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A method for appraising the stinging capacity of topically applied substances

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Synopsis

SUBSTANCES, which cause SUSTAINED STINGING, can be recognized by APPLICATION to the NASOLABIAL FOLDS and CHEEKS during PROFUSE SWEATING. The test was carried out on preselected individuals in whom a susceptibility to stinging had been demonstrated by exposure to 5 per cent aqueous lactic acid. Stinging proneness is greater in females than in males, and in whites more so than in blacks, and especially in light complexioned persons who tan poorly. Stinging is mainly perceived on the face.

Stinging and irritancy were not strictly correlated. Most primary irritants do not cause stinging. Weak irritants may sting badly.

INTRODUCTION

Producers of medicaments, cosmetics, and toiletries are attuned to the necessity of certifying the safety of their products. Generally speaking, adequate test procedures have been developed for assessing the likelihood of toxicity from irritation, contact sensitization, and photosensitization.

Nonetheless, products which pass these tests and which fulfill the purposes for which they were designed may still be unacceptable. The consumer will reject even effective formulations if disagreeable sensations arise after application. In this paper, we are concerned with a special type of subjective discomfort, namely, delayed stinging or smarting from topical agents applied to the skin. In contrast to substances like alcohol which cause immediate but transient stinging, those that induce delayed and sustained stinging are not so easily recognized. This adverse effect may not come to light until after the product has been in widespread use.

Our focus is on substances which begin to "sting" or "burn" within a minute or two. The discomfort intensifies over the next 5 to 10 min and may become so severe that frenetic attempts are made at removal. Intense stinging generally abates within about 15 min. Signs of irritation—redness, scaling, edema—do not develop.

It is this sustained, crescendo type stinging that we undertook to study. An embarrassing experience precipitated our research. Although, it had been previously noted (1) that amyl-dimethyl-p-aminobenzoic acid (ADP)* could cause stinging, our premarketing tests of a sunscreen containing this ingredient repeatedly failed to disclose sustained stinging even under thermal stress. It was only after widespread sale that complaints were made by a small proportion of users. In our opinion, the prevalence of disagreeable stinging from this product is less than that reported by Parrish *et al.* (2) Were this not so, the extensive field testing, which was done in hot climates, would have revealed this adverse reaction much earlier.

We are not the first to become conscious of the need to ascertain undesirable subjective reactions. Armstrong and his coworkers applied test substances to the base of cantharidin blisters in order to appraise their capacity to cause pain (3). This test is not relevant for materials that provoke stinging on normal skin. Laden (4) clearly perceived the problem and attempted to develop appropriate methodologies for detecting stinging potentiality.

He immersed the abraded tails of rats in test solutions and determined the time required for the rat to flick its tail out of the solution. He emphasized the variability of the results though useful information could be secured. For human testing, Laden applied test materials to scotch-tape stripped skin, obtaining reasonable agreement with the animal data. Again this procedure mainly measures pain (or does not clearly discriminate between pain and stinging); its applicability to stinging from application to normal skin is uncertain. Laden made the important observation that stinging and irritation could not be correlated. For example, substances such as citric and acetic acids caused intense stinging but scored quite low in tests of primary irritancy.

Recently, Shanahan and Ward (5) described an interesting animal model for estimating the stinging capacity of shampoos. They injected mice intraperitoneally and scored the intensity of the ensuing writhing. The results correlated well with human eye sting tests. Again, stinging and irritancy were not found to parallel each other. Some shampoos which proved to be nonirritating by the Draize rabbit eye test caused intense stinging.

MATERIALS AND METHODS

I. SELECTION OF SUBJECTS

We gradually came to appreciate that only a small proportion of persons would show a stinging response to ADP. Our first task then was to develop a screen to locate "stingers," persons who had this peculiar susceptibility. The following procedure proved reliable. A volunteer was brought to a state of profuse sweating in an environmental chamber at 110°F and 80 per cent relative humidity (R.H.). Then a 5 per cent aqueous solution of lactic acid was rubbed briskly over the nasolabial fold and cheek.

*Padimate, Eclipse, G. S. Herbert Co.

Those who experience sharp stinging for at least 3 to 5 min are identified as stingers. An alternative screening test, which can be carried out at room temperature, involves the application of 2 ml of 90 per cent aqueous dimethylsulfoxide (DMSO) in a small glass cup on the cheek for 5 min. This produces intense burning in stingers; however, the disadvantages of DMSO are disqualifying. The substance smells and tastes badly, not to mention that a solid tender wheal and persistent erythema develop. By contrast, lactic acid produces no visible changes.

II. STINGING ASSAY

This is carried out on groups of 5 to 10 stingers, depending on the degree of accuracy required. The subject enters the environmental chamber (110°F, 80 per cent R.H.) and after the face has begun to sweat copiously (generally after 15 min) a liberal amount of the test material on a cotton swab is thoroughly rubbed over the nasolabial fold and cheek. Stinging is evaluated immediately (10 sec) and at 2.5, 5.0, and 8.0 min on a 4-point scale: 0 = no stinging; 1 = slight; 2 = moderate; and 3 = severe. The time sequence was formed empirically. Some substances cause slight to severe stinging immediately after application with disappearance of the sensation within 5 to 30 sec. Delayed stinging is generally not preceded by a transient phase and usually becomes evident within a minute or two. The delayed stinging score for an individual is the mean of the three readings at 2.5, 5.0, and 8.0 min. We arbitrarily regard substances with average scores falling between 0.4 and 1.0 as having slight stinging potential. The range 1.1 to 2.0 signifies moderate stinging, and 2.1 to 3.0 signifies severe stinging.

A second agent can be simultaneously evaluated on the opposite cheek. Within limits, a series of agents can be evaluated during 1 sweating period.

EXPERIMENTAL OBSERVATIONS

I. PREVALENCE OF STINGERS

Thirty young adult student volunteers (20 whites, 10 blacks, equally divided as to sex) were evaluated using both lactic acid and DMSO probes. Both tests gave concordant results.

There were 4 stingers among the whites, only one of whom was a male. Only 1 black, again a female, exhibited stinging. With lactic acid stinging began after a few seconds and in 4 of 5 subjects persisted strongly for about another 5 min, subsiding slowly over the next 10. DMSO produced peak stinging within 3 min; this remained at high intensity for another 10.

The nonstingers did not perceive significant stinging with either test. It is worth noting that DMSO induced considerably less whealing and erythema in the latter. All 5 stingers thought they had unusually "sensitive" facial skin having had past trouble with cosmetics, soaps, etc. Three had a history of atopy (but not atopic dermatitis). The white stingers had light complexions, sun-burned easily, and tanned poorly. All 5 blushed easily.

The sample is too small for any but the most tentative judgments. Females seem to be more susceptible. It remains to be shown whether this is a true sex difference or

Table I
Comparison Between Stingers and Nonstingers

Agent	Lactic Acid Positive					Lactic Acid Negative				
	Subject	Score				Subject	Score			
		10 sec	2.5 min	5.0 min	8.0 min		10 sec	2.5 min	5.0 min	8.0 min
5 per cent lactic acid (Water)	1	1	3	3	0	1	0	0	0	0
	2	0	1	2	1	2	0	0	0	0
	3	3	2	2	1	3	0	1	0	0
	4	1	2	2	1	4	0	0	0	0
	5	2	1	1	0	5	0	0	0	0
	Mean ± S.E.	1.4 ± 0.51	1.8 ± 0.37	2.0 ± 0.32	0.6 ± 0.24	Mean ± S.E.	0	0.2 ± 0.2	0	0
5 per cent amyl-dimethyl paba (ethanol)	1	2	2	3	3	1	1	0	0	0
	2	0	2	3	2	2	0	0	0	0
	3	0	2	2	1	3	0	0	0	0
	4	1	1	2	0	4	1	0	0	0
	5	0	2	1	0	5	0	0	0	0
	Mean ± S.E.	0.6 ± 0.40	1.8 ± 0.20	2.2 ± 0.37	1.2 ± 0.58		0.4 ± 0.24	0	0	0
50 per cent dimethyl phthalate (ethanol)	1	2	1	0	0	1	1	0	0	0
	2	3	3	2	1	2	1	0	0	0
	3	3	1	1	0	3	0	0	0	0
	4	2	1	1	0	4	1	1	0	0
	5	1	2	2	0	5	0	0	0	0
	Mean ± S.E.	2.2 ± 0.37	1.6 ± 0.40	1.2 ± 0.37	0.2 ± 0.20		0.6 ± 0.24	0.2 ± 0.2	0	0
Propylene glycol	1	3	3	3	2	1	0	0	0	0
	2	2	1	1	0	2	0	0	0	0
	3	1	1	1	0	3	1	0	0	0
	4	2	2	1	0	4	0	0	0	0
	5	1	1	0	0	5	1	0	0	0
		1.8 ± 0.37	1.6 ± 0.40	1.2 ± 0.49	0.4 ± 0.4		0.4 ± 0.24	0	0	0
5 per cent phosphoric acid (water)	1	1	3	1	0	1	0	2	1	0
	2	1	2	3	2	2	0	2	0	0
	3	0	3	3	1	3	0	1	0	0
	4	2	3	2	0	4	1	2	1	0
	5	3	3	3	2	5	1	0	0	0
	Mean ± S.E.	1.4 ± 0.51	2.8 ± 0.20	2.4 ± 0.40	1.0 ± 0.45		0.4 ± 0.24	1.4 ± 0.40	0.4 ± 0.24	0

merely a reflection of the fact that more females had light complexions. Weigand and Mershon (6) evaluated tear gas (*o*-chlorobenzylidene malononitrile) for irritancy and stinging in various body regions of black and white subjects. They too found that on the cheek and retroauricular areas, stinging was weaker in blacks and appeared later.

II. COMPARISON BETWEEN "STINGERS" AND "NONSTINGERS"

ADP (5 per cent), dimethylphthalate (50 per cent in ethanol), propylene glycol (neat), and phosphoric acid (5 per cent) were applied during sweating to 5 lactic acid-positive and 5 lactic acid-negative persons, "stingers" and "nonstingers," respectively. The results are given in Table I.

Every stinger experienced moderate to severe stinging with all 4 test agents while stinging was virtually absent in the lactic acid-negative group, except for reduced reactions to phosphoric acid at 2 to 5 min. Five per cent ADP mainly produced delayed stinging, while 50 per cent dimethylphthalate caused strong transient stinging as well.

III. INFLUENCE OF SWEATING

A 5 member panel was tested with 5 per cent ADP in ethanol at room temperature. Tests on separate occasions were conducted at 5, 15, and 30 min after entering the environmental chamber.

Stinging did not occur at room temperature. However, stinging was already weakly evident when the application was made 5 min after entering the chamber, before sweating was at full flow. After 15 min, all 5 subjects experienced intense stinging at the 5-min reading. Rinsing the face with water without leaving the chamber was not followed by an immediate decrease in intensity; stinging persisted at the same level for about 10 min, fading to insignificance about 20 min after removal.

IV. ANHIDROSIS

The critical role of sweating was further demonstrated by testing skin that has been rendered anhidrotic. Sweating was completely suppressed on 1 cheek of 3 stingers by applying an occlusive patch of 5 per cent aqueous aluminum chloride hexahydrate for 24 h. Three h after removal of the patch, a 5 per cent ethanolic solution of ADP was applied to both cheeks after the subjects had been sweating for 15 min.

Stinging was markedly reduced on the anhidrotic side; the score was never greater than 1 compared to 3 on the sweating side.

V. MULTIPLE APPLICATIONS VERSUS 1 APPLICATION

We compared the intensity of stinging with 1 versus 5 applications of 5 per cent lactic acid and 5 per cent ADP in separate groups of 3 stingers each. The test material was applied to 1 side every 5 min for a total of 5 applications starting after the subject had been sweating for 10 min. The opposite side received 1 application at the time of the fifth application to the opposite cheek.

The results were most dramatic with lactic acid. The intensity of stinging increased with each application producing almost intolerable discomfort in all 3 subjects. These were hardy volunteers and it was only by dint of much persuasion that the test was completed. Indeed, superficial erosions subsequently developed in 2 of the 3. Signs of irritation have never been observed with 1 application.

The same pattern of progressive intensification of stinging was observed with ADP, but the severity was less.

VI. PRIOR DAMAGE

A. *Sunburn:* Using fluorescent sunlamp tubes* 2 MEDs were given to 1 cheek of 3 stingers. The next day stinging was assessed on both sides by applying 5 per cent ethanolic ADP after 15 min of sweating.

Stinging began earlier and was considerably more intense on the sunburned side in all 3 subjects. An inflammatory reaction thus accentuates the response.

B. *Chemical irritation:* A 5 per cent aqueous solution of a quaternary ammonium compound† was applied to 1 cheek by occlusive patches for 10 min twice daily for 2 days. Five per cent ADP was applied to both cheeks of 3 sweating stingers one day later when the treated skin showed moderate erythema and tiny pustules.

Stinging was sharply accentuated on irritated skin, emerging more rapidly and becoming very intense after a few minutes.

VII. STINGING ON STRIPPED SKIN

A. *Lactic acid:* The cheeks of 3 nonstingers were scotch-tape stripped to the glistening layer while half that number of strippings was made on the other side. After the subjects had been sweating for 15 min, 5 per cent lactic acid was applied to both cheeks.

Stinging appeared immediately and in high intensity on the completely stripped side. Stinging was less on the partially stripped opposite side, but was nonetheless appreciable. The duration on stripped skin was shorter than on the normal skin of "stingers." The stinging on completely stripped skin was very sharp upon application, but faded within 2.5 min. This result indicates that all persons will experience stinging after removal of the horny layer barrier.

In another test, 5 per cent lactic acid was applied to the stripped skin of the nonsweating back of 3 stingers and 3 nonstingers. Stinging, indeed pain, was equally intense in both groups immediately after application, declining rapidly within a few minutes. Discrimination is lost on stripped back skin.

B. *ADP:* A panel of 3 nonstingers was scotch-tape stripped to the glistening layer on 1 cheek and 1 side of the upper back. Five per cent ADP was applied to both normal and

*Westinghouse FS 20.
†Hyamine 3500, Rohm & Haas Co., Spring House, PA 19477.

stripped skin. The first test was carried out on the backs without sweating and on the cheeks after 15 min of sweating.

Stinging did not occur on normal skin. On the stripped sites of the back, as well as the cheek intense immediate stinging developed, disappearing within about a minute. Stripping practically eliminates the distinction between stingers and nonstingers. It should also be noted that almost as severe stinging occurred after application of the vehicle, 95 per cent alcohol. It had become evident by this time that the difference between stingers and nonstingers was merely quantitative. To test this idea, 5, 10, and 15 per cent ADP was applied on separate occasions to the profusely sweating cheeks of 3 stingers and 3 nonstingers. The stingers experienced sharp discomfort with 5 per cent ADP; the severity and duration of stinging increased progressively with the 10 and 15 per cent concentrations. With the latter, the distress was not appreciably reduced by leaving the chamber.

As expected, 5 per cent ADP had no effect on nonstingers. All 3, however, experienced slight stinging with 10 per cent, while with 15 per cent, 2 of the 3 had moderate stinging.

It is clear, then, that stingers merely have lower thresholds. When the stimulus is great enough, even nonstingers will experience mild to moderate stinging. We have repeatedly observed this phenomenon with other drugs at higher concentrations.

VIII. REGIONAL DIFFERENCES

Five per cent ADP was used on 3 stingers to compare the intensity of stinging in the following regions: central cheek, nasolabial fold, forehead, chin, infraorbital and retroauricular regions, axilla, antecubital fossa, upper back, and scalp. The applications were made after the subjects had been sweating for 15 min.

Clear-cut stinging was restricted to the face, being most pronounced on the nasolabial fold, followed by the cheek, chin, infraorbital, and retroauricular region. The forehead reaction was marginal. Stinging was not perceived on the scalp, back, arm, and axilla. These findings conform to those of Weigand and Mershon (6) using tear gas *o*-chlorobenzylidene malononitrile (CS) as the stinging agent. Stinging is predominantly a facial phenomenon.

IX. CORRELATION OF STINGING WITH IRRITANCY

A. *α-hydroxy acids:* The test agents were: lactic acid, pyruvic acid, tartaric acid, and glycolic acid. Five per cent aqueous solutions were evaluated in 3 stingers at different sweating sessions.

Comparative irritancy was determined by 24-h occlusive patch tests on the forearms with 5 and 15 per cent concentrations of the respective acids on the same subjects. The intensity of the reaction was scored on a 0 to 3 scale (0 = no erythema; 1 = slight; 2 = moderate; and 3 = severe). The rank order of descending irritancy was: pyruvic > glycolic > tartaric > lactic.

The first 2 produced severe erythema and vesiculation with the 15 per cent concentration. The rank order was the same in stinging tests; the correlation was thus very good despite the small size of the sample.

pH does not account for these differences (range 1.7 to 2.0). Laden (4) also found that acids of the same pH had quite different stinging capacities. Following his precedent, we compared equimolar (0.3 *N*) solutions (concentration percentages 2.25 to 2.70). The rank order of stinging was identical, although, stinging tended to be rather weak at these lower concentrations.

B. *Esters of p-aminobenzoic acid:* The test agents were glyceryl p-aminobenzoic acid (GP-Escalol 106), (ADP-Escalol 506), and octyl-dimethyl-paba (OCP-Escalol 507). Five per cent solutions in ethanol were tested for stinging in the usual manner on 3 subjects.

Five per cent solutions were evaluated for irritancy by occlusive patch tests on the forearm after criss–cross scarification of the skin with a 27-gauge needle. Applications were made daily for 3 consecutive days under cutaneous occlusion.

As usual, ADP induced sharp stinging. The other 2 esters lacked this property completely. GP caused modest redness while ADP and ODP were completely innocuous on sacrified skin.

So, in this instance, a stinging ester (ADP) was found to be nonirritating, while an irritating one (GP) was nonstinging.

C. *Metallic antiperspirants:* The test agents were aluminum chloride hexahydrate, aluminum chlohydroxide, aluminum bromhydroxide (basic aluminum bromide), and zirconium hydroxychloride. Thirty per cent aqueous solutions of these were evaluated for irritancy by 24-h occlusive patch tests on forearm skin. This same concentration was also used in stinging tests performed on 3 stingers. The rank order of irritancy was: aluminum chloride > zirconium hydroxychloride > aluminum chlorhydroxide equals basic aluminum bromide.

The last two caused no skin reaction, whereas the first two produced erythema and small pustules, aluminum chloride being somewhat more severe.

As with hydroxy acids, stinging capacity paralleled irritancy. Aluminum chloride caused slight stinging, followed closely by zirconium hydroxychloride, while the other 2 salts lacked this property altogether.

D. *Effect of strong irritants:* A 5 per cent aqueous solution of sodium lauryl sulfate (SLS) produced an intense dermatitis in a 24-h patch test on the forearm of 3 stingers, still this same solution did not induce stinging in any sweating subject when applied to the face.

Undiluted kerosene caused blisters in a 24-h patch test on the same 3 persons, but was utterly without stinging potentiality.

A 5 per cent solution of Hyamine 3500 produced a marked irritant reaction by a 24 hr occlusive patch test. It, too, failed to elicit stinging. It is thus apparent that strong irritants may be completely free of stinging capacity.

X. EFFECT OF VEHICLES

ADP was evaluated in 5 stingers using 5 per cent concentrations in the following vehicles: ethanol, hydrophilic ointment USP, carbowax USP, ethanol : propylene glycol (1:1) and vanishing cream USP.

The ethanol:propylene glycol solution stood out above all the others in stinging capacity, followed closely by ethanol. The onset and intensity of stinging was markedly reduced by vanishing cream. Stinging was virtually abolished with carbowax and hydrophilic ointment. Thus, solutions are more likely to cause stinging than creams and ointments. The effects are no doubt dependent on release from the vehicle.

XI. DOSE RESPONSES

ADP was tested at 1.0, 3.0, and 5.0 per cent concentrations in ethanol in 3 stingers. The 1 per cent concentration did not cause any stinging. Stinging was mild with 3 per cent in all 3 subjects. The 5 per cent solution produced intense stinging as usual; in addition, stinging came on earlier. Thus, clear-cut differences can be detected over a rather narrow concentration range.

XII. ASSAY OF MATERIALS OF DERMATOLOGIC INTEREST

Stinging tests were carried out on panels of 5 stingers with a variety of familiar substances. Stinging was not observed with 3 per cent hydrogen peroxide, 5 per cent ammonium hydroxide, saturated salt solution, vanishing cream USP, hydrophilic ointment USP, carbowax Ointment USP, "pool chlorine," and a variety of steroid creams and gels, 1 per cent aqueous neomycin sulfate, 5 per cent p-aminobenzoic acid in hydro-alcoholic vehicle*, 0.2 per cent Uvinul 539 in ethanol (2-ethylhexyl-2-cyano-3-3-diphenyl-acrylate), 8 per cent homosalate†. All the materials in Table II induced delayed stinging to varying degrees. These have been sorted into 3 groups (slight, moderate, and severe).

The following agents caused transient but not delayed stinging: methanol, ethanol, 5 per cent concentrations of ascorbic, acetic, citric and sorbic acids, retinoic acid.‡

DISCUSSION

The essential features of our stinging test are: (1) selection of volunteers who exhibit sharp stinging to 5 per cent lactic acid and (2) application of the test agent to the nasolabial fold after sweating has been induced. The results are highly repeatable; there is sufficient sensitivity to permit substances to be accurately rated as mild, moderate, or severe.

*Pre-Sun, Westwood Pharmaceuticals, Inc., Buffalo, N.Y. 14213.
†Coppertone Cream, Plough Inc., Memphis, TN 38151.
‡Retin A solution, 0.05 per cent, Johnson & Johnson Co., East Brunswick, N.J.

Table II
Agents That Induce Stinging

Agent	Concentration Per cent	pH	Vehicle	Severity
Benzene	1.0	7.5	Ethanol	Slight
Phenol	1.0	5.9	Ethanol	Slight
Salicylic acid	5.0	2.4	Ethanol	Slight
Resorcinol	5.0	6.4	Water	Slight
Phosphoric acid	1.0	2.1	Water	Slight
Sodium carbonate	15.0	11.2	Water	Moderate
Trisodium phosphate	5.0	12.0	Water	Moderate
Propylene glycol[a]	Neat	5.3		Moderate
Propylene carbonate[a]	Neat	8.05		Moderate
Propylene glycol diacetate[a]	Neat	3.8		Moderate
Dimethylacetamide	Neat	7.8		Moderate
Dimethylformamide	Neat	10.2		Moderate
Dimethylsulfoxide	Neat	14.0		Moderate
Diethyltoluamide[a] (Deet)	50.0	8.8	Ethanol	Moderate
Dimethyl phthalate[a]	50.0	4.0	Ethanol	Moderate
2-Ethyl-1, 3-hexanediol[a] (Rutgers 612)	50.0	10.5	Ethanol	Moderate
Benzoyl peroxide lotion[b]	5.0	9.9	Grease-free washable lotion base	Moderate
Benzoyl peroxide gel[c]	10.0	14.0	Polyoxyethylene lauryl ether gel	Moderate
Crude coal tar[a]	5.0	10.0	Dimethylformamide	Severe
Phosphoric acid	3.3 (1/3 mol)	1.9	Water	Severe
Hydrochloric acid[a]	1.2 (1/3 mol)	1.3	Water	Severe
Sodium hydroxide	1.3 (1/3 mol)	13.0	Water	Severe
2-ethoxyethyl p-methoxy-cinnamate[d]	2.0	7.4	Ethanol	Severe

[a]Immediate transient stinging as well as delayed type.
[b]Benoxyl 5 lotion, Stiefel Labs., Inc.
[c]Desquam-X 10 gel, Westwood Pharmaceuticals, Inc.
[d]Giv-Tan F®, Givaudan Corp.

The stinging phenomenon is peculiar to the face, particularly the nasolabial folds and cheeks, the latter being a little less sensitive. Our explanation for this localization relates to the high permeability of the nasolabial region (as determined by visible responses to vasoactive drugs), the high density of appendages (hair follicles and sweat glands), which can serve as penetration shunts into the dermis, and, not least, the elaborate sensory nerve network. In man, every vellus hair follicle is associated with specialized nerve endings; these along with the abundant dermal nerve network on the face confer an exceptional sensitivity to touch and pain.

Stinging seems to be a variant of pain and develops quickly after appropriate stimulation of sensory nerves. However, toxic and irritating chemicals, which can badly

damage skin, often lack the capacity to induce stinging. On the other hand, substances that are nonirritating may possess striking stinging capabilities.

As a class, acids tend to cause strong stinging. The differences in stinging potentiality among acids are not dependent on pH, but are probably related to their diffusional characteristics, the more troublesome ones penetrating more rapidly.

Likewise, strongly alkaline substances such as sodium carbonate, trisodium phosphate, and sodium hydroxide can induce marked stinging. Acids (below pH 2.0) and bases (above pH 11.0) probably excite nerve endings directly. The strong buffering capacity of the skin doubtlessly limits the damage from a single application.

While there is generally no clear correlation between irritancy and stinging, their properties may parallel each other within a specific class of substances, *viz.,* metallic antiperspirants and α-hydroxy acids.

That excitation of nerve endings is central to the stinging phenomenon is suggested by a number of observations. The intensity increases with each additional application of non-irritating materials. Hypertonic solutions do not cause stinging, but can cause its revocation after a stinging episode. For example, stinging will reappear when a saturated solution of sodium chloride is applied after the stinging induced by ADP or lactic acid has abated. Moreover, inflamed skin is far more prone toward stinging, even when the horny layer barrier is intact as in freshly sunburned skin. Of course, in chemically damaged skin the nerve endings are not only more excitable but increased permeability enables increased diffusion.

Stinging is perceived with 5 per cent ADP and 5 per cent lactic acid only when the subject is sweating; the intensity of the sensation is proportional to the amount and duration of sweating. The longer a person sweats, the easier and faster stinging can be elicited. In all probability, the principal role of sweating is to hydrate the skin, thus greatly increasing its permeability. It is well known that the flux of substances increases in moistened skin. Another is the associated increase in skin temperature, heightening the sensitivity of nerve endings. We found that stinging could be induced even at room temperature if a wet compress was applied to the cheek for 20 to 30 min before exposure to 5 per cent lactic acid. However, the intensity and duration of the stinging was always much less than with induced sweating. Then, too, diffusion through sweat-filled ducts promotes transport to dermal nerves. The sharp decrease in stinging on cheeks rendered anhidrotic by aluminum chloride is further evidence of the role of hydration.

Although, we have emphasized preselection of subjects, it is perfectly clear that stinging can be elicited in anyone by assuring access to nerve endings. Persons who do not sting, when lactic acid is applied to the normal cheek, will do so if the horny layer barrier is removed, partially or completely by scotch-tape stripping. Apparently, the peculiarity of "stingers" is more permeable skin. As a practical point, persons who are not stinging-prone may experience appreciable stinging if the skin has become inflamed through chemical or physical insults; for example, sunburned skin is considerably more reactive. We have preliminary evidence that the facial skin of stingers is more permeable. We have already mentioned that DMSO produced more intense whealing and erythema in stingers. It is our impression that stingers have more permeable skin everywhere.

At first glance, stripping might seem to offer the alternative of screening substances on areas other than the face and without the requirement of sweating. Unfortunately, this is not a feasible alternative. ADP, for example, did not elicit typical stinging in stripped skin of the back and was not different from its vehicle ethanol. Moreover, stinging in stripped skin was evanescent and not at all discriminating. Many irritating substances, which do not cause facial stinging, will produce pain and stinging on stripped skin.

GUIDELINES FOR PERFORMING STINGING TESTS

SUBJECTS

Stingers are likely to be white females with light complexions who give histories of easy sunburning. They frequently complain of having sensitive skin and of having trouble with cosmetics and soaps. The lesser susceptibility of blacks is doubtless due to their possession of a more effective barrier, the horny layer being both more dense and with a greater number of cell layers (7).

THERMAL STIMULATION

The facial sauna* is a useful substitute for an environmental chamber; the latter can hardly be viewed as standard equipment.

The subject places the face directly into the steam stream for at least 15 min or until sweating is brisk. The steam exposure must be continued for another 10 min after application of the test agent. We obtained comparable results with a variety of stinging formulations in a panel tested with both the chamber and the sauna. The intensity of stinging was a little less with the sauna.

REPEATED TESTING

If no stinging develops in 5 min, one can wipe off the substance with a wet towel and apply a new test material. This can be repeated at least twice on each cheek, allowing for the evaluation of 6 agents in a single session. The limiting factor in "no response" testing is the time that subjects can stay in the thermal chamber. They are generally wrung out by 45 min.

On the other hand, a positive response increases susceptibility to stinging. To avoid false positive readings, no further testing should be conducted on that cheek.

We have never observed tachyphylaxis over the short term, that is, unresponsiveness induced by repeated testing. However, we have witnessed the development of considerable resistance in 2 of 5 stingers, who were regularly used twice weekly for several months. One subject became virtually a nonstinger after 3 months of testing with stinging materials though irritation was never observed. This induced refratoriness may be a subliminal form of "hardening" that occurs with prolonged exposure to irritants. In any event, subjects who are used repeatedly, should be monitored from

*Facial Beautifying Mist, Model 60. Schick Electric, Inc., Lancaster, PA.

time to time for retention of sensitivity to lactic acid. Susceptibility returns after a rest period of several weeks.

We have recently developed a simpler method of selecting stingers for recruitment of test panels. Thermally induced sweating is not required. The application of 10 per cent lactic acid to the face will elicit stinging which is almost equivalent to 5 per cent acid on sweating skin. The same is true for 15 per cent ethanolic ADP. These higher concentrations have no effect on nonstingers. Let it be clearly noted, however, that tests of unknown materials must be carried out on sweating facial skin of preselected stingers. It should also be noted that substances with a high capacity to induce stinging can do so in the absence of sweating.

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