELECTION

We are persuaded that women are more likely to be stingers than men, but this difference has not been conclusively demonstrated. To eliminate ethnic differences, we recruit only normal white women. We restrict the age range to 25–50 since it is known that stinging diminishes in the elderly (6).

Selection must be a very careful process. Women are often confused about sensory descriptors. We take pains to explain the distinction between itching, burning, and stinging, using common-place examples of each. For example, itching is characterized by

the desire to scratch. Burning in the painful sensation that follows a thermal burn. Stinging occurs when alcohol is applied to a cut or abrasion. The reliability of the subject must also be checked. A woman who stings on the first exposure is subjected to a repeat test a week or two later, this time using distilled water. As many as 20% of initial stingers have to be disqualified because they report appreciable stinging to distilled water. Green *et al.* also identified false-positive reactors by prescreening with the vehicle only (4). Finally, we establish whether the candidate is able to discriminate between 5% and 10% lactic acid.

REPRODUCIBILITY

We applied 10% lactic acid to ten moderate stingers and repeated the exposure exactly one year later. The findings were virtually identical (Table I). We maintain a panel of experienced stingers. We found that stinging was greatest and most reproducible in the winter months (6). This also holds true for susceptibility to chemical irritants (11). We forego stinging tests in hot, humid summer months because of variability of results, especially if the subjects have recently sweated.

RESULTS AND DISCUSSION

EFFECT OF PRIOR DAMAGE

Frosch and Kligman found enhanced stinging when lactic acid was applied to facial skin one day after administering 2 MEDs of ultraviolet radiation (1). Likewise, sharper and faster stinging occurred on skin that had been mildly damaged by a chemical irritant, benzalkonium chloride.

We preselected a panel of mild-to-moderate stingers to evaluate the influence of prior

Table I
Reproducibility of the Lactic Acid Sting Cheek Test: Stinging Grades in Moderate Stingers Tested After a One-Year Interval

	Onset (minutes)		Peak intensity	
Subject	Nov. '92	Nov. '93	Nov. '92	Nov. '93
1	2	4	2	2
2	3	3	1	2
3	4	2	2	3
4	5	3	1	2
5	3	4	1	1
6	2	3	3	2
7	4	3	2	1
8	4	4	2	2
9	2	2	3	2
10	1	2	3	3
Median	3.0	3.0*	2.0	2.0**

Neither the median onset *, nor median intensity **, measured in 1993, was different from that measured in 1992. The Wilcoxon rank sum test was used for the statistical analysis.

chemical injury. Beginning on a Monday morning in February and repeated daily over the next four days, the face was washed with a highly detersive soap in the following way: A generous lather was worked up with the fingers on the moistened faces of ten subjects and was allowed to remain for five minutes before rinsing. By the fourth exposure the skin had become dry, scaly, and tight, with a variable mild redness.

Lactic acid was then applied one day after the last exposure (Table II). In every case the onset was shortened and stinging reached a peak within a few minutes. Half of the subjects showed erythema that lasted a few hours. Enhanced stinging, but to a lesser degree, was still evident when the test was repeated one week later.

We repeated this soaping procedure on a group of six nonstingers. An immediate slight stinging was experienced by four of the six after lactic acid was applied. However, there was little or no stinging after the first few minutes. Thus, nonstingers did not become stingers after being irritated by a detergent.

REGIONAL DIFFERENCES

The face is, of course, not a uniform territory. Among other differences, the density and size of the follicular orifices and sebum production vary in different regions, showing a mid-to-lateral decreasing gradient (12).

We evaluated stinging responses on ten moderate stingers in the following regions: the mid-forehead, the chin, the nasolabial fold, the lateral cheek (pre-auricular), and the malar eminence (Table III). One site was tested per day. The greatest stinging occurred on the malar eminence, followed closely by the nasolabial fold, with a sharper decrease on the chin. The forehead and lateral cheek were the least reactive. Accordingly, the malar eminence is our preferred test site. An explanation for these striking regional

Table II
Exacerbation of Stinging by Vigorous Twice-Daily Soap Washings on Four Successive Days: Effect of Soap Washings on Mild Stingers

	Baseline		Post-washings	
Subject	Onset (minutes)	Peak intensity	Onset (minutes)	Peak intensity
1	2	1	1	2
2	2	1	1	2
3	3	1	1	2
4	4	1	2	3
5	2	2	1	3
6	2	2	1	3
7	5	1	2	3
8	3	1	1	2
9	4	1	2	2
10	3	1	1	2
Median	3.0	1.0*	1.0*	2.0**

The onset time was significantly shortened *, and the peak intensity significantly increased **, after the soap washing regimen. Statistical analysis by Wilcoxon rank sum test.

Table III
Regional Differences in Stinging (peak intensity)

Subject	Forehead	Chin	Nasolabial	Lateral cheek	Malar eminence
1	1	2	2	0	2
2	0	2	3	1	3
3	1	2	2	0	3
4	0	2	3	1	3
5	1	2	2	1	2
6	1	3	2	0	1
7	0	2	2	0	2
8	0	3	3	0	3
9	1	2	2	1	3
10	0	2	2	0	2
Median	0.5	2.0*	2.0*	0	2.5*

The malar eminence*, nasolabial region*, and chin* show a modest rank order, but this was not statistically different. All were significantly greater than the forehead and lateral cheek. Statistical analysis by Wilcoxon rank sum test.

differences is not obvious. Innervation, blood supply, and permeability may all be playing a role.

CONCOMITANT TESTING ON OPPOSITE SIDES

Mayne *et al.* noted asymmetry of the reactions to swabbings with 5% lactic acid (10) on opposite sides. We examined disparate responses on opposite sides by performing chamber tests on ten moderate stingers on a single day. Ten percent lactic acid was applied to the right side of five subjects and to the left side in the remaining five. Two hours later, the test was repeated on the opposite sides. We found exceptional concordance in regard to onset and peak intensity (Table IV). The right and left cheeks are evidently symmetrical by chamber testing.

Table IV
Lactic Acid Stinging on Contralateral Cheeks: Symmetry of the Stinging Response

	Onset (minutes)		Peak intensity	
Subject	Right	Left	Right	Left
1	2	4	2	2
2	3	2	3	2
3	1	1	2	3
4	4	1	1	1
5	2	1	2	2
6	1	1	3	3
7	3	4	1	1
8	2	1	2	2
9	5	3	1	1
10	1	3	2	1
Median	2.0*	1.5*	2.0**	2.0**

The onset * and peak intensity ** were not statistically different.

Frosch and Kligman thought that concomitant testing on opposite sides was feasible (1). Thus, a comparison of two products or chemicals could be made at the same time (1). Experience has convinced us that concomitant testing yields inconsistent and misleading results. Striking interactions in perception occur when materials of differing stinging potential are applied to opposite sides. We demonstrated this by simultaneously applying 10% lactic acid on one side and distilled water on the opposite side of ten strong stingers. Three of these recorded moderate stinging on the water side. In another test on this same panel, 5% and 10% lactic acid were applied on opposite sides. The same three subjects reported equally severe stinging on both sides, and another three could not distinguish between the sides.

We have repeatedly found on simultaneous testing that the side which provokes more intense stinging elevates the stinging score on the opposite side. Moreover, some degree of perceptual confounding occurs even when the tests are separated by 30 to 60 minutes, if the material producing more intense stinging is applied first. We perform unilateral testing only.

STINGING AND IRRITANCY

Like Frosch and Kligman (1), we have not been able to establish a strict correlation between chemicals that induce inflammatory irritant reactions and their ability to provoke stinging. Some strong irritants such as sodium lauryl sulfate and benzalkonium chloride do not cause stinging, while many substances that are toxicologically innocuous can elicit stinging. A variety of acids, such as lactic, citric, and phosphoric acids, readily induce stinging, as do strongly alkaline agents such as sodium hydroxide. Stinging capacity cannot be reliably predicted by chemical structure. One has no recourse except to test empirically.

By contrast, Muizzudden *et al.* have found that facial stinging correlates well with hyperreactivity to irritants placed on the back and forearm (13). It is known that some persons have a generally hyperirritable skin sensitivity to chemical insults such as DMSO, ammonium hydroxide, and cantharidin (11). However, it remains to be demonstrated whether hyper-reactors are generally stingers.

EFFECT OF CONCENTRATION

Routine stinging tests were performed on ten subjects who were moderate stingers, using the following concentrations: 2.5, 5.0, 10.0, 15.0, and 20.0. We started with the lowest concentration, and exposed only one cheek per day. On each following day, the next higher concentration was applied, on the opposite cheek. The end point was the peak intensity score.

The results may be readily summarized. Two of ten subjects experienced slight stinging to 2.5% lactic acid. Four had mild stinging to 5%. With 15% the intensity scores were approximately the same as with 10%, though stinging tended to begin earlier. With 20%, four reported more severe stinging, while the remaining six showed no increase. These same four showed mild erythema that lasted for a few hours, followed by mild

scaling over the next few days. The onset of stinging was reduced by at least two minutes in eight of the ten subjects tested with 20%.

It thus appears that a near plateau in peak stinging is reached at 10%, with only modest increases with 20%. However, this last concentration can induce objective signs of irritation such as redness and desquamation.

We conducted a preliminary investigation with 50% lactic acid in five moderate stingers. Increases in the intensity scores were not impressive, though stinging began almost immediately. More noteworthy was the appearance of moderate erythema in four of the five, still present in three subjects 24 hours later. A mild spotty folliculitis was noted in two, 24 hours after exposure.

High concentrations thus can provoke a visible dermatitis after a ten-minute exposure.

INFLUENCE OF AREA

No attention has been paid to the size of the area of exposure. This turned out to be a surprisingly influential factor.

On separate days we compared the stinging scores of five subjects to 10% lactic acid using small Hilltop chambers having a diameter of 1.2 cm and larger ones of 1.7-cm diameter. The subjects included mild and moderate stingers. In nearly every case, both the onset of stinging and peak intensity was greater with the larger chamber. Stinging was even further enhanced when we placed 3 ml of 10% lactic acid in a 3-cm-diameter glass cylinder hand held to the skin of these five subjects.

Swabbing the entire face of these subjects with 10% lactic acid enormously increased the stinging response. The onset was almost immediate, increasing to peak intensity within one minute. In four, stinging was so severe as to require rinsing with water to obtain relief. Moreover, objective signs of irritation, notably erythema, appeared, lasting for hours or even the following day. We routinely use 1.7-cm -diameter Hilltop chambers.

This amplification of subjective and objective signs of irritancy by application to larger areas is an intriguing phenomenon that deserves further study. Incidentally, in unrelated studies we were able to demonstrate more intensive inflammatory reactions to 24-hour occlusive applications of irritants like sodium lauryl sulfate and benzalkonium chloride when the patch test area was increased fourfold.

RESERVOIR EFFECTS

Stinging ceases quickly when the test area is washed and rinsed. The consequences are quite different if the area is not washed after removing the chambers. Stinging diminishes slowly, usually within one to two hours. Then, reapplication of a chamber filled with distilled water will elicit stinging, though of somewhat lesser intensity. Water can elicit this response in some persons even one day later. Reactivation of stinging by water does not occur when the test area is washed and rinsed in the routine way.

Clearly, without rinsing, a reservoir of lactic acid is established in the stratum corneum. Water results in a release of the stored acid. We have made similar observations with strong soaps applied in a chamber. When the site was not washed, reexposure to water caused reappearance of erythema. Testers need to be aware of this phenomenon and insist

on a wash-out period before assessing subjective and objective reactions to potentially irritating materials.

TACHYPHYLAXIS?

This esoteric term applies to a well known phenomenon in which a predicted response to a chemical fails to occur upon repeated challenge. For example, the vasoconstriction induced by a six-hour exposure to a corticosteroid will not occur after three to four such exposures daily. The site becomes refractory. We were cautious not to repeat the lactic acid test too frequently on the same site for fear of intensifying the stinging response. Previous reports have warned against repeated exposures in order to prevent intensified reactions (9).

However, reexposures every other day or every week on the same site did not, as we expected, augment the response. The scores either remained nearly the same or the reactions actually diminished, suggesting tachyphylaxis. More work is needed to define the dimensions of this phenomenon. How tachyphylaxis occurs is unknown. Perhaps receptors become saturated, the horny layer becomes less permeable, or follicular shunts become blocked in some way. Desensitization to the sensations induced by repeated exposures to capsaicin is well known (4).

CONCLUSIONS

The ability to identify stingers and classify them into mild, moderate, and severe opens up new clinical applications. There is tentative evidence that stingers are more likely to have "sensitive" skin. Muizzuddin *et al.* found that women who reported higher intolerance to various products had lactic acid stinging scores that were more than three times that of persons without "sensitive" skin (13). These same individuals were also much more likely to develop irritation after exposure to Balsam of Peru. It would seem worthwhile to test new products on stingers prior to marketing. Our practice is to apply finished formulations twice daily for two weeks to the entire face of moderate stingers. Lactic acid stinging is scored at baseline and one to two days after two weeks of product usage. An appreciable increase in stinging scores would signal a potential for the occurrence of disagreeable reactions in users who have "sensitive" skin. On the other hand, a reduction in stinging scores would indicate a beneficial, protective effect. We have witnessed both outcomes in preliminary trials of proprietary "moisturizers."

The stinging phenomenon is still mysterious. We are ignorant of the neurophysiologic mechanisms that underlie it. Why some apparently normal women sting severely and others not at all is a question worthy of serious investigation. Stingers cannot be recognized by phenotype or by clinical examination. Many factors are undoubtedly at play and can only be brought to light by focused research. Among the variables that might affect stinging are age, ethnicity, sex, atopic background, barrier function, cosmetic practices, and degree of oiliness or dryness. We are looking into all of these.

A huge variety of skin care products and cosmetics have been designed for facial application. The predictive value of the stinging test must take into account the intended use of the product. For example, Grove *et al.* determined that a commercial formulation did not cause adverse effects in a panel of stingers (9). Nonetheless, the

manufacturer had to recall the product after marketing because of disagreeable reactions. It was then learned that the product was designed for the eye area. It is well known that the thinner eyelids are more susceptible to irritation. Eye area products should therefore be tested directly on that area. Products designed for a specific area of the face should be tested directly on that area.

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