

Temperature dependent primary irritant dermatitis from lemon perfume

HANS W. ROTHENBORG¹, TORKIL MENNÉ¹ AND KNUD-ERIK SJØLIN²

¹ Department of Dermato-Venerology, Gentofte Hospital, Hellerup, Denmark

² Institute of Pathology, Sundby Hospital, Copenhagen, Denmark

In a recent outbreak of hand eczema amongst cleaning personnel after the introduction of a new, lemon-scented detergent, it was noted that the patients complained of a burning, stinging sensation when their hands were submerged in hot detergent solutions. Since routine patch testing with the Standard Series and perfume components was of no help in pinpointing the responsible agent, testing with selected perfume components was repeated at higher temperatures. Identical tests were placed on both forearms for 20 min, one arm being exposed to 43°C, the other to 23-25°C. Little or no reaction was seen on the "cold" arm, whereas the lemon perfume component Citral proved to be a strong primary irritant at higher temperatures.

Histological examination of the test sites showed the reaction to be of a toxic (primary irritant) nature. Surprisingly, the toxic character could still be recognized in biopsies taken as late as 48 h after exposure.

It is suggested that:

1. Heat plays an important part in the outbreak of primary irritant (toxic) dermatitis and can be used as an accelerating factor when testing for primary irritants.
2. It is important to be sure that detergents and detergent perfumes do not contain substances which act as irritants at the temperatures at which they are habitually used (45-50°C).
3. We probably ought to use lukewarm rather than hot water for manual dishwashing and cleaning whenever it is possible.

Key words: Citral - "housewives' eczema" - lemon perfume - primary irritant dermatitis - temperature dependent - toxic contact dermatitis.

Received for publication October 4, 1976

Shortly after the introduction of a lemon-scented detergent in two 900-bed hospitals in Copenhagen County, a cluster of eczemas occurred amongst the cleaning personnel. The detergent in question was suspected right from the start, but the responsible agent proved difficult to pin down. Initially, it was thought that we were dealing with an allergic contact dermatitis, but as the preliminary investigations proceeded, we came to realize that we were dealing with a

hitherto unrecognized primary irritant dermatitis mechanism.

The local manufacturer provided samples of the whole perfume as well as of the unscented detergent. The main components of the latter were sodium lauryl sulphate and sodium dodecyl benzene sulphonate both anionic detergents. Primarily, patch tests were made with the whole perfume (2 % in petrolatum), the scented and unscented detergent (1 % and 10 % in water) as well

as the Scandinavian Standard Patch Test Series (Wilkinson et al. 1970) on all exposed patients referred. Readings at 48 h gave no clear indication of relevant sensitivity; in fact, 18 out of 20 patients (18/20) were completely negative.

When testing for perfume allergies, tests with whole perfume or with solutions of scented soaps or detergents often give false positive or negative reactions (Rothenborg & Hjorth 1968). The reason is, that when using whole perfume for testing the relevant allergen may appear in too low a concentration to evoke a reaction with the present patch test technique. If patch tests are made with solutions of scented soaps or detergents, the irritant properties of the soap or detergent components may result in false positive tests. In consequence, it is necessary, as far as possible, to get the perfume components for testing.

Accordingly, samples of the perfume broken down into six fractions were obtained from the perfume manufacturer and further skin testing was undertaken with 5 % and 10 % solutions of these fractions in alcohol. Again, no relevant sensitivity pattern was found when the test were read at 48 h, but it became apparent that a number of the patients had an immediate type wheal and flare reaction to some of the ingredients of the perfume.

During this initial phase of the examinations new patients, who had been exposed to lemon-scented detergent, continued to come to the clinic. Quite frequently they volunteered the information that they had experienced burning and stinging sensations when putting their hands in hot water containing the detergent under suspicion. None of the patients had used gloves while cleaning.

Only at this point did we realize the significance of what seems to be the accepted cleaning and washing-up procedure: In most cases people use water as hot as their hands

can possibly tolerate for dish washing and cleaning. There seems to be a firm belief, which to our knowledge is unfounded in experimental fact, that the hotter the water, the better the cleaning. This belief is probably based on the experience that many greasy materials cannot be removed by cold water with or without detergent.

Combining the information spontaneously supplied by patients of burning sensations in the hands submerged into hot detergent/water solutions, with the finding of a number of immediate type, urticaria-like reactions to some of the perfume components, we came to the conclusion that the perfume tests needed to be repeated at a higher temperature than is normally done.

Material and Methods

From the preliminary test battery of perfume components and a few chemically pure ingredients known to occur in the perfume, those fractions were selected which had given rise to any reaction at all during the preliminary tests. To this list were added the scented detergent (20 % in water) and the non-scented (also 20 % in water) in all 12 substances to be tested (Table 1). The test substances were transferred to Finn chamber discs (Pirilä 1975) by means of capillary tubes. No filter paper was used for fear that the filter paper might absorb or withhold some of the active ingredients (Fregert et al. 1968). The tests were applied to the flexor side of the forearms on 12 patients (Fig. 1) symmetrically on both arms. In order not to spill the test substances, the plaster with the discs were applied on the patients with the open side of the discs and the adhesive side of the tape turning upwards, while the patients held their forearms horizontally, palm down and elbow bent (Fig. 1). Tests were placed on both forearms simultaneously. The arms were then placed horizontally on plastic containers filled with water at 23–27°C and

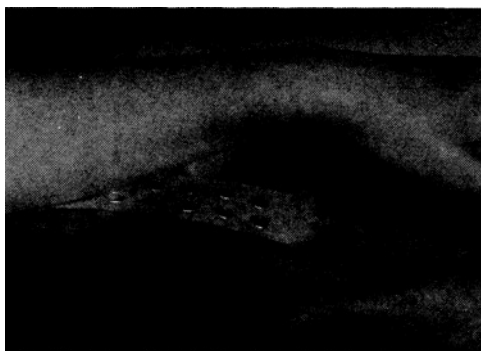


Fig. 1. Application of Finn chambers on palmar aspect of forearm. The cup-like Finn chambers are placed, face upwards to avoid loss of liquid test material.



Fig. 3. Strapping down of arm to avoid accidental movement during test period.

43°C respectively (Fig. 2). The arms were strapped reasonably tight to the plastic containers to prevent accidental movement during the 20-min exposure time (Fig. 3). Thermocouples were placed between skin and plastic container to monitor the temperatures during the experimental period. Temperatures were read on a Withof Trans-

ocomp® 288 potentiometric recorder, temperature range 0–115°C, paper speed 240 mm/h. Ambient temperature was held at $21^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

After 20 min the tapes with the aluminum chambers containing the test material were removed, and the test sites were examined immediately through a looking glass with two-fold magnification. As Coenraads (Coenraads et al. 1975), but unlike Bjørnberg (1968), the tests were read as positive or negative without any attempt at grading. The tests were read again at 3 and 24 h whenever possible, and in all cases at 48 or 72 h. Patients with positive reactions were photographed in a standardized setup at the time of observation.

To get an impression of the time relation of the histological changes the patients were divided into four groups, which were biopsied respectively 20 min, 3, 24, and 48 h after exposure, but only one biopsy from each patient.

Results

Of the ingredients tested only Citral caused a significant number of manifest reactions (Table 1), and this only when the test sites



Fig. 2. Test subject with forearms placed on waterfilled plastic container, right arm on container 43°C, left arm on container 23–27°C. Lower left corner temperature monitor.

Table 1. Immediate and late reactions to detergent components

		20 min	24 h	48 h
1. Anthone 10 % in alc. (citrufix):	28°C	0/12	0/12	0/12
	43°C	0/12	0/12	0/12
2. Terpenes de limette 5 % in alc.:	28°C	0/12	0/12	0/12
	43°C	1/12	1/12	0/12
3. Terpenes de citron 5 % in alc.:	28°C	0/12	0/12	0/12
	43°C	1/12	4/12	1/12
4. Acetate P.A. 10 % in alc. (citrufix):	28°C	0/12	0/12	0/12
	43°C	0/12	0/12	0/12
5. Terpenes d'oranges 5 % in alc.:	28°C	0/12	0/12	0/12
	43°C	1/12	3/12	0/12
6. Rumehal 10 % in alc. (citrufix):	28°C	3/12	0/12	0/12
	43°C	3/12	1/12	0/12
7. Citral A 10 % in alc.:	28°C	6/12	1/12	0/12
	43°C	9/12	9/12	8/12
8. Citral N 10 % in alc.:	28°C	7/12	1/12	0/12
	43°C	9/12	9/12	9/12
9. Citral zur synthese (Merck) 10 % in alc.:	28°C	6/12	1/12	0/12
	43°C	9/12	8/12	10/12
10. Detergent with perfume 20 % in water:	28°C	1/12	0/12	0/12
	43°C	2/12	0/12	0/12
11. Detergent without perfume 20 % in water:	28°C	1/12	1/12	0/12
	43°C	1/12	0/12	0/12
12. Essences et fractionnes Citral:	28°C	0/12	0/12	0/12
	43°C	0/12	0/12	0/12

3/12 means 3 patients out of 12 tested, etc.

were heated to 43°C during the test period. At 23°C testing with pure Citral (items no. 7, 8, and 9) gave in all 19 slightly positive reactions at 20 min; at 24 h these positive reactions were reduced to 3, and at the reading after 48 h all signs of reaction had

disappeared without trace (Fig. 4 A–D, left arm).

At 43°C, however, the macroscopic reactions produced by Citral were already quite pronounced at 20 min with redness, swelling, and a desiccated surface in 9/12 of the

Fig. 4 A. Patient no. 10 photographed 30 min after exposure to perfume components, right arm at 43°C, left arm at 23°C. Note that at this initial point there is a slight redness also on the unheated left arm.

Fig. 4 B. Patient no. 10 1 h after exposure. Note that tests on unheated left arm have almost disappeared, whereas they have increased on the heated arm.

Fig. 4 C+D. Patient no. 10 respectively 24 and 48 h after exposure. Reactions on the unheated, left arm have disappeared, but are firmly established on the right heated arm.

Fig. 4 E. Patient no. 10 8 days after exposure. There is still a trace of the reactions to perfume components on the heated arm, whereas the skin on the unheated left arm is normal. The inflamed spot on the ulnar side of the right arm is due to the biopsy taken 6 days before.

Fig. 4 F. Patient no. 2 7 days after exposure to perfume components. The three strongly positive reactions on the right heated arm are from Citral. There are no traces on the left arm, which has been exposed simultaneously to the same perfume components, but at normal temperature during testing.

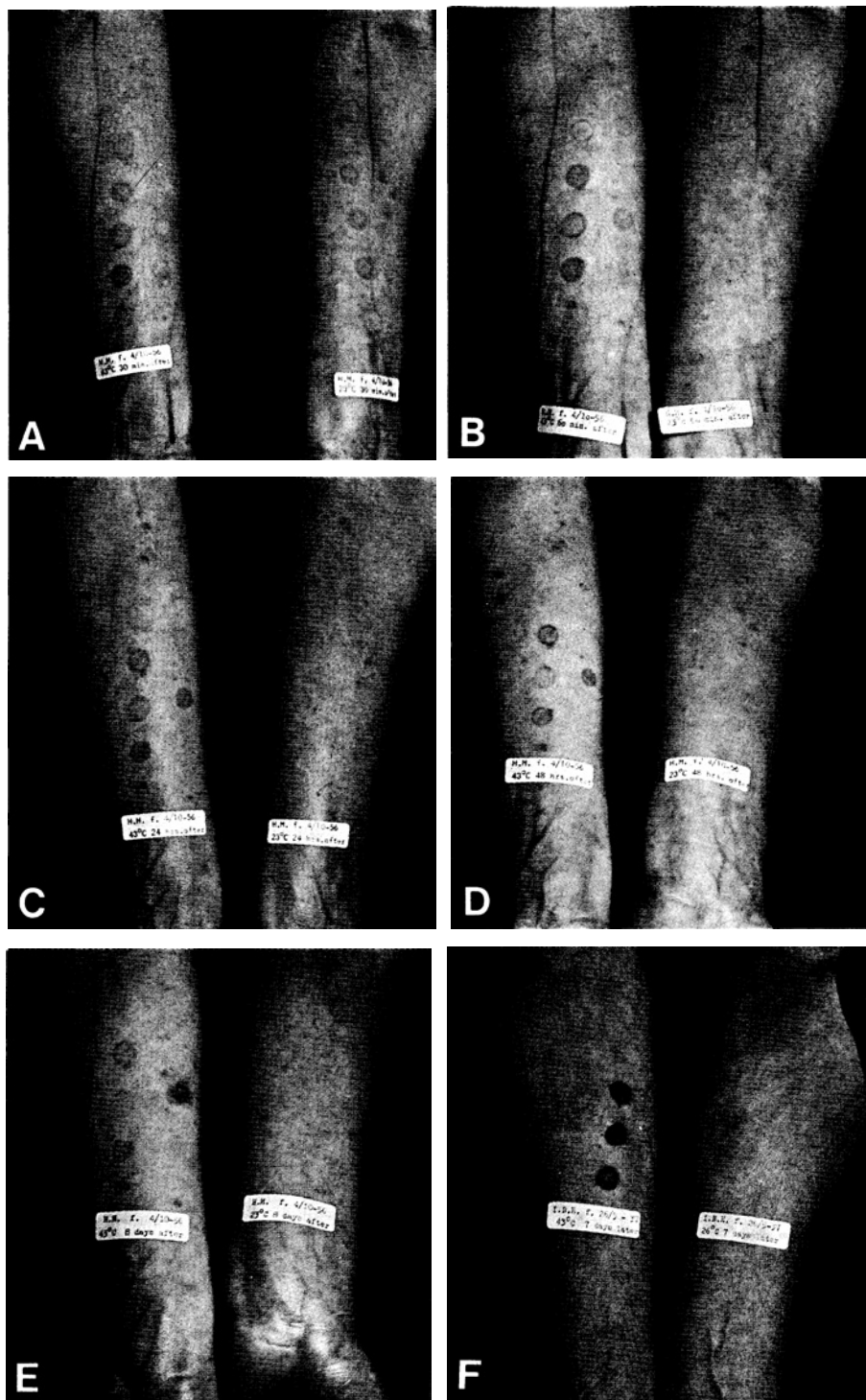


Table 2. Histological findings in skin biopsies from positive test sites of 11 patients exposed to perfume components at 43°C

Patient No.	Time of biopsy*	Age years	Epidermis			Cellular infiltration	Bullae	Dilated capillaries	Dermis		Diagnosis
			Keratosis	Necrobiosis	Spongiosis				Edema	Leucocytes	
1.	10 min	57	normal	+	+	—	—	+	+	+	Acute dermatitis, toxic
2.	10 min	38	normal	+	+	+	subepidermal cleft	+	+	+	Acute dermatitis, toxic
3.	2½ h	65	normal	(+)	—	—	—	+	+	+	Acute dermatitis, toxic, very sl.
4.	3 h	52	normal	(+)	(+)	(+)	—	+	(+)	+	Acute dermatitis, toxic, slight
5.	3 h	21	normal	(+)	(+)	—	—	—	+	—	Acute dermatitis, toxic, slight
6.	24 h	32	normal	+	+	+	—	+	+	+	Acute dermatitis, toxic, severe
7.	24 h	18	normal	+	+	+	—	+	+	+	Acute dermatitis, toxic
8.	24 h	19	normal	+	+	+	—	+	+	+	Acute dermatitis, toxic
9.	43 h	39	normal	+	+	+	—	+	+	+	Acute dermatitis, toxic, severe
10.	48 h	19	normal	+	+	+	—	+	+	+	Acute dermatitis, toxic, severe
11.	48 h	44	normal	+	+	+	—	+	+	+	Acute dermatitis, toxic, severe

* Time elapsed from removal of test substances to biopsy.

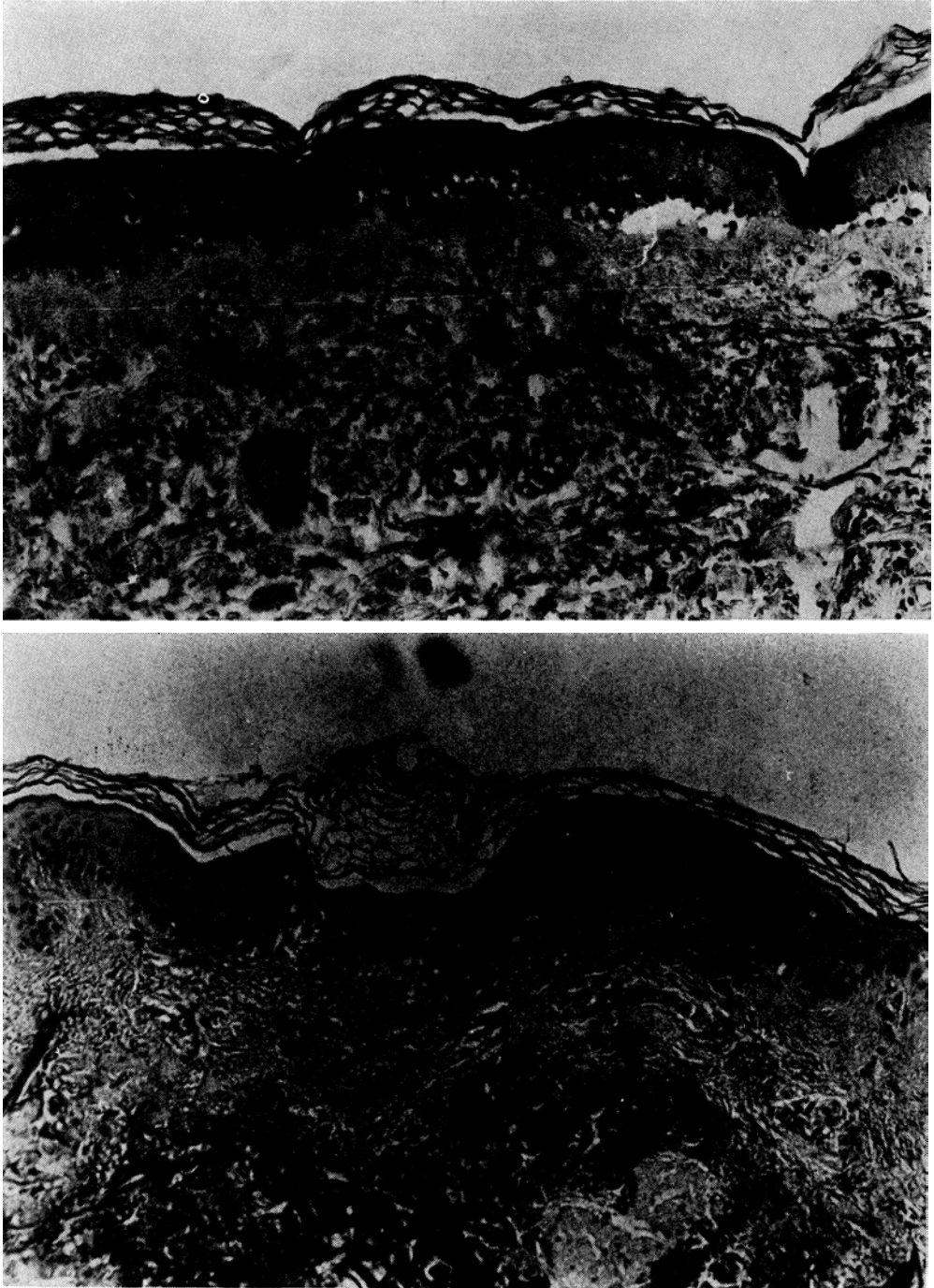


Fig. 5 A. (Above) Patient no. 2. Biopsy from heated test site with Citral. The biopsy taken a few minutes after the removal of the Finn chambers. For details, see Table 2 and text. ($\times 100$).

Fig. 5 B. (Below) Patient no. 5. Biopsy from heated test site with Citral. The biopsy taken 3 h after the removal of the Finn chambers. For details, see Table 2 and text. ($\times 100$).

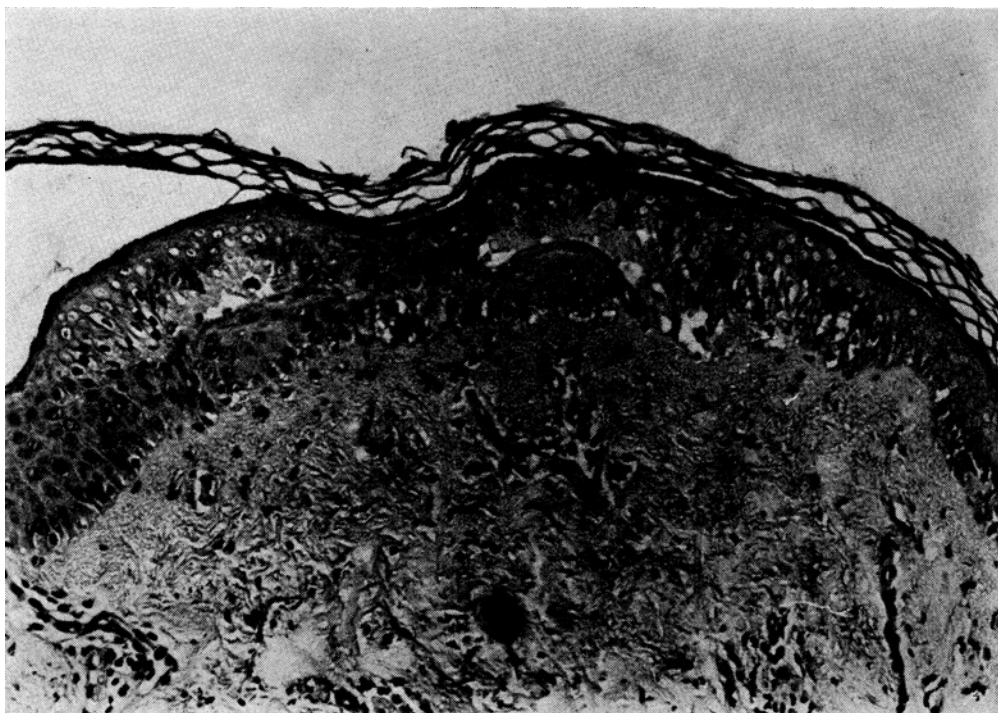
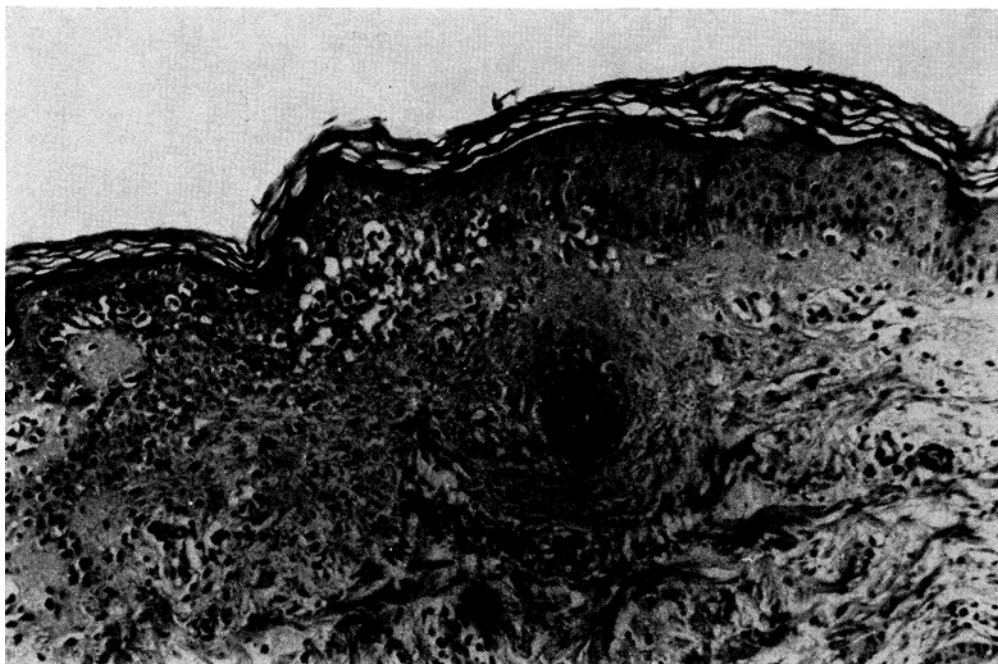


Fig. 5 C. (Above) Patient no. 8. Biopsy from heated test site with Citral. The biopsy taken 24 h after the removal of the Finn chambers. For details, see Table 2 and text. ($\times 100$).

Fig. 5 D. (Below) Patient no. 11. Biopsy from heated test with Citral. The biopsy taken 48 h after the removal of the Finn chambers. For details, see Table 2 and text. ($\times 100$).

cases tested; at 48 h 8/12, 9/12, and 10/12 respectively were red and infiltrated with well-pronounced crusts (Fig. 4 A-D, right arm). Moreover, the reactions produced by Citral at 43°C were longlasting, and in many cases could still be seen after 7 to 10 days (Fig. 4 E-F). However, they healed without scarring, implicating rather superficial skin damage, a supposition confirmed by the histological examinations.

The natural product Terpenes de citron, 5 % in alcohol, which contains some Citral, also caused some slight reaction after 24 h when applied at 43°C in 4/12 of the cases, and in 1/12 of the cases after 48 h, indicating that irritation could still be produced with the natural product, although in fewer cases and less pronounced.

Histology

The results of the histological examinations are summarized in Table 2. The biopsies have been divided into four groups: the first (patients nos. 1 and 2) where the biopsies were taken immediately after removal of the Finn chambers, and the second, third, and fourth groups where the biopsies were taken respectively 2½-3 h, 24 h, and 43-48 h after application of the Finn chambers.

Fig. 5 A-D depicts examples of the histological changes found in each of the mentioned groups.

Fig. 5 A is from patient no. 2 (biopsy no. 10298/75). To the right the epidermis is pale and the structure blurred. The nuclei can be recognized as shadows. Below the epidermis there is a spongiotic split, but no acantholysis. The corneal layer is preserved, but a little disintegrated. Towards the left the changes are gradually decreasing, but still with spongiosis and a few neutrocytes and lymphocytes at the base, and necrobiosis at the top of epidermis. The papillary layer of the epidermis shows slight edema, a few neutrocytes, and lymphocytes around the vessels. The other pa-

tient in this group had even slighter histological changes.

Fig. 5 B is from patient no. 5 (biopsy no. 11952/75). To the left the superficial part of epidermis is pale. The corneal layer is a little disintegrated. Focal areas of spongiosis, neutrocytes, and lymphocytes are seen. The papillary layer of the dermis shows some edema, but only a few leucocytes around the capillaries.

Fig. 5 C is from patient no. 8 (biopsy no. 9341/75). The superficial part of the epidermis is pale with a blurred structure. The corneal layer is a little disintegrated. The basal part of epidermis shows severe spongiosis and some infiltration with neutrocytes and lymphocytes. The papillary and superficial parts of the dermis are edematous with some perivascular neutrocytic and lymphocytic infiltration.

Fig. 5 D is from patient no. 11 (biopsy no. 12732/75). Extensive necrobiosis of the epidermis is found. The corneal layer is a little disintegrated. There is spongiosis and infiltration with a neutrocytes and lymphocytes. The papillary dermis is edematous mainly with perivascular infiltration of neutrocytes and lymphocytes.

Discussion

The histological examination of the skin changes in this material leaves no doubt that we are dealing with a toxic dermatitis (Bandmann 1970, Lever 1967, Miescher 1961, Pinkus & Mehregan 1969). The damage can be seen within 20 min after exposure in the superficial part of the epidermis, later spreading to the deeper parts of the epidermis where spongiosis and immigration of neutrocytes and lymphocytes are seen. The changes in the dermis are limited to a vascular reaction. It is remarkable that the lesion could still be recognized as toxic 48 h after the exposure to Citral.

It is surprising that the smallest histological changes are found in the second group of patients ($2\frac{1}{2}$ –3 h), but this could be a statistical phenomenon. Such a phenomenon could be explained either by variability in the test procedure or in an individual variation in the resistance to irritants. Apparently there is no relationship between age and degree of tissue reaction (Table 2).

The mechanism behind the increased irritancy at higher temperatures of Citral and of the lemon perfume as such in the detergent solution is obscure. The following mechanisms have been considered:

1. Increased penetration of toxic substances
2. A simple increase in chemical reaction involving the skin and following the usual laws of chemistry (which according to Arrhenius' equation means a two-fold increase in speed of chemical reactions for every temperature increase of 10°C)
3. Increased irritancy of the anionic detergent components due to the content of Citral
4. Contact urticaria.

Anionic detergents are usually considered more hazardous to the skin than non-ionic or cationic (Schwartz et al. 1957). Some evidence exists of increased percutaneous absorption at higher temperatures (Arita et al. 1970, Blank et al. 1967, Stoughton 1969). All show that percutaneous penetration is increased as the temperature rises, but only Blank et al. have worked at temperatures which approach the water temperature normally used for cleaning.

Although little is known about the chemical interactions between the skin and chemicals or topical medicaments, it seems fairly evident that such a reaction must follow simple physical and chemical laws. It is hard to explain the observed well-pronounced reaction to Citral persisting for more than a week without it being some form of direct chemical damage. Such damage is

most likely due to an increase in both penetration and local chemical reactions at the higher temperature.

The lemon perfume components have a slightly acid reaction with a pH of 4–5 in aqueous solution, whereas the anionic detergents are weakly alkaline. According to Barrett (1969) and Higuchi (1960) weakly acid and alkaline compounds show maximum penetration if buffered to weakly acid respectively alkaline pH. Thus a weakly alkaline compound will penetrate better if buffered to an alkaline pH than if buffered to an acid pH. If the weakly acid lemon perfume has any influence on the anionic detergent, it ought therefore to render the detergent less toxic to the skin. However, neither the perfume nor the detergent have any pronounced buffering capacity in the concentrations normally used for cleaning. Furthermore, with the test procedure described, no lasting positive reactions have been evoked with pure detergent/water solutions irrespective of concentration or temperature. It would therefore seem, that whatever effect has been caused by the lemon perfume, it must be a direct one – an irritation caused by the lemon perfume as such, not through an influence on the detergent.

Suspicion of eczema due to contact urticaria (Hjorth & Roed-Petersen 1976, Maibach & Johnson, 1975) was aroused by the clinical symptoms described by the patients, i.e. burning and stinging of hands submerged in hot detergent solution as well as by the wheal and flare reactions initially observed on the test sites on some of the patients during the early stages of the investigation. The longlasting reactions produced in many of the test subjects, as well as the result of the histological examinations (Fig. 5 A–D), however, seem to repudiate the idea of contact urticaria in our patients. Wheal and flare was observed only at the time when tests were made with the whole

perfume applied at body temperature, not during testing with perfume fractions at higher temperatures. However, burning and stinging at those heated test sites, which later turned out to be positive, was a frequent finding.

It is only fair to emphasize that we have found no case of sensitization to lemon perfumes, and no ill effects of their application at normal body temperature. The lemon perfumes, therefore, should be quite in order for soaps, deodorants and other products used at normal body temperature. But we believe that they have no place in detergents.

Conclusion

The investigation has clearly shown Citral, a constituent of lemon perfume, to be a strong irritant if applied in connection with heat. Thus the investigation underscores two concepts new to contact dermatology:

1. Temperature is a factor to be taken into consideration in the development of primary irritant hand eczemas (*dermatitis contactus toxica manuum*).
2. Detergent perfumes should not contain potentially irritant substances, substances which, although harmless at normal body temperature, become irritant at higher temperatures.

Probably we ought to advise our patients to use cold or lukewarm water rather than hot whenever possible for manual dish washing, cleaning, and laundry. However, housewives and cleaning personnel love hot water, water as hot as their hands can possibly tolerate, and old habits are difficult to change. Therefore, it is most unfortunate to use perfumes with potentially irritant substances as detergent scents. Lemon perfume is a point in question. It undoubtedly contains substances which become irritant

at higher temperatures (45–50°C), probably even in low concentrations. If we must have perfumed detergents the perfumes must be free of irritants.

Acknowledgments

The authors wish to express their gratitude to Verner Faurby, MSc. and Niels Jakob Nielsen, MSc., both of the Central Pharmacy, Gentofte Hospital, 2900 Hellerup, Denmark for their assistance with pH measurements of detergent and lemon perfume components and solutions, and to Glaxo Pharmaceuticals, Ltd., Vermlandsgade 67, 2300 Copenhagen S, Denmark for their assistance in the production of the color prints in this article.

References

- Arita, T., Hori, R., Anmo, T., Washitake, M., Akatsu, M. & Yajima, T. (1970) Studies on percutaneous absorption of drugs. *Chemical and Pharmaceutical Bulletin* 18, 1045–1049.
- Bandmann, H.-J. (1970) Das histopathologische bild der kontaktallergischen und alkalitoxischen ekzem. *Therapeutische Umschau* 27, 488.
- Barrett, C. W. (1969) Skin penetration. *Journal of the Society of Cosmetic Chemistry* 20, 487–499.
- Björnberg, A. (1968) *Skin Reactions to Primary Irritants in Patients with Hand Eczema*, pp. 76–82. Gothenburg: Oscar Isacson Tryckeri AB.
- Blank, I. H. (1964) Penetration of low-molecular-weight alcohols into skin. *Journal of Investigative Dermatology* 43, 415–420.
- Blank, I. H., Scheuplin, R. J. & Macfarlane, D. J. (1967) Mechanism of percutaneous absorption. *Journal of Investigative Dermatology* 49, 582–589.
- Coenraads, P. J., Bleumink, E. & Nater, J. P. (1975) Susceptibility to primary irritants. *Contact Dermatitis* 1, 377–381.
- Fregert, S., Hjorth, N. & Schulz, K.-H. (1968) Patch testing with synthetic primin in persons sensitive to primula obconica. *Archives of Dermatology* 98, 144–147.
- Higuchi, T. (1960) Physical chemical analysis

- of percutaneous absorption process from creams and ointments. *Journal of the Society of Cosmetic Chemistry* 11, 85-97.
- Hjorth, N. & Roed-Petersen, J. (1976) Occupational protein contact dermatitis in food handlers. *Contact Dermatitis* 2, 28-42.
- Lever, W. F. (1967) *Histopathology of the Skin*, 4th ed., pp. 102-103. Philadelphia, Toronto: J. B. Lippincott Company.
- Maibach, H. J. & Johnson, H. L. (1975) Contact urticaria syndrome. *Archives of Dermatology* 111, 726-730.
- Miescher, G. (1961) Abgrenzung des allergischen und toxischen geschehens in morphologischer und funktioneller sicht. *Archiv für klinische und experimentelle Dermatologie* 213, 297.
- Pinkus, H. & Mehregan, A. H. (1969) *A Guide to Dermatohistopathology*, pp. 95-100. New York: Appleton-Century-Crofts, Meredith Corporation.
- Pirilä, V. (1975) Chamber test versus patch test for epicutaneous testing. *Contact Dermatitis* 1, 48-52.
- Rothenborg, H. W. & Hjorth, N. (1968) Allergy to perfumes from toilet soaps and detergents in patients with dermatitis. *Archives of Dermatology* 97, 417-421.
- Schwartz, L., Tulipan, L. & Birmingham, D. J. (1957) *Occupational Diseases of the Skin*, p. 422. Philadelphia: Lea & Febiger.
- Stoughton, R. B. (1969) Pharmacology of the skin. In *Advances in Biology of the Skin*, ed. Montagna, W., Stoughton, R. B. & Scott, E. J., pp. 537-539. New York: Appleton-Century-Crofts, Meredith Corporation.
- Wilkinson, D. S., Bandmann, H.-J., Calnan, C. D., Cronin, E., Fregert, S., Hjorth, N., Magnusson, B., Maibach, H. I., Malten, K. E., Meneghini, C. L. & Pirilä, V. (1970) The role of contact allergy in hand eczema. *Transactions of the St. John's Hospital Dermatological Society* 56, 19-25.

Address:

H. W. Rothenborg, M.D.
 Department of Dermatology
 Gentofte Hospital
 Niels Andersenvej 65
 2900 Hellerup
 Denmark