

The chamber-scarification test for irritancy*

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A procedure has been designed (the chamber-scarification test), which possesses greatly increased sensitivity for assessing the irritancy of topically applied materials. A forearm test site is criss-cross scarified by drawing a 30-gauge needle over the skin with just enough pressure to cleave the epidermis without drawing blood. The test agent is applied in an aluminum chamber once daily for 3 days.

The advantages of the method over conventional patch testing are: enhanced capacity to measure mild irritants, reduced time (3 days versus 10 to 21 days), less effort, less cost, less discomfort for the volunteers and greater reproducibility.

Key words: Antiperspirants – chamber method – cosmetics – irritancy testing – propylene glycol – scarification – surfactants.

Received for publication July 19, 1976

Concern for the safety of topically applied substances is now greater than ever before. Manufacturers are under considerable pressure to provide assurances that their products will do no harm. This holds especially true for toiletries and cosmetics where the benefits are generally not deemed important enough to justify even modest risks.

Conventional methods of irritancy testing utilize daily occlusive patch tests for 10 to 21 days (Kligman & Wooding 1967, Lanman et al. 1968, Finkelstein et al. 1963). These procedures are no longer adequate for many reasons. They take too long, are burdensome to the subjects and are costly of effort and money. By far their greatest shortcoming is lack of sensitivity; they simply fail to detect agents which possess only a modest potentiality for producing irritant

reactions. We would cite from our own experience difficulties in evaluating the irritancy of antiperspirants containing aluminum chlorhydroxide, non-ionic surfactants such as the Tweens® (Atlas Chemicals) and Tritons® (Rohm & Haas), esters of fatty acids and others. Even more persuasive to us was the insistence of some adult women with mild acne that their faces would become irritated after a few days' use of certain popular creams which are generally regarded as bland. These creams failed to produce reactions when applied occlusively to human skin daily for 3 weeks. Yet twice-daily application to the faces of these particular women did indeed produce an irritant reaction in a week or less.

Added to this was our increasing disaffection with the patch test as a device for

* Presented at the Third Conference on Cutaneous Toxicity, Washington, D.C., 18 May 1976.

presenting test materials to the skin. Patches have numerous disadvantages: they are hard to keep in place, and often slide, loosen or wrinkle. Intimate, uniform exposure cannot be guaranteed, hence, reproducibility is only fair. Loss of the test material to the surrounding skin is an ever-present problem.

Instead of patches we now place the test substances in a chamber which is a modification of the one designed by Pirilä in Finland for use in tests of contact sensitization (Pirilä 1975). Details of the Duhring Chamber will be described elsewhere (Frosh & Kligman, in press). The Duhring Chamber is larger and deeper than Pirilä's, having an inner diameter of 12 mm compared to 8 mm. The capacity is, therefore, very much greater for measuring. Exposure in chambers permits quantification to a degree not possible with patches. There is no loss of material (completeness of sealing is evident by a circular indentation of the skin at the time of removal) and contact with the skin is uniform.

The insensitivity of conventional methods can largely be ascribed to the horny layer which presents a formidable barrier to the diffusion of applied substances. A means had to be found which would assure penetration of the test substance to the viable tissue where an inflammatory response could be mounted. We tried many techniques for breaching the barrier; abrasion, scotch-tape stripping, pretreatment with anionic surfactants and use of vehicles which enhance diffusion (DMSO). The paramount difficulty with all of these was lack of reproducibility; in addition, the inflammatory response from the procedure itself obscured the response to the test agent.

After many false starts and maunderings, we discovered a truly ancient procedure for introducing substances into the skin, namely scarification.

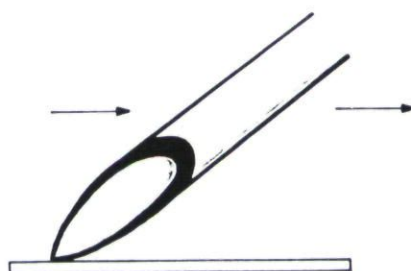
In this paper we shall describe a new procedure for assessing irritancy. Its note-

worthy features are: shortening of the period of exposure to 3 days, application to scarified skin and use of a chamber instead of a patch.

Method

Subjects

These are light-skinned, young adult Caucasoids. For very discriminating work we select "hyper-reactors"; these persons are especially vulnerable to irritants and can



1+ Erythema confined to scratches.



2+ Broader bands of increased erythema with or without rows of vesicles, pustules or erosions.



3+ Severe erythema with partial confluency with or without other lesions.



4+ Confluent, severe erythema sometimes associated with edema, necrosis or bulla formation.

Fig. 1. Above. Position of needle for scarifying skin. The bevel faces sideways and the needle is at a 45° angle.

Below. Scoring system.

be identified by a simple test, namely, elicitation of a brisk inflammatory reaction (severe erythema with edema and tiny vesicles) by a 24-hour forearm chamber exposure to 5 % aqueous sodium lauryl sulfate. Preselection lessens the variance but is not obligatory for routine work. Tanned skin is less reactive and should be avoided. Five to 10 volunteers constitute a test panel. The larger number naturally provide greater accuracy.

Application by chambers

Fixed quantities of the test agents are applied, viz. 0.1 mg. of ointments, creams and powders is loaded into the chambers. Solutions are handled differently; 0.1 ml is pipetted on to a disc of two thicknesses of non-woven cotton cloth (Webril®, Curity)

snugly fitting the chamber. The chamber is sealed to the skin with non-occlusive tape, Elastoplast® Beiersdorf Inc.) or Dermicel® (Johnson & Johnson).

Six to eight chambers can be applied to the mid-volar forearm. The back is even more reactive (Magnusson & Hersle 1965), but the chambers are more difficult to secure. Then, too, the arm is more convenient for the volunteers. By encircling the extremity with tape, the chambers can be securely fastened, minimizing leakage.

Water-soluble substances are dissolved in water; lipid-soluble ones in 95 % ethanol (if soluble) or in U.S.P. mineral oil. Quite insoluble materials can be finely suspended in bland oil-in-water emulsions such as U.S.P. vanishing cream. One must first ascertain that the vehicle is not irritating.

Table 1. Scarification indices* (S.I.)

Agent	Solvent	Threshold concentration (%)		S.I.
		Normal skin	Scarified skin	
<i>Surfactants:</i>				
Triethanolamine	ethanol	100.0	5.0	20
Octoxynol – 9 ¹	water	50.0	1.0	50
Sodium lauryl sulfate	water	0.5	0.05	10
<i>Inorganic salts:</i>				
Nickel sulfate	water	20.0	0.13	154
Aluminum chloride	water	30.0	2.5	12
Potassium chloride	water	60.0	5.0	12
<i>Antimicrobials:</i>				
Formalin	water	2.0	0.05	40
Triclosan ²	ethanol	1.5	0.25	6
Benzalkonium chloride	water	0.2	0.05	4
Hexachlorophene	ethanol	2.5	1.0	2.5
<i>Acids:</i>				
Benzoic acid	ethanol	30.0	7.5	4
Lauric acid	ethanol	4.0	1.0	4
Linolenic acid	ethanol	20.0	5.0	4
Oleic acid	ethanol	30.0	5.0	6

¹ Triton X-100 (Rohm & Haas)

² Irgasan DP 300 (Ciba-Geigy)

* The scarification index (S.I.) represents the ratio of threshold concentration on normal skin and threshold concentration on scarified skin.

Exposure technique

Eight criss-cross scratches are made with a 30-gauge, one-half inch needle (four scratches in one direction and another four at right angles). We tried various mechanical devices for controlling the depth of the scratches but none were as dependable as a deft technician. The bevel of the needle faces sideways (neither up nor down) and is drawn across the skin at a 45° angle with just enough pressure to cleave the epidermis without drawing blood (Fig. 1).

The test material is applied once daily for 3 days with readings made at 72 h, 30

min after removal. The reactions were graded on a five-point scale from 0 to 4 (Fig. 1). With inert materials, one can barely make out the scratch marks after 3 days.

Results*1. Irritant responses on normal versus scarified skin*

One can quickly perceive the enhancement of sensitivity by comparing the threshold irritating concentrations on normal and scarified skin, a ratio designated as the scarification index (S.I.) (see Table 1). For ex-

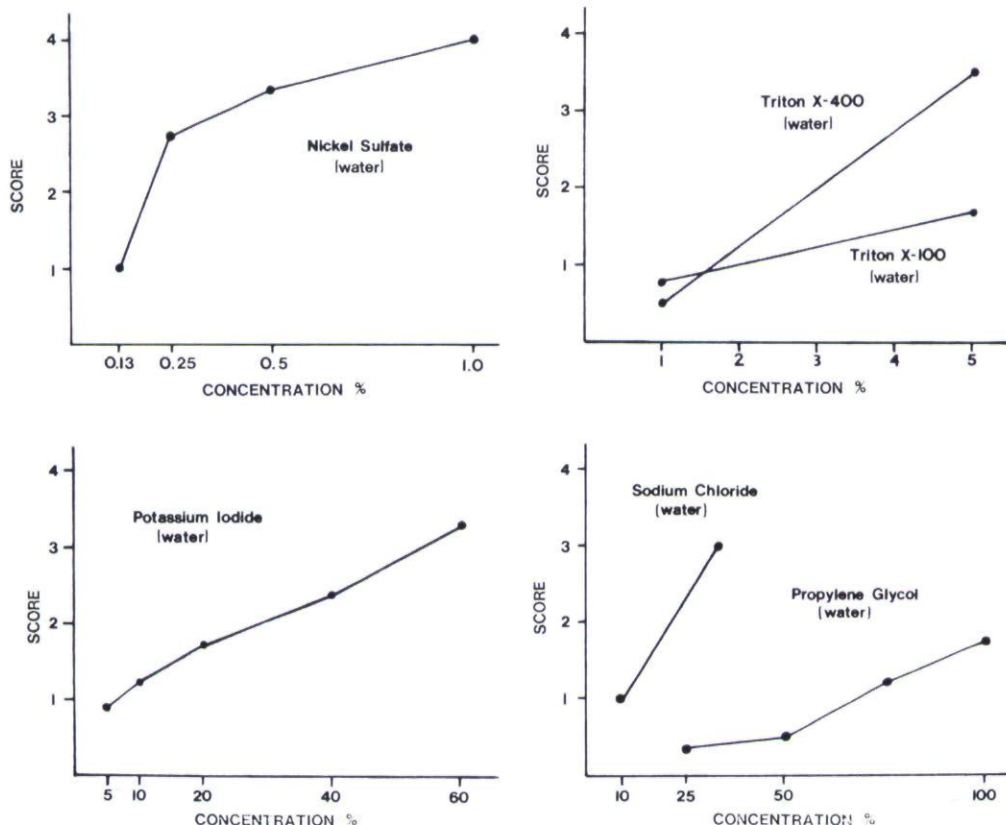


Fig. 2. Dosage response curves for different substances; the slopes vary greatly but are more or less linear. The steeper slope of Triton X-400 indicates that this substance is more irritating than Triton X-100, although these appear equivalent at 1%.

ample, it takes more than a hundred-fold increase in concentration of nickel sulfate to produce just an irritant reaction in 3 days on normal skin and a 40-fold increase for formalin. For comparatively toxic lipid-soluble materials which penetrate readily the S.I.'s may be quite low, as with hexachlorophene (S.I. = 2.5).

The S.I. is only a ratio of threshold concentrations and by itself is not a useful measure of cutaneous toxicity. Two substances that genuinely differ in irritancy could possess the same S.I., for example formalin and Triton X-100® (Table 1). Needless to say, formalin is far more toxic in absolute terms.

Ranking substances by their S.I.'s is therefore not appropriate.

On the other hand, if the S.I. of a substance is found to be very high, this suggests poor penetration through normal skin but substantial toxicity once the agent reaches viable tissue as a result of damage to the barrier. The S.I. is therefore of value in characterizing certain qualities of a substance.

2. Dosage response

As expected, the intensity of the reaction is dose-dependent. Fig. 2 illustrates that the slope of the dose-response curves may be very different for different substances. For example, the plot is very steep for nickel sulfate. It is a marginal irritant at about 0.1 % but a ferocious one at 1.0 %. Potas-

Table 2. Irritancy ratings of topical materials

Agent	Concentration (%)	Vehicle	Classification			
			Low (0-0.4)	Slight (0.5 1.4)	Moderate (1.5 2.4)	Marked (2.5 4.0)
<i>Antiperspirants:</i>						
Aluminum chloride	2.5	water		*		
(AlCl ₃ .6H ₂ O)	5.0	water				(P++)
Aluminum chlorhydroxide	50.0	water	*			
Aluminum bromhydroxide	30.0	water	*			
Zirconium hydroxychloride	15.0	water			*	
<i>Acids:</i>						
Stearic acid	25.0	ethanol	*			
Palmitic acid	25.0	ethanol		*		
Linoleic acid	25.0	ethanol			*	
Myristic acid	25.0	ethanol			*	
Benzoic acid	7.5	ethanol			*	
	15.0	ethanol				(Er++)
Lauric acid	1.0	ethanol			*	
	6.0	ethanol				(P+++)
Linoleic acid	5.0	ethanol		*		
	12.5	ethanol				(Er++)
Oleic acid	5.0	ethanol			*	
	25.0	ethanol				*
<i>Antimicrobials:</i>						
Boric acid	5.0	water-ethanol (1:1)	*			

Table 2. (cont.)

Agent	Concentration (%)	Vehicle	Classification			
			Low (0-0.4)	Slight (0.5 1.4)	Moderate (1.5 2.4)	Marked (2.5 4.0)
Chlorhexidine	0.5	water	*			
Hexachlorophene	1.0	ethanol		*		
	5.0	ethanol				(Er++)
Triclosan ¹	0.25	ethanol		*		
	1.0	ethanol				(Er++)
Quaternium - 1 ²	0.1	water			*	
	0.25	water				(P+++)
Benzalkonium chloride	0.05	water		*		
	0.1	water				(P+++)
Formalin	0.05	water		*		
	0.5	water				(P+++)
Urea	7.5	water		*		
	30.0	water				*
Basic fuchsin	1.0	water	*			
Neomycin sulfate	2.0	water	*			
Glutaraldehyde	2.0	water				*
Gentian violet	1.0	water			*	
<i>Solvents:</i>						
Diethylene glycol	neat		*			
Isopropyl myristate	neat		*			
Isopropyl palmitate	neat			*		
Oleyl alcohol	25.0	mineral oil	*			
Hexadecyl alcohol	25.0	mineral oil		*		
Tetradecyl alcohol	25.0	mineral oil			*	
Dodecyl alcohol	25.0	mineral oil				*
Decyl alcohol	25.0	mineral oil				*
Propylene glycol diacetate	neat			*		
Propylene glycol	neat				*	
Propylene carbonate	neat				*	
<i>Vegetable oils:</i>						
Safflower oil			*			
Peanut oil			*			
Sesame oil			*			
Olive oil			*			
Linseed oil					*	
Corn oil				*		
<i>Bland ointments and creams:</i>						
Lanolin (hydrous) USP			*			
Petrolatum USP			*			
Carbowax ointment USP			*			
Cold cream USP			*			
Vanishing cream USP			*			
Keri lotion (Westwood)			*			
Lubriderm (Texas Pharmacal)			*			

Table 2. (cont.)

Agent	Concentration (%)	Vehicle	Classification			
			Low (0-0.4)	Slight (0.5 1.4)	Moderate (1.5 2.4)	Marked (2.5 4.0)
Noxzema (Noxell)					*	
Aquacare cream (Allergan)			*			
Aquacare HP - 10 % urea (Allergan)				*		
Nivea cream (Beiersdorf)				*		
Vanishing cream (Pond's)					*	
Purpose cream (Johnson & Johnson)					*	
Dermassage cream (Colgate-Palmolive)					*	
Zinc oxide ointment USP			*			
<i>Powders:</i>						
Talcum powder USP			*			
Kaolin NF			*			
Corn starch USP			*			
Zinc oxide powder USP			*			
Magnesium trisilicate powder USP			*			
Titanium dioxide powder			*			
Sodium bicarbonate USP						
- saturated solution	10	water		*		
- powder	10	talc		*		
- powder	neat					*
<i>Sunscreens:</i>						
Amyl-dimethyl p-amino benzoic acid	5	ethanol	*			
Octyl-dimethyl p-amino benzoic acid	5	ethanol	*			
Glyceryl p-amino benzoic acid	5	ethanol		*		
<i>Surfactants:</i>						
Polysorbate - 20 ³	20	water		*		
Polysorbate - 80 ⁴	5	water	*			
Polysorbate - 85 ⁵	5	water		*		
Amphoteric ⁶	20	water			*	
Isostearamidopropyl morpholine lactate ⁷	2.5	water		*		
	5.0	water			*	
	25.0	water				*
Stearamidopropyl dimethylamine lactate ⁸	0.5	water			*	
	2.5	water				*
	5.0	water				(P++)
Triethanolamine	5.0	ethanol		*		
	10.0	ethanol				(P++)
Stearalkonium chloride ⁹	1.0	water		*		
	5.0	water				*

Table 2. (cont.)

Agent	Concentration (%)	Vehicle	Classification			
			Low (0-0.4)	Slight (0.5 1.4)	Moderate (1.5 2.4)	Marked (2.5 4.0)
Octoxynol - 9 ¹⁰	1.0	water		*		
	5.0	water			*	
Nonoxynol - 9 ¹¹	5.0	water		*		
	10.0	water			*	
Laureth - 4 ¹²	2.0	water		*		
	10.0	water				*
<i>Miscellaneous:</i>						
Crude coal tar	5.0	vanishing cream U.S.P.			*	
Vitamin A acid cream ¹³	0.1				*	

Key: P = Postules, Er = Erosions.

¹ Irgasan DP 300 (Ciba-Geigy)

² Hyamine 3500 (Rohm & Haas)

³ Tween 20 (ICI America)

⁴ Tween 80 (ICI America)

⁵ Tween 85 (ICI America)

⁶ Miranol C2M CONC (Miranol)

⁷ Richamate ISML (Richardson)

⁸ Richamate 3780 (Richardson)

⁹ Triton X-400 (Rohm & Haas)

¹⁰ Triton X-100 (Rohm & Haas)

¹¹ Tergitol TP 9 (Union Carbide)

¹² Brij 30 (ICI America)

¹³ Retin-A cream (Johnson & Johnson)

sium iodide and propylene glycol on the other hand exhibit shallow curves.

For comparative assessments we emphasize the importance of testing both a threshold and supra-threshold dose. Triton X-100 and Triton X-400® are examples of substances whose irritating thresholds are very similar. Yet Triton X-400 is a much more toxic substance, as becomes evident when the two are compared at 5 % concentrations.

3. Scarification scores for common topicals

Table 2 summarizes the results for a variety of familiar substances. We designated irritancy potential as low when the mean scores for five subjects fell between 0-0.4, slight between 0.5-1.4, moderate between 1.5-2.4 and marked between 2.5 and 4.0.

That the scarification test has adequate specificity and is not prone to false positive reactions is shown by the low scores attained by such notably bland substances as petrolatum, carbowax ointment U.S.P., zinc

oxide ointment, lanolin, U.S.P. cold cream, etc. Interestingly, a few proprietary "moisturizing" creams had a moderate irritancy potential.

Not all U.S.P. creams lived up to their "bland" reputation. Hydrophilic ointment, as Bergstresser & Eaglestein (1973) had shown earlier, was moderately irritating. This can be attributed to the presence of 1 % sodium lauryl sulfate as the emulsifier. Another result which deserves attention was obtained with 1 % gentian violet which was strongly irritating. This dye has been long and lavishly used as an antimicrobial treatment for pyoderms and burns. It is a testimony to the horny layer barrier that this toxic substance has not caused more harm than it has. In damages, as on scarified skin, it is highly toxic, while on normal skin saturated solutions have no effect owing to lack of penetration. Björnberg & Mo-backen (1972) observed necrotic skin reactions with therapeutic applications of 1 % gentian violet. The incidence of adverse

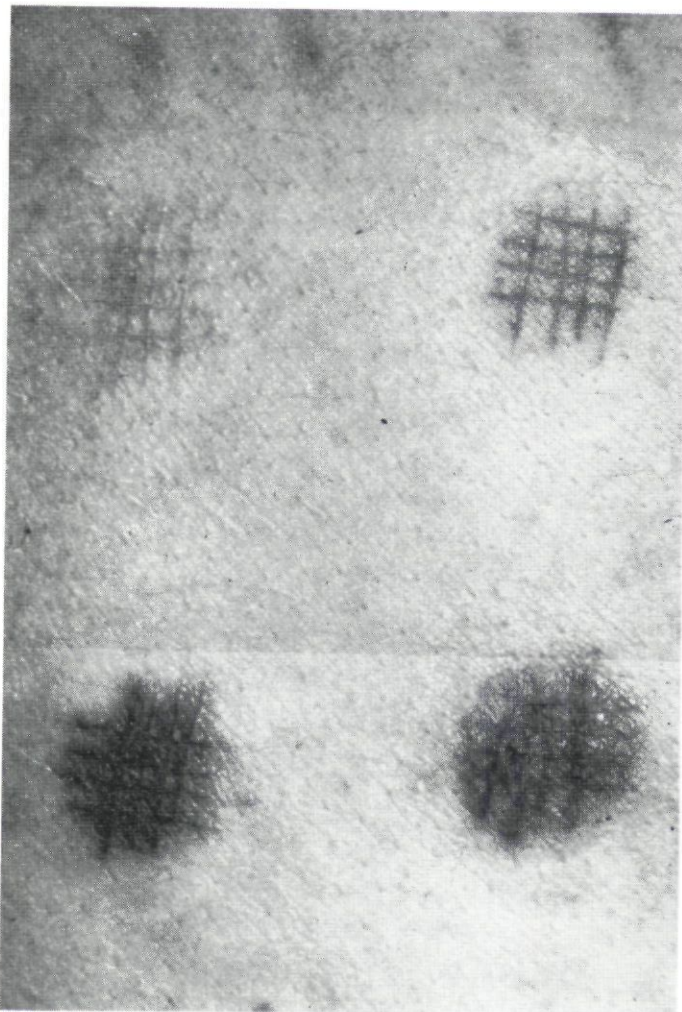


Fig. 3. Reactions to nickel sulfate in a series of two-fold increases in concentration (0.06, 0.13, 0.25, 0.5). The intensity of the response is sharply dependent on dose.

reactions is doubtless very much greater than physicians generally believe.

Even powders can be tested by the scarification technique. While talc, starch and zinc oxide were inert, sodium bicarbonate was not. While hypertonicity is a factor as with other salts, 38 % sodium chloride being quite irritating, a physical factor is also playing a role. Greater reactions were obtained with larger crystals of sodium bicarbonate.

Propylene glycol at concentrations above

50 % is a moderate irritant as others have previously recognized (Warshaw & Hermann 1952). Propylene glycol has peculiar effects which we are now studying. For one thing, the threshold concentrations are the same on scarified and normal skin. Certain persons seem to react excessively, even to concentrations well below 50 %. Propylene glycol may be an interesting substance to identify unusual integuments.

We desired to know whether hyper-reactors to propylene glycol would have ir-

Table 3. Comparison in individuals, respectively hypo- and hyper-reactive, to propylene glycol (readings at 48 hours)

Agent	Hyper-reactors		Hypo-reactors	
	Scarified	Normal skin	Scarified	Normal skin
Propylene glycol (neat)	1.2	1.2	0.2	0.2
Vanishing cream (Pond's)	1.4	1.2	0.3	0.2
0.1 % Vit. A acid cream (J & J)	2.2	0.6	1.4	0
5 % Decyl alcohol (mineral oil)	2.2	1.0	1.6	0
5 % Triton X-100 (water)	2.0	0	1.2	0
30 % Urea (water)	2.4	0	0.8	0
5 % Aluminum chloride (water)	3.2	0	2.4	0
10 % Potassium iodide (water)	2.2	0	1.0	0

ritable skin in general. Table 3 compares hypo- and hyper-reactors using a variety of irritants. As can be seen, hypo-reactors do indeed have less irritable skin. This cannot be attributed to differences in the horny layer barrier since the tests were carried out on scarified skin.

Discussion

Like all bioassay methods, interpretation of the results requires circumspection. Estimating the actual hazard will depend on many variables: frequency of use (including misuse), body region, season, climate, etc. The scores refer only to *potential* irritancy. One does not automatically discard irritating substances or products if these possess other highly desirable features. On the other hand, one can feel very confident that toxic reactions will not occur with appreciable frequency with materials that cause no reaction on scarified skin. A negative response is therefore all the more meaningful.

To date, the scarification technique has been used to test hundreds of substances on many hundreds of volunteers. No untoward effects have been noted. Scarring has never been encountered in routine testing. Se-

condary infection (*S. aureus*) is a rare complication which responds readily to topical antibiotics.

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