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# NON-IMMUNOLOGIC CONTACT URTICARIA

by

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*To my family*

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## ABSTRACT

Non-immunologic contact urticaria (NICU) was studied in man using the open and chamber skin test methods and peroral challenge tests. The open test method was more sensitive than the chamber test, but the latter was more suitable for testing many substances at the same time because only a small skin area is needed.

The substances tested were benzoic acid (BA), sodium benzoate (SB), sorbic acid (SA), cinnamic acid (CA), cinnamic chloride, acetic acid, sodium acetate, ethyl alcohol, butyric acid, butyl alcohol, lactic acid, sodium lactate, citric acid, sodium sitrate, methyl-, ethyl-, and propyl-para-oxy-benzoates, tartrazin, salicylic acid, acetosalicylic acid, perfume mixture, balsam of Peru, polymyxin B sulfate, and propylene glycol. Immediate skin reactions were seen to BA, SB, SA, CA, balsam of Peru, acetic acid, ethyl alcohol, butyric acid, and butyl alcohol.

NICU was most frequently caused by BA 5.0 % in petrolatum in 88 %, CA 5.0 % in 85 % and SA 2.5 % in 58 % of the 103 persons tested.

The lowest concentrations of BA eliciting wheal and flare reactions in the chamber test were 0.050 % in water and 0.10 % in petrolatum, and those of SA 0.050 % in water, 0.10 % in W/O emulsion, 0.25 % in petrolatum, and 0.50 % in O/W emulsion. Neither stripping nor scratching the skin enhanced the reaction. Atopics were no more liable to get NICU than non-atopics.

Most of the skin reactions in the open test to BA 5.0 %, SA 2.5 %, and CA 5.0 % in petrolatum appeared within 45 min and disappeared within two hours. The optimum for recording the results was 40 to 45 min after the application of the test substance both in the open test and in the chamber test with 20 minutes' occlusion. The back and the dorsal sides of the upper and lower arm were most sensitive and suitable for testing substances producing NICU.

The administration of antihistamine (hydroxyzine) perorally or emptying the histamine storage of the skin mast cells with compound 48/80 did not prevent the contact urticarial reaction to BA. However, repeated applications of BA abolished the whealing totally in most cases. The findings suggested that the reaction was mediated by vasoactive substances other than histamine.

In peroral challenge tests with BA, SA, CA, and SB, objective symptoms were seen in 15 % and subjective symptoms in 33 % of the 106 patients tested. Most, if not all, of the reactions were obviously non-specific and comparable to placebo reactions. No correlation was seen between the reactivity in the skin test and that in the peroral test.

Recognizing NICU from common additives in foods and drugs is important, as they are used in large amounts worldwide. NICU from them seems to be more common than previously believed. However, the exact mechanism of the wheal and flare reaction remains obscure, indicating the necessity of further studies.

*Key words:* Contact urticaria; Food and drug additives; Skin testing

## ABBREVIATIONS

| | |
|---|---|
| ACD: | allergic contact dermatitis |
| AD: | atopic dermatitis |
| AR: | allergic rhinitis |
| BA: | benzoic acid |
| CA: | cinnamic acid |
| DMSO: | dimethyl sulfoxide |
| ICU: | immunologic contact urticaria |
| NICU: | non-immunologic contact urticaria |
| SA: | sorbic acid |
| SB: | sodium benzoate |

CONTENTS

## INTRODUCTION

The term contact urticaria is given to a wheal and flare reaction which appears when certain agents make external contact with the intact skin, usually within a few minutes to half an hour. The strength of a reaction varies from local redness and/or oedema to generalized urticaria and anaphylactic symptoms, asthma and shock.

Contact urticarial reactions are divided into two main types on the basis of possible aetiological mechanisms: firstly, non-immunologic contact urticaria (NICU), in which urticariagenic agents produce the reaction without any previous sensitization in most or almost all exposed persons; and secondly, immunologic (immediate type hypersensitivity) contact urticaria (ICU), in which the reaction appears on previously sensitized skin. In addition, there is a third group, in which the mechanism is uncertain, and in which features of non-immunologic and immunologic mechanisms can often be found.

NICU is well known e.g. from contact with nettle (*Urtica*), medusae and sea anemones, but some chemicals used in the food and drug industries can also elicit contact urticaria of this type. Preservatives are important food and drug additives which are used in large quantities throughout the world. Some reports have been published on contact urticaria caused by certain preservatives. In this study, these substances were investigated more extensively, with main emphasis being placed on test methods, the natural course of the reactions, the mechanisms underlying the urticarial response of the skin, and the correlation between the results of a peroral challenge test and a skin test.

## REVIEW OF THE LITERATURE

Contact urticaria has been a recognized phenomenon for quite some time. Urticarial lesions produced by nettle and certain hairy caterpillars were reported back in the 19th century (60). In the first few decades of the current century, pollens (59), silk (87), jelly fish (117), wool (63), and cat hair (48) were added to the list.

Foodstuffs aroused the interest of investigators especially in the thirties and forties. Water melon (122), orange, grape fruit, wheat flour, carrot, fish (43), and lemon peel (118) were found to elicit contact urticaria.

The mechanisms of contact urticarial response were also the subject of speculation. Hopkins & Kesten (48) divided contact urticaria into two types: toxic, which was produced by substances with the pharmacologic property of eliciting wheals in normal skin, and allergic, in which the wheals were produced by an allergic reaction occurring only in the skin of sensitized persons.

### TYPES OF CONTACT URTICARIA

The most common of the three types used for classification today exhibits a *non-immunologic* mechanism (82). Agents eliciting contact urticaria of this type do so in most or almost all exposed persons. The passive transfer test is negative. The exact mechanism is not known but it is suggested to be a direct release of histamine, a slow reacting substance of anaphylaxis (SRS-A), bradykinin, or some other vasoactive substance (82). This mechanism has been investigated using e.g. antihistamines (7, 28, 35, 57, 67, 78, 107, 111), compound 48/80 (7, 28, 82, 107), topical corticosteroid (78), and local analgesic (57, 67).

An *immunologic* urticarial reaction is evoked upon external contact with previously sensitized skin. In this type of contact urticaria, the passive transfer test is often positive. A broad spectrum of symptoms is seen, ranging from localized urticaria to generalized urticaria with anaphylactic symptoms.

Neither an immunologic mechanism nor a direct releasing action of vasoactive substances can be clearly shown for contact urticaria of *uncertain* mechanism (67, 82).

### AGENTS PRODUCING NON-IMMUNOLOGIC CONTACT URTICARIA

*Dimethyl sulfoxide* (DMSO) is a local analgesic, an anti-inflammatory agent, and a good solvent (22). It also enhances the percutaneous absorption of certain chemicals.

Stoughton & Fritsch (110) reported that DMSO 20 % caused transient erythema at the site of application in most subjects. Real contact urticarial lesions are frequently caused by DMSO at higher concentrations (57, 82). As the response may occur upon first contact, the mechanism is thought to be non-immunologic in nature.

*Tetrahydrofurfuryl ester of nicotinic acid* (Trafuril®, Ciba-Geigy, Basel, Switzerland) has been used as a rubefacient in inflammatory joint diseases (103) and in the management of mild disorders of the blood circulation of

the hands and feet (33). It has also been used in the treatment of alopecia areata (72), acrocyanosis and ulcus cruris (109).

Vaillancourt (119) tested 72 apparently healthy subjects with Trafuril® and found a local hyperaemic reaction with or without oedema on the application site within five to 10 min in 69 subjects. This was considered to be a normal reaction. Patients suffering from atopic dermatitis, acute rheumatic fever, and rheumatoid arthritis had a blanching reaction instead of urticaria and erythema (78).

*Cobalt chloride* is often used as a colour indicator in experimental sweat tests. It produces allergic contact dermatitis, tuberculin-like reactions, and urticaria when injected intradermally (22).

Smith et al. (107) reported contact urticaria from cobalt chloride, and suggested that histamine or other vasoactive substances are at least partially responsible for the reaction.

Strictly speaking, contact urticaria from *arthropods, nettle,* and *marine life* does not meet the criteria of contact urticaria, because urticariagenic agents are stung into the skin. However, these agents are usually discussed under the heading of contact urticaria (22, 82).

The list of such agents consists of venomous, bristly spines or hairs of certain caterpillars and moths (44, 124), nettle, and many species of tropical, salt-water marine life. Usually these species producing contact urticaria are animals, such as jellyfish, sea anemones, and corals, but plants can also elicit a reaction of this kind. In Hawaii, Grauer & Arnold (34) reported 125 cases of sea weed dermatitis from a blue green alga, *Lyngbya majuscula* Gomont.

A »Portuguese man-of-war» (*Physalia physalis* (L.)) is a colony of floating hydroid coelenterates with nematocysts or stinging capsules characteristic of all species within the phylum *Coelenterata*. Contact with it may produce severe urticarial reactions when toxins are released from nematocysts in the fishing tentacles (22, 52).

*Benzoic acid* (BA) occurs in balsam of Peru and balsam of Tolu, in many essential oils from flowers and spices, and in berries (cranberries, cowberries, bilberries) (45, 53). It has antibacterial and antifungal properties and is commonly used as a preservative in acidic food products. Whitfield's ointment, containing BA 6 % as an antifungal agent and salicylic acid 3 %, can be used in the treatment of fungous infections of the skin (69).

Hjorth & Trolle-Lassen (47) studied sensitizing properties of preservatives of creams and noticed that BA often caused erythematous skin reactions. Maibach & Johnson (67) mentioned BA as a possible cause of contact urticaria, and Forsbeck & Skog (28) reported contact urticarial reactions from BA 5 % in three patients with immediate skin reactions to balsam of Peru. Temesvári et al. (112) reported a patient with an immediate contact reaction to both balsam of Peru and BA.

*Sorbic acid* (SA), like BA, is a natural preservative occurring e.g. in berries of the mountain ash (*Sorbus*) (74). It is active against moulds and yeasts and to a lesser degree against bacteria. Below pH 6.5 (optimal pH 4.5) it is used as a preservative for many pharmaceutical products and foods at a concentration up to 0.2 % (69).

Contact urticaria from SA is thought to be rare, and only a few reports can be found in the literature. Fryklöf (31) noticed that creams and ointments containing SA caused erythema and slight itching and sometimes slight oedema on the face in about half of the 20 persons tested. Hjorth & Trolle-Lassen (47) confirmed the results of Fryklöf and reported erythema reactions in 18 out of 26 persons tested. Rietschel (89) saw a female patient with contact urticaria from shampoo containing SA.

*Cinnamic aldehyde* is a constituent of cinnamon and one of the substances responsible for the typical odour and flavour of this spice. Its oxidation occurs readily on exposure to air, yielding cinnamic acid. Cinnamic aldehyde is used as a flavouring agent in soft drinks, chewing gum, ice cream, baked goods, dentifrices, mouthwashes, soaps, etc. (12).

Contact urticaria from cinnamic aldehyde has been reported by some authors. Nater et al. (80) found erythema and oedema in three patients and erythema alone in three other patients from cinnamic aldehyde 10 % in alcohol. Forsbeck & Skog (28) found localized urticaria from cinnamic aldehyde 2 % in petrolatum in four out of five patients with immediate skin reactions to balsam of Peru. Rudzki & Grzywa (99) noted immediate reactions to both balsam of Peru and cinnamic aldehyde in two patients.

Cinnamic aldehyde is also a potent sensitizer, and delayed type allergic contact dermatitis caused by this substance has been described by many authors (6, 18, 45, 64, 83, 104).

*Cinnamic acid* (CA) has been found among the constituents of the essential oils of basil, Chinese cinnamon, styrax, oil of cinnamon, coca leaves, and balsam of Peru (74, 84). It is used as a flavouring ingredient in pharmaceutical preparations, in food products, and in perfumery (12, 84). CA has antibacterial and antifungal properties similar to those of BA (69). Contact urticaria from CA 5 % in petrolatum was found by Forsbeck & Skog (28) in three out of five patients with immediate skin reactions to balsam of Peru.

*Balsam of Peru* originates from a tree (Myroxylon balsamum (L.) Harms var. Peireirae (Royle) Baillon) growing e.g. in El Salvador. Earlier, it was commonly used both in pharmacy and in the cosmetic and flavouring industries (45). It is a well known delayed-type sensitizer, and it also produces contact urticaria (28, 30, 97, 99, 112, 117).

*Localized heat* urticaria is also considered to be non-immunologic in nature (82). In spite of numerous investigations, the pathophysiology of this syndrome has not been delineated. De Moragas et al. (16) implicated the kininogen-kinin system and possibly histamine, and Daman et al. (14) the activation of the alternative complement pathway in the genesis of increased vascular permeability in this rare type of contact urticaria.

## AGENTS PRODUCING IMMUNOLOGIC CONTACT URTICARIA

Several different agents are able to produce ICU. The skin of the patient is previously sensitized, and a local urticarial reaction appears on contact with the same agent. The most sensitive patients may also get generalized anaphylactic symptoms. Selected substances eliciting ICU are listed in Table I.

Table I. *Selected agents eliciting immunologic contact urticaria (numbers in parentheses refer to references)*

| | |
|---|---|
| *Foods* | |
| Potato | (23, 39, 86, 93) |
| Carrot | (22, 39) |
| Spices | (22) |
| Fish, lobster and chicken | (46) |
| Wheat flour, lamb, turkey skin | (65) |
| Apple | (39) |
| Lettuce and endive | (58) |
| Egg | (98, 99) |
| *Textiles* | |
| Perlon | (79) |
| Wool | (22) |
| Silk | (96) |
| *Animals* | |
| Animal hair | (94, 99, 105) |
| Dog and cat saliva | (5, 105) |
| Animal danders | (22) |
| Cow placenta | (102) |
| Cockroaches | (125) |
| *Medicaments* | |
| Estrogenic cream | (11) |
| Tetanus antitoxin | (108) |
| Menthol | (85) |

| | |
|---|---|
| Cetyl and stearyl alcohol | (32) |
| Streptomycin | (62) |
| Neomycin | (71) |
| Gentamycin | (71) |
| Penicillin G | (41, 71) |
| Benzophenone | (88) |
| Mechlorethamine hydrochloride | (15, 36) |
| Aspirin | (22) |
| Chlorpromazine | (22) |
| Cod liver oil | (22) |
| Horse serum | (22) |
| Monoamylamine | (113) |
| Diethyltoluamide | (67) |
| Cephalosporins | (116) |
| Promethazine hydrochloride | (41) |
| Aminophenazone | (8, 41) |
| Polyethylene glycol | (24, 26) |
| Polysorbate 60 | (66) |
| *Industrial exposure* | |
| Platinum salts | (51, 61) |
| Acrylic monomer | (22) |
| Aliphatic polyamide | (22) |
| Aminothiazole | (22) |
| Ammonia | (22) |
| Castor bean | (22) |
| Lindane | (22) |
| Sodium sulfide | (22) |
| Sulphur dioxide | (22) |
| Formaldehyde | (42, 73) |
| Teak | (101) |
| Terpinyl acetate | (73) |
| *Cosmetics* | |
| Hair sprays | (22) |
| Nail polish | (22) |
| Perfumes | (22) |
| *Others* | |
| Seminal fluid | (25, 37) |
| Phenylmercuric propionate | (70) |
| Algae and lichens | (10) |
| Pollens | (95) |
| Rubber | (81) |

Many *proteinaceous substances*, especially in foodstuffs, can cause immediate allergic reactions on contact with the skin. Maibach (65) described an atopic patient who had hand eczema and wheal and flare reactions within 10 to 20 min of the application of turkey skin, ground lamb and wheat flour to the dermatitic skin of the forearm. Hjorth & Roed-Petersen (46) described wheal and flare reactions from e.g. fish and shellfish not only on dermatitic skin but also on normal skin in food handlers. It has been reported that immediate allergy to proteinaceous materials is not always related to the atopic diathesis (46, 82). Schmidt (102) described a non-atopic veterinary surgeon who got contact urticaria on his hands from cow placenta.

Immediate protein contact dermatitis is either a form of contact urticaria or else at least closely related to it. It usually appears as dermatitis, but urticaria and vesiculation are also often seen.

*Essential acquired contact cold urticaria* has also been regarded to be immunologic in nature (82). Lesions usually appear within minutes after contact e.g. with an ice cube. The passive transfer test is positive in about 50 % of reported cases (82). The transferable agent has been suggested to be IgE (50, 55) or IgM (120).

## AGENTS PRODUCING CONTACT URTICARIA BY AN UNCERTAIN MECHANISM

*Ammonium persulfate*, an ingredient previously used in hair bleaches, has been reported to cause contact urticaria and anaphylactic reactions (4, 7, 27). The exact mechanism is unknown. The fact that only a small number of individuals react to it, and the severity of systemic reactions suggest an immunologic mechanism. On the other hand, the negative passive transfer test, and the finding that some persons react on their very first exposure to it speak against an antibody mechanism.

*Solar urticaria* is a relatively rare disease. Various clinical, biophysical, biochemical, and immunological studies have shown that the disease can be classified into several different types. Allergic, unknown, and protoporfyrin mechanisms have been suggested (40, 49).

*Aquagenic urticaria* also belongs to this group; its mechanism has not yet been clarified (9, 106, 115, 123).

## SYMPTOMS AND SIGNS FROM SUBSTANCES CAUSING NON-IMMUNOLOGIC CONTACT URTICARIA IN PERORAL CHALLENGE TESTS

There are only a few reports in the literature on peroral challenge tests with contact urticariagenic agents other than SB. Juhlin et al. (54) reported urticaria, asthma, or pharyngeal oedema from a peroral challenge test with 250 mg or 500 mg of SB. Asthma from peroral SB has also been found by Rosenhall & Zetterström (91) and Freedman (29); urticaria by Michaëlsson & Juhlin (75), Doeglas (17), Thune & Granholt (114), and Ros et al. (90); and aggravated allergic vasculitis with purpura by Michaëlsson et al. (76).

Other symptoms from SB such as headache, palpitations, fatigue, a feeling of tightness in the chest, redness of the skin, itching of the skin, conjunctival injection, increased tear secretion, nasal congestion, cough, hoarseness, and hot flushes have been found in peroral challenge tests by Michaëlsson & Juhlin (75), Rosenhall & Zetterström (91), Doeglas (17), and Thune & Granholt (114).

Rosenhall & Zetterström (92) challenged 100 asthma patients with BA and elicited asthma, rhinitis, or urticaria in 47 of them. Klaschka & Beiersdorff (56) gave SA to three patients with positive delayed reactions to SA in epicutaneous tests; none of them had symptoms in the peroral challenge test. Forsbeck & Skog (28) were not able to elicit any symptoms in peroral challenge with 25 mg of balsam of Peru and 25 mg of CA in five patients with contact urticaria from balsam of Peru.

## AIMS OF THE STUDY

The aims of the present study were:

1. To find out which of the substances earlier known to cause urticarial or NICU reactions and which of the substances chemically related to them are able to elicit NICU most frequently.
2. To compare the open and chamber test methods for the study of contact urticarial reactions.
3. To investigate the natural course of the NICU response to BA, SA, and CA.
4. To find out whether atopic persons are more liable to get reactions from contact urticariagenic substances than non-atopic persons.
5. To study the effect of the following variations in the test procedure on the NICU reaction:
   - — the concentration of the contact urticariagenic agent in various vehicles,
   - — scratching or stripping the skin before testing,
   - — the test site, and
   - — repeating the test on the same skin site.
6. To study mechanisms possibly underlying NICU.
7. To find out if there is any correlation between the reactivity to substances causing NICU in the skin test and that in the peroral test.

## PATIENTS AND METHODS

The test subjects in this study were inpatients at the Department of Dermatology, Helsinki University Central Hospital, and inpatients at the Department of Dermatology, Oulu University Central Hospital, unless stated otherwise. Patients with dermatitis of the test area and those on systemic antihistamine, corticosteroid, or psychopharmaca therapy were excluded from the series. Persons who had asthma, allergic rhinitis (AR) and/or atopic dermatitis (AD) at the moment of investigation, or had suffered from these diseases in the past, are called atopics in this study.

The skin tests were performed using the chamber and open test techniques. Test chambers (Finn Chamber®, Epitest Ltd, Helsinki, Finland) were fixed on the skin with porous tape (Scanpor®, Norgesplaster A/S, Oslo, Norway). The occlusion time was 20 min and the result was recorded after various intervals, as described in different parts of the study. The volume of the test substance in the chamber test was 20 $\mu$l, measured with a disposable plastic syringe.

### THE ABILITY OF SELECTED SUBSTANCES TO CAUSE NON-IMMUNOLOGIC CONTACT URTICARIA

One hundred and ten dermatological patients were chosen for the study. The patients belonged to one of the following four groups: atopics, urticaria, non-atopic dermatitis, and non-allergic comparison series.

*Atopics.* Thirty-six patients: eight women, mean age 24.3 (14—36) yrs, and 28 men, mean age 19.1 (2—35) yrs. Twenty-two patients had AD, 12 AR, and two bronchial asthma.

*Urticaria.* Twenty-three patients: nine women, mean age 32.3 (11—61) yrs, and 14 men, mean age 27.1 (13—62) yrs. All had chronic urticaria of unknown aetiology.

*Non-atopic dermatitis.* Twenty-six patients: 13 women, mean age 43.3 (17—65) yrs, and 13 men, mean age 51.4 (34—69) yrs. Ten patients had allergic contact dermatitis (ACD), six nummular eczema, four primary irritant hand eczema, three infectious eczema, and three chronic hand eczema of unknown aetiology.

*Comparison series.* Twenty-five non-allergic patients: 13 women, mean age 32.8 (8—70) yrs, and 12 men, mean age 41.6 (5—68) yrs. Nineteen patients had psoriasis, three primary syphilis, two infections in paranasal sinuses, and one facial herpes simplex infection.

The test substances were BA 5.0 % (Pharmacopoea Nordica (Editio Fennica)) (Ph. Nord. (Ed. F.)), SB 10 % (Ph. Nord. (Ed. F.)), SA 2.5 % (Tanabe Seiyaku Co. Ltd, Osaka, Japan), methyl-, ethyl-, and propyl-para-oxybenzoates in mixture, 5.0 % of each (Ph. Nord. (Ed. F.)), tartrazin 5.0 % (Chroma Gesellschaft, Stuttgart, Germany), salicylic acid 5.0 % (Ph. Nord. (Ed. F.)), perfume mixture 3.0 % (the perfume of Tabac® after-shave lotion, Firmenich S.A., Geneva, Switzerland), balsam of Peru 25 % (Ph. Nord. (Ed. F.)), polymyxin B sulfate 20 % (European Pharmacopoeia), propylene glycol (Ph. Nord. (Ed. F.)), hydrophilic ointment containing methyl-para-

oxy-benzoate 0.1 % (Novalan®, Orion, Helsinki, Finland), hydrophilic ointment containing SA 0.2 % (Ambilan®, Orion, Helsinki, Finland) (see appendix).

Yellow petrolatum (Ph. Nord. (Ed. F.)) was used as the vehicle for all but three of the test substances; propylene glycol and the two hydrophilic ointments were tested as such.

The test chambers were fixed on the patients' upper back and removed after 20 min. The results were then recorded immediately.

Salicylic acid 5.0 % (Ph. Nord. (Ed. F.)) and acetosalicylic acid 5.0 % (Ph. Nord. (Ed. F.)) in petrolatum were tested in 138 dermatological patients, 75 of whom were women and 63 men. The mean age of the whole group was 27.5 (4—91) yrs. Eighty-four of the patients were atopics and 54 non-atopics. The test was carried out with the chamber method on the skin of the back using 20 minutes' occlusion. The result was recorded 10 min after the tests were removed.

*Statistical methods:* $\chi^2$-test, 2 × 2 contingency table without Yates' correction and Fisher's exact probability test if there are values less than 5 in the 2 × 2 contingency table.

## CHEMICALLY RELATED TEST SUBSTANCES

Forty-nine atopic and 56 non-atopic patients were tested in this part of the study. The atopic patients comprised 35 women, mean age 27.3 (8—59) yrs, and 14 men, mean age 25.8 (16—54) yrs. The non-atopic patients comprised 31 women, mean age 49.7 (15—78) yrs, and 25 men, mean age 48.3 (13—84) yrs.

Thirty of the atopics had AR, seven AD, four urticaria and past AD, three angio-oedema and past AD, one bronchial asthma, two AD and AR, and two bronchial asthma and AR.

Eleven of the non-atopics had infectious eczema, eight ACD, five psoriasis, three solar eczema, three circumscribed neurodermatitis, two venous leg ulcer, two erysipelas, two chronic pharyngitis, and two exanthem of unknown aetiology. The following diseases were recorded in one patient each: tarsal neurinoma, chronic conjunctivitis, primary irritant hand dermatitis, chronic infection in paranasal sinuses, erythema induratum of Bazin, sacral bedsore, dequalon ulcers, palmoplantar pustulosis, necrobiosis lipoidica, herpes zoster, stasis dermatitis, disseminated lupus erythematosus, tinea of the toe nails, dermographism, seborrhoeic dermatitis, erosive balanitis, acne, and vasculitis with purpura.

The test chambers were removed from the upper back after 20 min and the results were recorded immediately. The test substances were BA 5.0 %, SB 10 %, CA 5.0 %, cinnamic chloride 5.0 % (Fluka A.G., Buchs, Switzerland), acetic acid 0.50 % (Ph. Nord. (Ed. F.)), sodium acetate 5.0 % (E. Merck, Darmstadt, Germany), ethyl alcohol 70 % (Alko Oy., Koskenkorva, Finland), butyric acid 2.5 % (E. Merck, Darmstadt, Germany), butyl alcohol (E. Merck, Darmstadt, Germany), lactic acid 2.5 % (Ph. Nord. (Ed. F.)), sodium lactate 10 % (BDH Chemicals Ltd, Poole, England), citric acid 2.5 % (Ph. Nord. (Ed. F.)), and sodium citrate 10 % (Ph. Nord. (Ed. F.)).

Butyl alcohol was tested as such. Petrolatum was the vehicle for BA, SB, CA, and cinnamic chloride, and water for the other substances.

*Statistical methods:* 2 × 2 contingency table and Fisher's exact probability test.

## OPEN TEST VERSUS CLOSED TEST

Atopic and non-atopic subjects were tested in this part of the study. The 51 atopic patients comprised 33 women, mean age 38.8 (14—66) yrs, and 18 men, mean age 29.3 (14—69) yrs. The 55 non-atopic patients comprised

30 women, mean age 47.3 (23—64) yrs, and 25 men, mean age 39.6 (16—59) yrs.

Thirty-six of the atopics had AD, three AR, and 12 some other skin disorders in addition to past AD or AR. I.e., six had chronic urticaria of unknown aetiology, two psoriasis, one angio-oedema, one exanthem of unknown aetiology, one ACD, and one chronic hand eczema of unknown aetiology.

Twelve of the non-atopics had infectious eczema, five psoriasis, four ACD, three primary irritant hand eczema, three palmoplantar pustulosis, three acne, two a fixed eruption, two itching of the skin of unknown aetiology, two intrinsic rhinitis, two chronic urticaria of unknown aetiology, and two venous leg ulcer. The following diseases were diagnosed in one patient each: angio-oedema, dermographism, lichen ruber planus, exanthem of unknown aetiology, nummular eczema, perioral dermatitis, echtyma of the leg, hereditary palmoplantar hyperkeratosis, diabetic ulcer of the leg, folliculitis of the scalp, anal pruritus, chronic cheilitis, chronic hand eczema of unknown aetiology, tinea of the foot, and facial erysipelas (already healed).

The chamber and open tests were performed on the upper back. The chamber tests were removed after 20 min and the results were read 10 min later. Open tests were carried out by spreading about 0.1 ml of the test substance on a 3 × 3 cm area and the results were read 30 min after the application. The test substances were BA 5.0 %, SA 2.5 %, and CA 5.0 % (E. Merck, Darmstadt, Germany).

*Statistical methods:* 2 × 2 contingency table.

## THE NATURAL COURSE OF OPEN TEST REACTIONS

Test subjects in this part of the study were apparently healthy physicians and nurses and their relatives. Of the 103 persons tested, 29 currently or previously had atopic symptoms; 16 of them were women, mean age 33.1 (24—58) yrs, and 13 men, mean age 31.0 (12—48) yrs. The remaining 74 persons were non-atopics: 48 women, mean age 32.6 (11—61) yrs, and 26 men, mean age 31.4 (16—46) yrs.

About 0.1 ml of the test substances was applied on 3 × 3 cm areas on the volar side of the forearm. The results were recorded every 15 min for six hours. The test substances were BA 5.0 %, SA 2.5 %, and CA 5.0 % in petrolatum.

*Statistical methods:* 2 × 2 contingency table, Fisher's exact probability test and Student's t-test of two means after log transformation.

## THE EFFECTS OF VARIOUS TEST PROCEDURES, VEHICLES, AND PRETREATMENTS ON CONTACT URTICARIAL REACTIONS

### Vehicle and concentration of the test substance

Only patients with an oedema and redness reaction to BA 5.0 % or to SA 2.5 % in the chamber test were chosen for this part of the study. The chamber test method was used on the upper back with an occlusion time of 20 min. The result was read 10 min after the tests were removed.

*Benzoic acid*

These series consisted of 16 atopic and 16 non-atopic patients. The atopics comprised nine women, mean age 31.1 (8—52) yrs, and seven men, mean age 28.4 (9—50) yrs. Six atopics had AD, six AR, three chronic urticaria with past AD, and one bronchial asthma.

The non-atopic group comprised eight women, mean age 44.4 (27—51) yrs, and eight men, mean age 44.8 (34—55) yrs. Three sub-

jects had infectious eczema, three intrinsic rhinitis, and two palmoplantar hyperkeratosis. The other patients each had one of the following diseases: chronic urticaria of unknown aetiology, palmoplantar pustulosis, primary irritant hand eczema, chronic cheilitis, perioral dermatitis, folliculitis of the scalp, exanthem of unknown aetiology, and pruritus of the skin of unknown aetiology.

The test substances were BA 5.0 %, 2.5 %, 1.0 %, 0.50 %, 0.25 %, 0.10 %, 0.050 %, 0.025 %, and 0.010 % in petrolatum; and BA 0.25 %, 0.10 %, 0.050 %, 0.025 %, and 0.010 % in water.

*Sorbic acid*

The subjects in these tests were 13 atopics and 13 non-atopics. The atopic patients consisted of six women, mean age 30.5 (18—44) yrs, and seven men, mean age 28.4 (9—50) yrs. Five of the atopics had AD, five AR, and three chronic urticaria and past AD.

The non-atopic group comprised six women, mean age 43.5 (27—48) yrs, and seven men, mean age 44.7 (34—55) yrs. Three subjects had infectious eczema, three intrinsic rhinitis. The other patients each had one of the following diseases: palmoplantar hyperkeratosis, primary irritant hand eczema, punctate palmar hyperkeratosis, chronic cheilitis, folliculitis of the scalp, exanthem of unknown aetiology, and pruritus of the skin of unknown aetiology.

The test substances were SA 2.5 %, 1.0 %, 0.50 %, 0.25 %, 0.10 %, 0.050 %, 0.025 %, and 0.010 % in petrolatum; SA 0.10 %, 0.050 %, 0.025 %, and 0.010 % in water; SA 2.5 %, 1.0 %, 0.50 %, 0.25 %, 0.10 %, 0.050 %, 0.025 %, and 0.010 % in a lipophilic ointment containing methyl-para-oxy-benzoate 0.07 % and propyl-para-oxy-benzoate 0.03 % as preservative agents (Hydran®, Orion, Helsinki, Finland); and SA 2.5 %, 1.0 %, 0.50 %, 0.25 %, 0.10 %, 0.050 %, 0.025 %, and 0.010 % in a hydrophilic ointment (Novalan®, Orion, Helsinki, Finland) (see appendix).

*Statistical methods:* 2 × 2 contingency table and Fisher's exact probability test.

**Scratching the skin**

Subjects with an oedema and redness reaction to BA 5.0 % in the chamber test were chosen for this test. The 11 atopics comprised seven women, mean age 32.6 (8—52) yrs, and four men, mean age 21.5 (9—48) yrs. The 11 non-atopics comprised five women, mean age 41.6 (27—47) yrs, and six men, mean age 46.5 (40—55) yrs.

Six atopic patients had AR, three AD, one bronchial asthma, and one chronic urticaria of unknown aetiology and past AD. Three non-atopics had infectious hand eczema, three intrinsic rhinitis. Chronic urticaria of unknown aetiology, pruritus of the skin of unknown aetiology, punctate palmar hyperkeratosis, palmoplantar pustulosis, and folliculitis of the scalp were each recorded in one case.

The chamber and scratch-chamber tests were used parallelly on the upper back. In the scratch-chamber test, a 5 mm long scratch was made with a blood lancet in the epidermis, and the chamber was fixed on the scratch. The occlusion time was 20 min, and the result was recorded 10 min later.

The test substances were BA 5.0 %, 2.5 %, 1.0 %, 0.50 %, 0.25 %, 0.10 %, 0.050 %, 0.025 %, and 0.010 % in petrolatum.

*Statistical methods:* Fisher's exact probability test.

**Stripping the skin**

Seven patients were chosen for this part of the study: four women, mean age 37.0 (22—53) yrs, and three men, mean age 42.3 (17—65) yrs, with an oedema and redness reaction to BA 5.0 % in the chamber test. Psoriasis, acne, and AD were diagnosed in two cases, and venous leg ulcer in one case.

The chamber test with 20 minutes' occlusion was used on both the stripped and the control areas. The results were read 40 min after the application. Stripping was performed 20 to 24 hours before the test. A 20 cm long cellophane tape strip (Tesa® tape, Kauppakumppanit Oy., Turku, Finland) was pressed on the left scapular area 8 to 10 cm lateral to the spinal processes and was taken off after about 15 seconds. The stripping was repeated 10 times. The right scapular area was the control area.

The test substances were BA 5.0 %, 2.5 %, 1.0 %, 0.50 %, 0.25 %, 0.10 %, 0.050 %, 0.025 %, and 0.010 % in petrolatum.

*Statistical methods:* Fisher's exact probability test.

**Test site**

The test subjects were six women, mean age 39.5 (22—56) yrs, and six men, mean age 34.7 (12—65) yrs. Three subjects had acne, three AD, three psoriasis, two venous leg ulcer, and one seborrhoeic dermatitis.

About 10 ml of BA 5.0 % in petrolatum was applied to the left half of the body with the exception of the head, neck, buttocks, and genitals. The result was recorded 40 min later.

*Statistical methods:* Fisher's exact probability test.

**Peroral antihistamine**

Five patients with an oedema and redness reaction to BA 5.0 % in the chamber test on the upper back took part in this investigation. Their mean age was 47.2 (22—65) yrs. Two patients had psoriasis, one acne, one AD, and one venous leg ulcer.

On the first day, the tests with serial dilutions of BA were carried out between 8.00 and 9.00 a.m. with the chamber method on the left scapular area using 20 minutes' occlusion and reading the results 20 min after the tests were removed. A histamine scratch with 10 mg/ml (Dome Laboratories, Division of Miles laboratories Ltd, Slough, England) was also made on the left scapular area. The result of the histamine scratch was recorded after 15 min by measuring the longest diameter of the wheal and the longest diameter perpendicular to it. At 8.00 p.m. the patients were given a 25 mg hydroxyzine chloride tablet (Atarax®, UCB S.A. Pharmaceutical Division, Brussels, Belgium).

On the following day between 8.00 and 9.00 a.m. the patients were tested again in the same way as on the first day on the right scapular area. The concentrations of BA were 5.0 %, 2.5 %, 1.0 %, 0.50 %, 0.25 %, 0.10 %, 0.050 %, 0.025 %, and 0.010 % in petrolatum.

*Statistical methods:* Fisher's exact probability test and t-test of paired observations.

**Compound 48/80, betamethasone dipropionate, and lidocaine**

The prerequisite for the selection of patients was an oedema and redness reaction in the chamber test to BA 5.0 % on the proximal radial edge on the volar side of the left forearm. The occlusion time was 20 min and the result was read 20 min after the tests were removed.

Eight women, mean age 36.6 (22—53) yrs, and 12 men, mean age 38.0 (21—64) yrs, took part in the study. Three patients had AD, three acne, two psoriasis, and two AR. The following diseases were diagnosed in one patient each: infectious hand eczema, nummular eczema, genital pruritus of unknown aetiology, condyloma acuminatum, circumscribed neurodermatitis, exanthem of unknown aetiology, rosacea, folliculitis, venous leg ulcer, and chronic urticaria of unknown aetiology.

In order to examine the influence of a

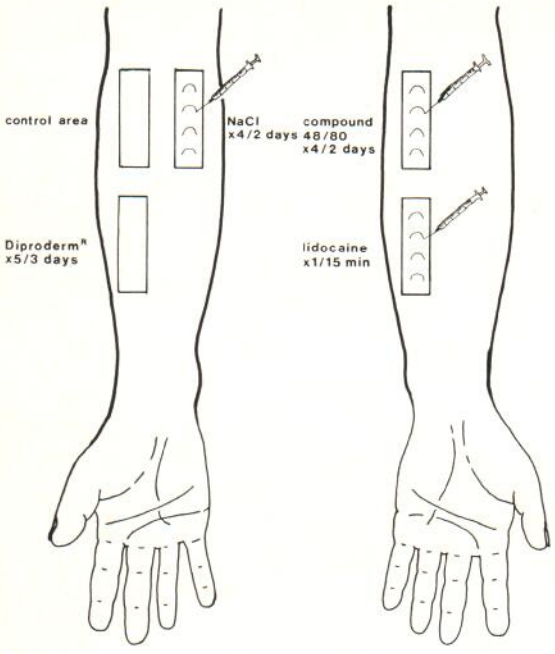


*Fig. 1.* Pretreating the skin of the forearms with compound 48/80, lidocaine, and betamethasone dipropionate cream (Diproderm®) prior to the tests with benzoic acid.

histamine releaser, compound 48/80 (a condensation product of N-methyl-homoanisylamineformaldehyde), betamethasone dipropionate, and lidocaine on the contact urticarial reaction to BA, the test areas were pretreated with the substances as follows:

*Compound 48/80* (Sigma® Chemical Company, St. Louis, U.S.A.) was dissolved at 0.1 mg/ml in NaCl 0.9 %. The solution was sterilized with a Millipore® filter (Type GS 0.22 μm, Millipore S.A., Molsheim, France) and the sterility was checked with a bacterial culture. Four 0.1 ml doses of compound 48/80 solution were injected intradermally in a row at 2 cm intervals on the proximal ulnar side of the left volar forearm four times at the same sites on two subsequent days, namely between 1.00 and 2.00 p.m. and between 9.00 and 10.00 p.m. on the first day, and between 8.00 and 8.30 a.m. and between 10.00 and 10.30 a.m. on the second day. Correspondingly, NaCl 0.9 % was injected into the right forearm as a control solution (Fig. 1). The test was performed 20 to 30 min after the last injection.

Another test procedure was used in order to empty the histamine storage of skin mast cells. A dose of 0.1 ml of the NaCl 0.9 % solution containing compound 48/80 1.0 mg/ml was injected twice with a 24-hour interval intradermally into the dorsal sides of the forearms of three women and three men (mean age 53.8, range 41—67 yrs) with an oedema and redness reaction to benzoic acid 5.0 % in petrolatum in the open test. Correspondingly, NaCl 0.9 % was injected into the other forearm as a control solution. The open test with benzoic acid 5.0 % in petrolatum was performed on the test sites about 48 hours after the second injection. The results were recorded 40 min later.

About 0.1 ml of Diproderm® cream (Schering Corporation, Bloomfield, U.S.A.), containing *betamethasone dipropionate* 0.64 mg/g, was spread on a 2 × 6 cm area on the radial edge of the right volar forearm (Fig. 1) five times before the test, namely between 8.00 and 8.30 a.m. and between 8.00 and 8.30 p.m. on two subsequent days and between 8.00 and 8.30 a.m. on the third day. The test with BA was performed between 10.30 and 11.00 a.m.

0.1 ml of *lidocaine* 10 mg/ml with methyl-para-oxy-benzoate 0.1 % as the preservative (Lidocain®, Orion, Helsinki, Finland) was injected intradermally in a row of four injections at 2 cm intervals on the distal ulnar edge of the left volar forearm 15 min before the test.

The test was carried out on the pretreated skin areas and on the control areas with the chamber technique using 20 minutes' occlusion and recording the results 20 min later. The test substances were BA 5.0 %, 1.0 %, 0.25 %, and 0.10 % in petrolatum.

*Statistical methods:* 2 × 2 contingency table and Fisher's exact probability test.

**Repeating the test on the same test site**

The test subjects in this part of the study were 17 nurses and physicians, and one patient with a venous leg ulcer. There were 13 women and five men whose mean age was 35.6 (26—54) yrs.

The test was performed with the open test technique on the dorsal side of the forearm. An area of 3 × 3 cm was marked on the skin with a ball-point pen. About 0.1 ml of BA 5.0 % in petrolatum was spread on the marked area and left on the skin for 40 min. It was then gently wiped off with a piece of cotton and the result was recorded. The test was repeated on the same site 14 times at two-hour intervals on two subsequent days. The first application was carried out at 8.00 a.m. and the last at 8.00 p.m. on both days.

In two subjects, a scratch test with the solution containing histamine 10 mg/ml was performed on both the test site and the control site on the second day when the skin no longer reacted to BA.

*Statistical methods:* 2 × 2 contingency table and Fisher's exact probability test.

## THE CORRELATION BETWEEN PERORAL CHALLENGE TEST AND SKIN TEST RESULTS

The test subjects and skin test techniques were the same in this part of the study as in the section »Open test versus closed test».

Peroral challenge tests with 200 mg of BA, 500 mg of SA, 200 mg of CA, and 500 mg of SB, packed in colourless, transparent, gelatinous capsules, 6 × 19 mm in size (Lilly, Indianapolis, U.S.A.), were performed on the ward on four subsequent days. The capsules were given between 8.00 and 8.30 a.m. with a glass of water. Axillar body temperature was measured once an hour and all objective and subjective symptoms were recorded over a period of 12 hours.

The test substances in the skin tests were BA 5.0 %, SA 2.5 %, CA 5.0 %, and SB 10 % in petrolatum.

*Statistical methods:* 2 × 2 contingency table, Fisher's exact probability test and Student's t-test of two means.

## RESULTS AND COMMENTS

### THE ABILITY OF SELECTED SUBSTANCES TO CAUSE NON-IMMUNOLOGIC CONTACT URTICARIA

Immediate reactions were seen in 47 (43 %) out of 110 patients. Immediate reactions from one or more of the test substances were elicited in 11/36 (31 %) of the atopics, 10/23 (44 %) in the urticaria group, 15/26 (58 %) in the non-atopic dermatitis group, and 11/25 (44 %) in the comparison group. The differences were not statistically significant. Forty-five per cent of the patients with immediate reactions responded to one substance only, whereas the other patients had contact urticarial reactions to two or three substances. One patient reacted to all four substances. BA produced immediate reactions more often (39 %) than SA (14 %) ($p < 0.001$) and balsam of Peru (24 %) ($p < 0.05$) (Table II). No immediate reactions were seen to SB, para-oxy-benzoates, tartrazin, salicylic acid, perfume mixture, polymyxin B sulfate, propylene glycol, and hydrophilic ointment containing methyl-para-oxy-benzoate.

There was no correlation between the age of the patients and the occurrence of immediate reactions, but reactions were seen more frequently in males, 35/67 (52 %), than in females, 12/43 (28 %) ($p < 0.05$).

Later on, salicylic acid (5.0 %) and aceto-salicylic acid (5.0 %) in petrolatum were tested in 138 subjects with the chamber test using 20 minutes' occlusion; the results were recorded 10 min later. No immediate skin reactions were seen.

Table II. *Contact urticarial reactions to benzoic acid, sorbic acid, and balsam of Peru in four patient groups: atopics, urticaria patients, non-atopic dermatitis patients, and comparison patients*

Chamber method with 20 minutes' occlusion. Results recorded immediately after the tests were removed

| Test substance | Concentration % | Atopics (36 patients) Positive No. (%) | Urticaria (23 patients) Positive No. (%) | Non-atopic dermatitis (26 patients) Positive No. (%) | Comparison series (25 patients) Positive No. (%) | Total (110 patients) Positive No. (%) |
|---|---|---|---|---|---|---|
| Benzoic acid | 5.0 | 10 (28) | 9 (39) | 14 (54) | 10 (40) | 43 (39) |
| Sorbic acid | 2.5 | 3 (8) | 2 (9) | 6 (23) | 4 (16) | 15 (14) |
| Balsam of Peru | 25 | 7 (19) | 4 (17) | 6 (23) | 9 (36) | 26 (24) |
| Hydrophilic ointment (cont. sorbic acid 0.2 %) | as is | 0 | 0 | 1 (4) | 1 (4) | 2 (2) |

Table III. *20-min reactions to chemically related test substances in the chamber test*

| Test substance | Concentration % | Tested No. | Positive reactions: Redness and oedema No. | Positive reactions: Redness No. | Positive reactions: Total No. |
|---|---|---|---|---|---|
| Benzoic acid | 5.0 | 105 | 7 | 48 | 55 |
| Sodium benzoate | 10 | 105 | 2 | 9 | 11 |
| Cinnamic acid | 5.0 | 97 | 1 | 28 | 29 |
| Cinnamic chloride | 5.0 | 76 | 0 | 0 | 0 |
| Acetic acid | 0.50 | 105 | 0 | 2 | 2 |
| Sodium acetate | 5.0 | 105 | 0 | 0 | 0 |
| Ethyl alcohol | 70 | 105 | 0 | 1 | 1 |
| Butyric acid | 2.5 | 105 | 2 | 55 | 57 |
| Butyl alcohol | as is | 105 | 0 | 4 | 4 |

Agents eliciting NICU are numerous, varying from DMSO to plants, larvae of insects and marine life. DMSO, tetrahydrofurfuryl ester of nicotinic acid, BA, SA, CA, and cinnamic aldehyde are substances found in dermatological preparations previously reported to be capable of producing NICU (see pages 8—10). No new substances of this kind were found in this part of the study. BA was the most potent contact urticariagenic agent of the substances tested; it elicited reactions in about one third of the patients. However, later on during the study, it was found that recording the results immediately after the removal of the test chambers did not give the optimal results. The chamber method is, however, well suited to this kind of testing.

## IMMEDIATE REACTIONS TO CHEMICALLY RELATED SUBSTANCES

In order to see whether the acid, the salt, or the alcohol of the same basic chemical structure is the most potent urticariagenic agent, various derivatives of BA, CA, acetic acid, butyric acid, lactic acid, and citric acid were tested.

Immediate reactions were seen more frequently to BA than to SB ($p < 0.001$) (Table III). CA gave reactions while cinnamic chloride did not. Acetic acid elicited an immediate reaction in two patients while sodium acetate failed to do so. Butyric acid elicited immediate reactions more frequently than butyl alcohol ($p < 0.001$). Lactic acid, sodium lactate, citric acid, and sodium citrate gave no immediate reactions in the test subjects.

Some studies on NICU have been made using acids, e.g. BA, SA, and CA (28, 31, 47), but cinnamic aldehyde is also known to produce urticarial reactions in some subjects (28, 80, 99). It is not clear which chemical properties make a substance capable of producing NICU. It is not likely to be the acidity of the substance in itself because such substances as salicylic acid, acetosalicylic acid, lactic acid, and citric acid were not able to elicit immediate skin reactions.

## A COMPARISON OF OPEN AND CLOSED TEST METHODS

BA and CA gave a redness and oedema reaction more often in the open test than in the

Table IV. *Comparison of open and closed tests in atopics and non-atopics*

30-min reactions to benzoic acid, sorbic acid, and cinnamic acid. Occlusion time 20 min

| Test substance | Concentration % | Open test | | Closed test | |
|---|---|---|---|---|---|
| | | Redness and oedema Positive No. (%) | Redness Positive No. (%) | Redness and oedema Positive No. (%) | Redness Positive No. (%) |
| *Atopics* (51 patients) | | | | | |
| Benzoic acid | 5.0 | 30 (59) | 8 (16) | 16 (31) | 17 (33) |
| Sorbic acid | 2.5 | 14 (28) | 8 (16) | 9 (18) | 7 (14) |
| Cinnamic acid | 5.0 | 27 (53) | 10 (20) | 12 (24) | 17 (33) |
| *Non-atopics* (55 patients) | | | | | |
| Benzoic acid | 5.0 | 43 (78) | 8 (15) | 33 (62) | 15 (27) |
| Sorbic acid | 2.5 | 27 (49) | 12 (22) | 20 (36) | 12 (22) |
| Cinnamic acid | 5.0 | 38 (69) | 10 (18) | 26 (47) | 16 (29) |

closed test ($p < 0.001$), but with SA the difference was not significant (Table IV). In the chamber test, BA gave redness and oedema reactions significantly more frequently in the non-atopics than in the atopics ($p < 0.01$). The difference in the open test was almost significant ($p < 0.05$). In the open and chamber tests, SA elicited a redness and oedema reaction almost significantly more frequently in the non-atopic than in the atopic patient group ($p < 0.05$). In the closed test, CA produced redness and oedema reactions almost significantly more often in the non-atopics than in the atopics ($p < 0.05$). The difference in the open test, however, was not significant. Usually, there were fewer redness reactions in the open test than in the closed test.

Today the closed patch test is the routine procedure for detecting delayed type hypersensitivity. The open test method has been used to study contact urticarias for decades. The open test was more sensitive than the closed test concerning the strength of the reactions to BA and CA when the commonly recommended test procedure for contact urticaria was used and the results were read 30 min after the application of the test substance. Few corresponding investigations with open and closed tests have previously been published; Haustein (41) noticed allergic contact urticarial reactions in the patch test to aminophenazone, promethazine hydrochloride, and penicillin G, in one patient each. He confirmed these results in open tests and stated that the open test is a safer method when suspected allergic contact urticarial reactions are being studied. Haustein noted that occlusion enhances the percutaneous absorption and serious anaphylactic reactions can occur, as they did in one of his patients.

In this part of the study, in which the results were read after 30 min, non-atopics seemed to be more sensitive to BA, SA, and CA than atopics, especially when the chamber method was used. However, no such difference was noticed when a study was made of the natural course of contact urticarial reactions to these agents in the open test (p. 24). Thus, occlusion influenced atopic skin in a different way than non-atopic skin. The mechanism might well be vasoconstriction, which is more pronounced in atopic skin than in non-atopic skin.

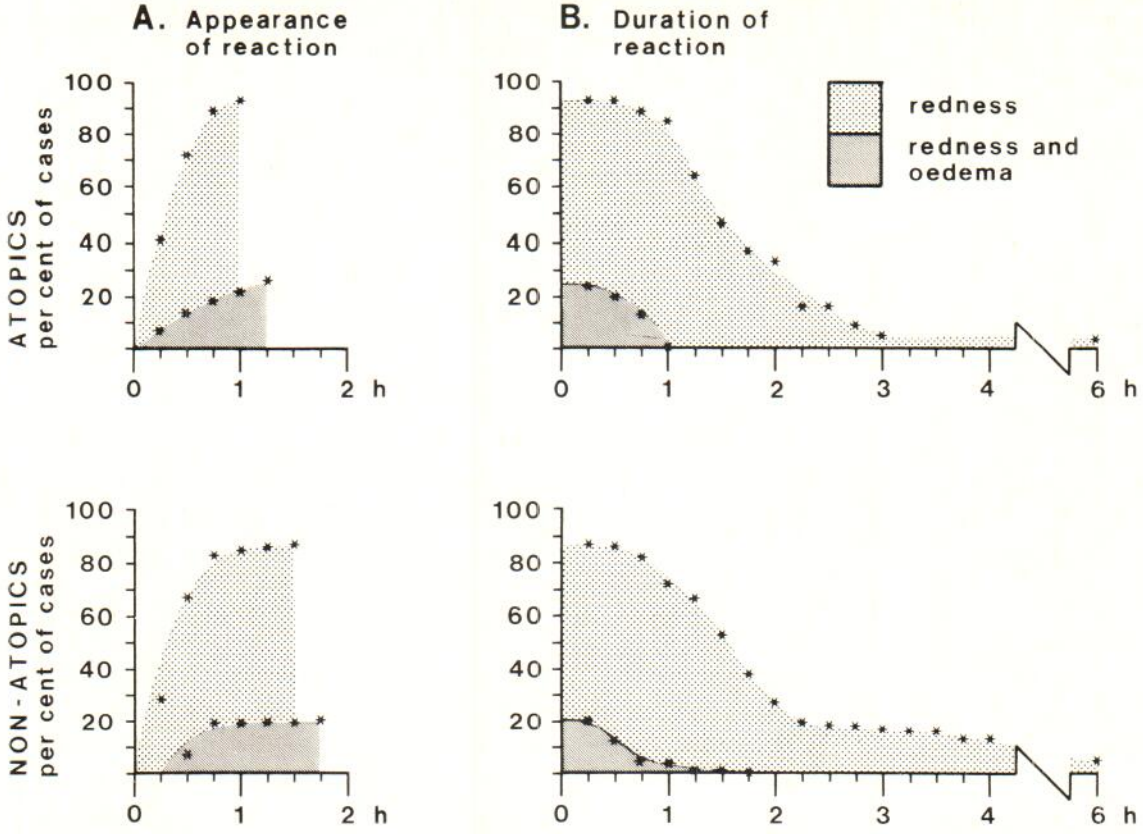


*Fig. 2.* A. Cumulative indices of the appearance of contact urticarial reactions in the open tests to benzoic acid 5.0 % in petrolatum in 29 atopic and 74 non-atopic persons (0 = application of the test substance).

B. Duration of reactions (0 = appearance of the reaction).

## THE NATURAL COURSE OF OPEN TEST REACTIONS

Contact urticarial reactions to BA were seen in 27/29 (93 %) of the atopics and in 64/74 (87 %) of the non-atopics, to SA in 20/29 (69 %) and in 40/74 (54 %), and to CA in 24/29 (83 %) and in 63/74 (85 %), respectively. Most of the reactions to BA, SA, and CA appeared within 45 min and disappeared within two hours (Figs. 2, 3 and 4). Oedema reactions did not last over one hour 45 min, but sometimes redness reactions to BA and CA persisted to the end of the six-hour observation time. The strength, frequency, and course of the reactions to BA and CA were about the same, but SA elicited fewer ($p < 0.001$) and weaker ($p < 0.001$) reactions than the other test substances. Also, redness reactions to SA were of shorter duration than those produced by BA or CA ($p < 0.001$).

Subjective symptoms in the test areas were also recorded. BA elicited tingling or itching in 12 (41 %) of the atopic and 24 (32 %) of the non-atopic persons, SA in five (17 %) and nine (12 %), and CA in 12 (41 %) and 22 (30 %), respectively.

In atopics, tingling and itching were induced by BA on average 29 (15—60) min after application and disappeared after 65 (15—285) min. These symptoms were induced by SA in 27 (15—45) min and disappeared after

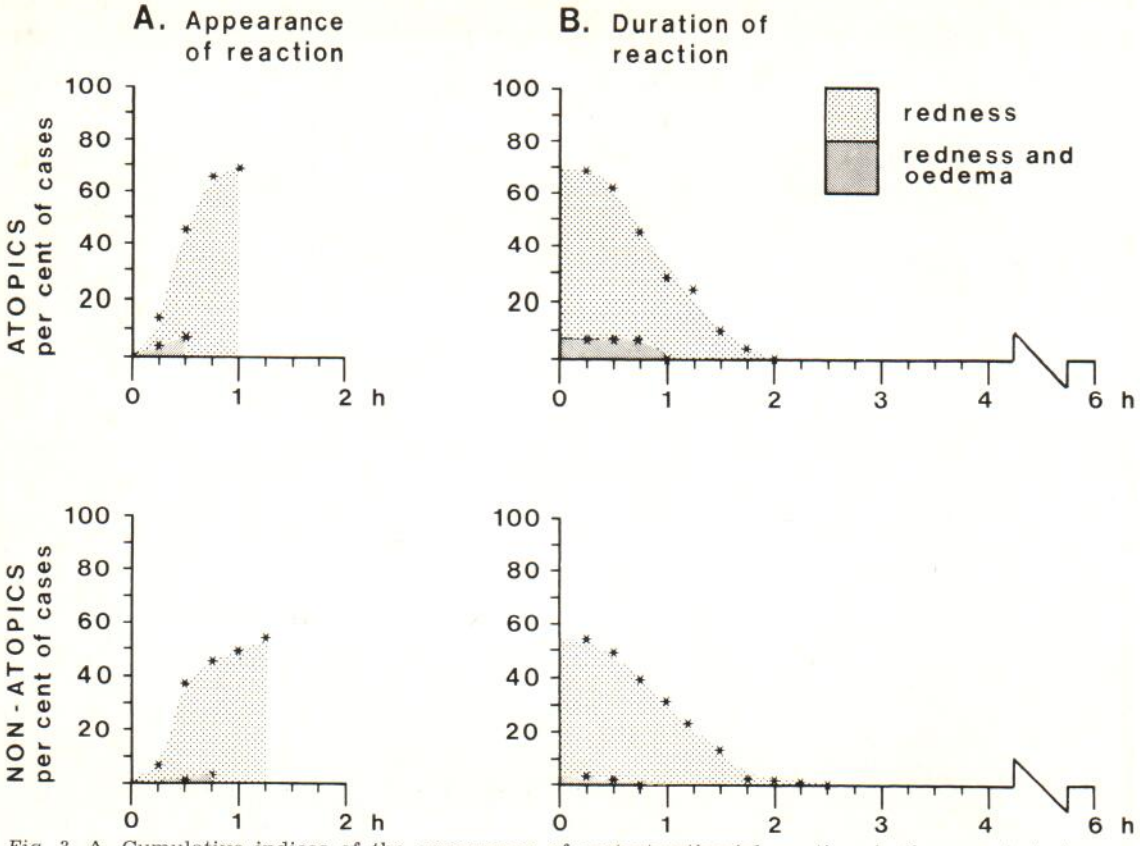


*Fig. 3.* A. Cumulative indices of the appearance of contact urticarial reactions in the open tests to sorbic acid 2.5 % in petrolatum in 29 atopic and 74 non-atopic persons (0 = application of the test substance).

B. Duration of reactions (0 = appearance of the reaction).

39 (15—60) min. Tingling and itching were produced by CA in 28 (15—60) min and disappeared after 64 (15—285) min.

In non-atopics, the corresponding times for BA were 28 (15—45) and 33 (15—90) min, those for SA 32 (30—45) and 37 (15—75) min, and those for CA 36 (15—75) and 33 (15—105) min.

The differences between atopic and non-atopic persons in the frequency, appearance and duration of subjective symptoms were not statistically significant. Neither were there significant differences between atopics and non-atopics as to the frequency, strength, or natural course of the contact urticarial reactions to the test substances.

Tetrahydrofurfuryl ester of nicotinic acid (Trafuril®) is one of the most widely investigated substances producing NICU. The cutaneous reaction, which resembles that produced by BA, SA, and CA, has been described in detail (35, 109, 111). Erythema with or without oedema appears on the site of application within 10 to 30 min, accompanied by a feeling of tingling, itching, or burning which usually disappears in one to three hours. The reaction often appears in a follicular pattern, and the skin looks like local »gooseflesh» (35). The reactivity varies from complete unresponsiveness to marked local oedema and redness.

In the present study, most of the reactions

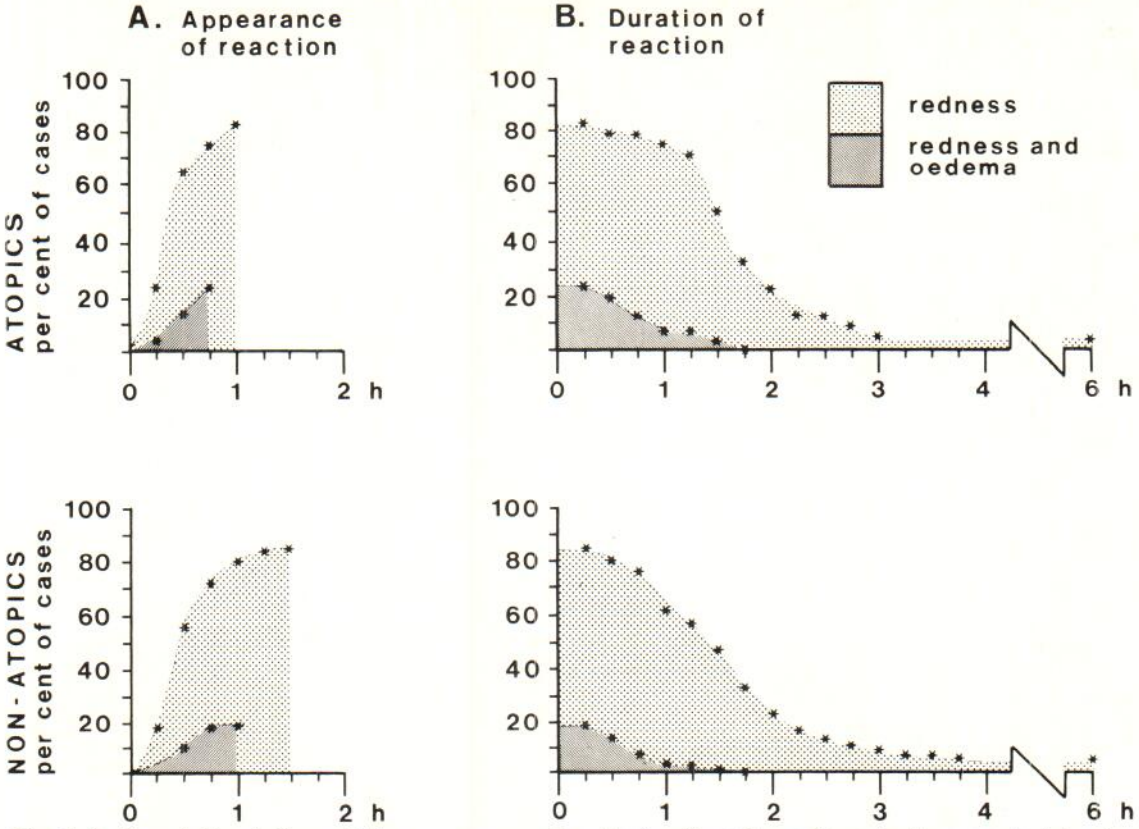


*Fig. 4.* A. Cumulative indices of the appearance of contact urticarial reactions in the open test to cinnamic acid 5.0 % in petrolatum in 29 atopic and 74 non-atopic persons (0 = application of the test substance).
B. Duration of reactions (0 = appearance of the reaction).

to BA, SA, and CA appeared within 45 min to one hour and disappeared within one to two hours, though they sometimes persisted for 24 hours or more. The follicular pattern was frequently seen in the reactions. The great variation in the reactivity between different persons was also clear. Immediate contact reactions to DMSO have also been reported to appear in 10 to 15 min (57) and disappear within six hours at the latest (110). The corresponding figures for balsam of Peru have been reported to be 10 to 20 min and two to three hours (28, 97, 99). The figures for cinnamic aldehyde are 10 to 20 min and 45 min (80), and those for SA five to 30 min and one to two hours (31, 47).

The natural courses of contact urticarial reactions from different chemical substances are quite similar, thus suggesting that the same mediator(s) is responsible for these reactions.

## THE EFFECTS OF VARIOUS TEST PROCEDURES, VEHICLES, AND PRETREATMENTS ON CONTACT URTICARIAL REACTIONS

### Vehicle and concentration of the test substance

BA produced reactions at lower concentrations in water than in petrolatum (Fig. 5). The

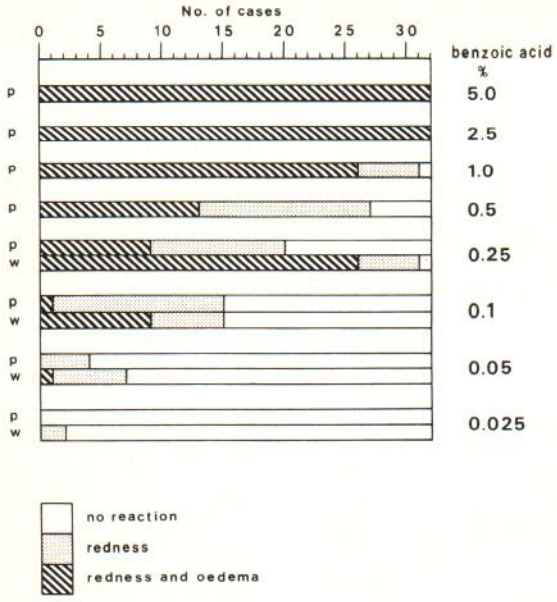


*Fig. 5.* Contact urticaria from benzoic acid in petrolatum (p) and water (w). Chamber method with 20 minutes' occlusion. Results recorded 10 min after the tests were removed.

frequency of urticarial reactions to BA 0.25 % in water and to BA 1.0 % in petrolatum was the same. BA at 0.10 % showed the same frequency of reactions in petrolatum and in water, but more oedema reactions were seen when water was the vehicle ($p < 0.01$).

The results of the tests with SA in petrolatum, Hydran®, Novalan®, and water are presented in Fig. 6. Reactions to SA 0.10 % were found more frequently in water than in Hydran® ($p < 0.05$), petrolatum ($p < 0.01$), and Novalan® ($p < 0.001$). Oedema reactions were seen more frequently in water than in other vehicles ($p < 0.01$). No difference in the frequency or strength of the reactions to BA or to SA was seen between atopic and non-atopic patients.

Both the vehicle and the concentration of the contact urticariagenic substance influence the strength and frequency of the reactions. In Fryklöf's (31) work, SA elicited contact urticarial reactions at concentrations of 0.025 % to 0.05 % in cold cream (W/O) (water in oil) and at concentrations of 0.01 % to 0.02 % in water. Hjorth & Trolle-Lassen (47) found that SA in W/O emulsion produced stronger reactions than SA in O/W (oil in water) emulsion or in petrolatum. The results of the present author concerning SA were in good agreement with the findings mentioned above. Also, BA in water produced reactions more easily than BA in petrolatum.

The concentration of the substance producing NICU influences the strength of the reaction. One per cent Trafuril® solution has been reported to cause erythema and 10 % a wheal and flare reaction (109). Cinnamic aldehyde at concentrations below 1 % does not elicit immediate reactions but 1 % elicits local erythema, 3 % produces a weak wheal and flare response, and 10 % a clear response of this kind (80). Twenty per cent DMSO pro-

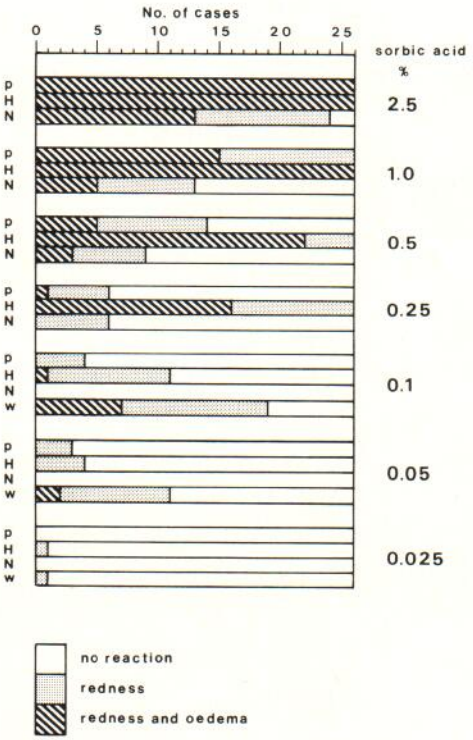


*Fig. 6.* Contact urticaria from sorbic acid in petrolatum (p), Hydran® (H), Novalan® (N) and water (w). Chamber method with 20 minutes' occlusion. Results recorded 10 min after the tests were removed.

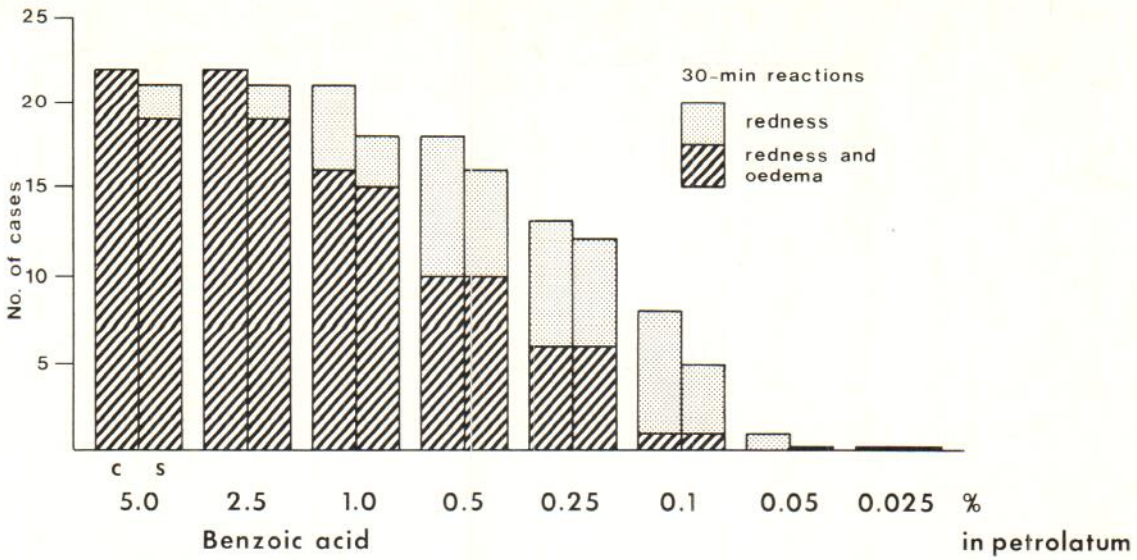


*Fig. 7.* The effect of scratching the skin on the contact urticarial reaction to benzoic acid. 20 minutes' occlusion (c = chamber test, s = scratch-chamber test).

duces erythema (110) and 70 % erythema and oedema on the skin (57). Balsam of Peru elicits more immediate reactions at concentrations of 12.5 % and 25 % than at concentrations beyond these limits (28).

According to results presented, BA and SA at concentrations usually used for preservatives (up to 0.2 %) are able to elicit immediate skin reactions, which vary from erythema to a clear contact urticarial wheal and flare response in some persons. In most test subjects reactions were found only at higher concentrations.

### Scratching the skin

The results for all 22 patients are presented together in Fig. 7 because there was no difference in reactivity between atopics and non-atopics. There was no statistical difference between the chamber and scratch-chamber tests in the frequency or strength of the contact urticarial reactions to different concentrations of BA. In some patients, scratching the skin before the application of the test substance either reduced the strength of the reaction or else abolished it altogether.

A mechanical trauma of the corneal layer of the skin sufficient to cause interruptions in barrier continuity increases percutaneous absorption of different substances up to several hundred-fold (68). According to the results of the present study, scratching the skin did not strengthen the reactions to BA. Obviously, the absorption of BA from intact skin of the back is sufficient for maximal contact urticarial reactivity, and therefore damaging the barrier of the corneal layer did not have any enhancing influence. The mechanical trauma itself could be the reason why the contact urticarial response was diminished in some patients.

### Stripping the skin

The results of the 40-min reactions are presented in Fig. 8. No reactions to BA were seen below a concentration of 0.10 %. Stripping the skin weakened the strength of the contact urticarial reaction in some pa-

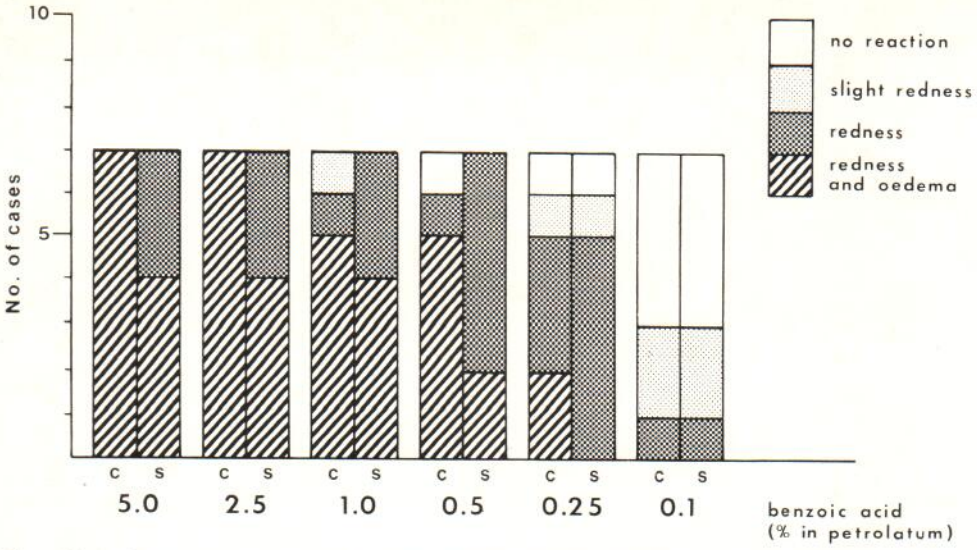


*Fig. 8.* The effect of stripping the skin on 40-min reactions to benzoic acid. Chamber method with 20 minutes' occlusion (c = control area, s = stripped area).

tients, but did not significantly alter the frequency of the reactions to BA at different concentrations.

Blank et al. (3) noticed that stripping the skin seven to 12 times was needed for a significant increase in the permeability of the skin to water and to an anticholinesterase agent (sarin). Ten strippings were used in the present study, a number which usually elicited red dermal papillae. Because neither scratching nor stripping the skin enhanced the contact urticarial reaction to BA, the absorption of the acid from the intact skin must be optimal for the contact urticarial response. As might be the case with scratching the skin, the mechanical manipulation of the skin in the stripping procedure could explain why the strength of reactions observed diminished in some patients.

**Test site**

The upper back and the extensor sides of the upper extremities were the most sensitive areas to BA 5.0 % in petrolatum, while the hands showed few reactions and the soles did not react at all (Fig. 9). The dorsal side of the forearm was more sensitive than the volar side ($p < 0.01$) (slight redness not included).

Reactions to Trafuril® have been reported to be weak or absent on the palms and soles (35) and stronger on the volar sides of the arms, on the bend of the elbow, and on the neck (111). Regional differences have also been reported in contact urticaria caused by ethyl nicotinate, privin base, and histamine base (13). The site of maximum response varied from one compound to another but the minimum response was seen on the lower leg with all three compounds tested. The punctum maximum with ethyl nicotinate was the presternal area, with privin base the forehead, and with histamine base the back. Maibach & Conant (66) reported a case with contact urticaria caused by polysorbate 60 in a corticosteroid cream on the forehead but not on the arm or back. Rietschel (89) had a patient with contact urticaria from shampoo containing SA on the face but not on other areas of the body.

In addition to the chemical structure of the substance and the vehicle, the thickness of

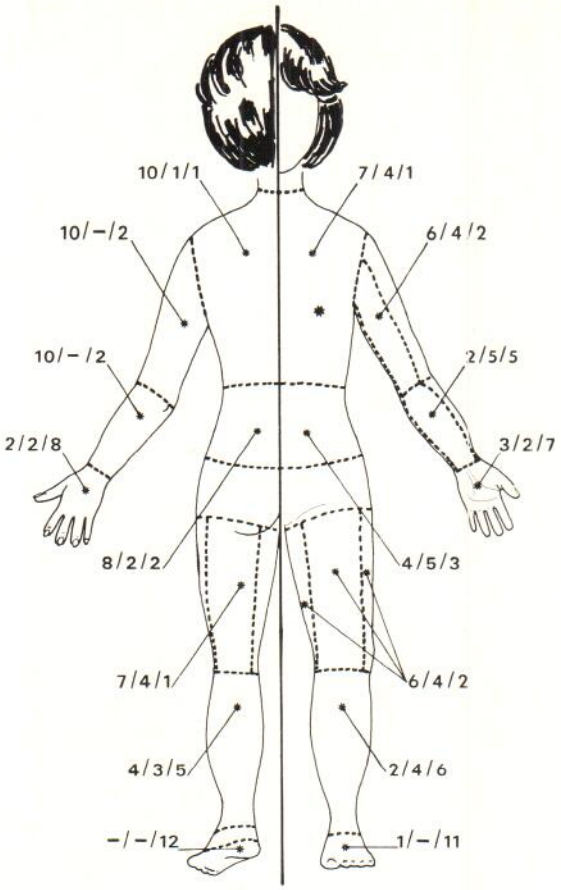


*Fig. 9.* 40-min reactions to benzoic acid 5.0 % in petrolatum on different skin areas in 12 patients (redness/slight redness/no reaction).

the intact stratum corneum and, to a lesser degree, the density of cutaneous appendages also influence the absorption of the substance into the skin (68). However, the regional variations in the permeability of the skin are not the only reason for the skin's varying contact urticarial reactivity. There are still questions to be answered, e.g. regarding the reactivity of small vessels and the number of mast cells in different skin areas. These aspects are discussed in detail on page 40.

**Peroral antihistamine**

Hydroxyzine did not have any significant influence on the strength or the frequency of BA reactions (Fig. 10), but the wheal in the histamine scratch test diminished significantly from 17.4 mm ± 2.2 mm to 7.4 mm ± 0.9 mm (mean of D + d ± SD) ($p < 0.001$). No reactions to BA were seen below a concentration of 0.10 %.

Forsbeck & Skog (28) used clemastine before the test with balsam of Peru and some of its components. They reported the blocking effect in three out of four patients with immediate reactions to balsam of Peru, in

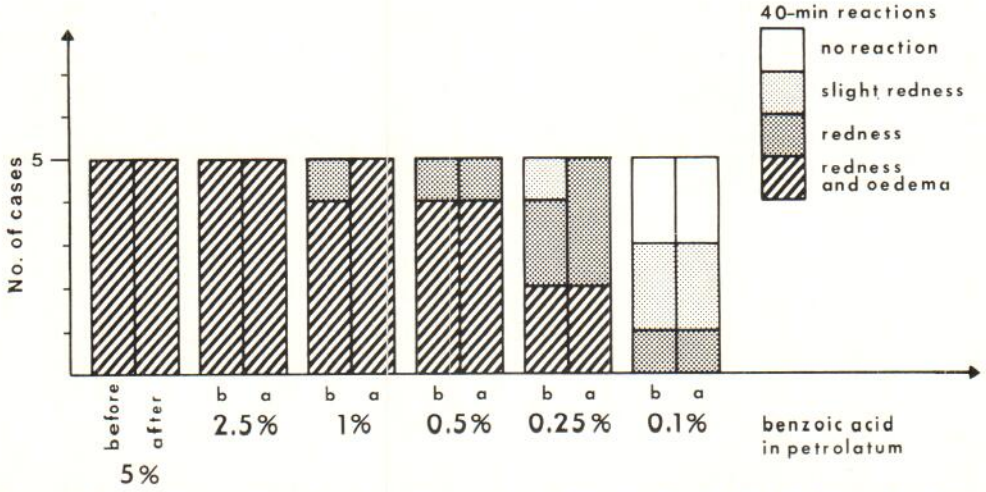


*Fig. 10.* Contact urticarial reactions to benzoic acid in the chamber test before and after peroral antihistamine (hydroxyzine, 25 mg, 12 hours before the test).

both patients with immediate reactions to BA, and in two out of three patients with such reactions to CA. No effect was noted on contact urticarial reactions to cinnamic aldehyde in two patients.

Other investigators have used different antihistaminic drugs with varying results. Gross & Merz (35) used an antihistaminic drug (Antistin) intradermally, and Strehler (111) systemically to study NICU from Trafuril®. None of these authors observed any blocking effect on the reaction. Murrel & Taylor (78) also failed to find that peroral diphenhydramine had any significant influence on contact urticaria from Trafuril.® Smith et al. (107) succeeded in blocking the contact urticarial reaction to cobalt chloride and Kligman (57) the reaction to DMSO using intradermally injected diphenhydramine. Maibach & Johnson (67) reported that diethyltoluamide caused ICU which they were not able to block with locally injected diphenhydramine or chlorpheniramine. Calnan & Shuster (7) studied immediate reactions to ammonium persulfate and noticed that promethazine hydrochloride or chlorpheniramine maleate injected intramuscularly did not influence the urticarial reaction in one patient, reduced it in two, and inhibited it in one of the four patients tested.

The influence of antihistaminic drugs thus seems to depend on the substance producing contact urticaria. The results of the current study would indicate that histamine plays no major role in the production of a contact urticarial response to BA. The reaction may be mediated by agents other than histamine. Alternatively, BA may have the same kind of direct influence on dermal vessels which Stark-Mittelholzer (109) suggested for Trafuril®.

**Compound 48/80, betamethasone dipropionate, and lidocaine**

Compound 48/80 did not significantly influence the strength or the frequency of the reactions elicited by BA in the chamber test (Fig. 11). It also failed to diminish the urticarial reaction to BA 5.0 % in the open test in all but one of the six persons tested.

Betamethasone dipropionate (Diproderm®) decreased oedema ($p < 0.001$ with BA in concentrations of both 5.0 % and 1.0 %) but did not show a significant influence on the erythema.

Local anaesthesia induced by lidocaine decreased the reactivity to BA almost significantly ($p < 0.05$ with concentrations of both 1.0 % and 0.25 %).

Compound 48/80, injected intracutaneously, is a potent local liberator of histamine from mast cells in the skin. It is commonly used to study the role of histamine in contact urticarial reactions. The reaction is considered to be mediated at least in part by histamine if it can be blocked by pretreatment with compound 48/80 (82). The refractory period of reactions mediated by histamine produced by compound 48/80 has been reported to last two to six days (20).

Forsbeck & Skog (28) used solution containing compound 48/80 0.1 mg/ml and injected 0.1 ml three times intradermally into the same site of the forearm skin at about 10-hour intervals. This blocked the contact urticarial response to balsam of Peru applied in a closed patch test to the injection site. Four injections instead of three were used in the current study, and the BA test was performed after 20 to 22 hours instead of 30 hours after the first injection. No wheal was seen after the fourth injection to indicate that the mast cell histamine storage was emptied, but this did not significantly influence the reactivity of the skin to BA applied in chambers. The stronger solution of compound 48/80 (1.0 mg/ml) also had no significant effect on the contact urticarial reaction to BA in the open test. These results suggest that histamine is not essential for the production of the reaction.

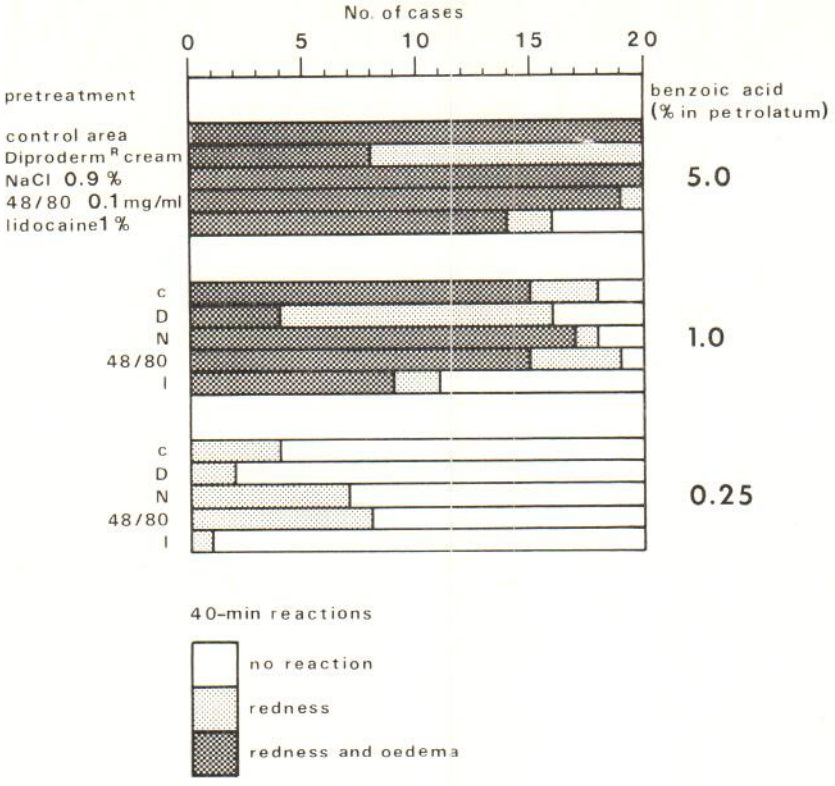


*Fig. 11.* The effect of pretreatments with compound 48/80, betamethasone dipropionate (Diproderm®) and lidocaine on the contact urticarial reaction to benzoic acid. Chamber method with 20 minutes' occlusion. Results recorded 20 min after the tests were removed.

Other methods have also been used to empty the mast cell histamine storage. Calnan & Shuster (7) injected a dose of 0.2 mg of compound 48/80 intracutaneously at each corner of a triangle with 2-cm sides on the forearm skin; Smith et al. (107) used three intradermal doses of 2.5 mg. Maibach & Johnson (67) used three 0.1 ml injections of compound 48/80 solution containing 0.05 mg/ml at eight-hour intervals.

All methods presented seemed to empty histamine storages of the skin and inhibit contact urticarial reactions at least to ammonium persulfate (7), diethyltoluamide (67), cobalt chloride (107), and balsam of Peru (28).

Murrel & Taylor (78) used hydrocortisone 2.5 % locally 10 min before Trafuril® application, but did not notice any influence on the contact urticarial response to this substance. In the current study, betamethasone dipropionate, a strong fluorinated corticosteroid, did not significantly influence the frequency of reactions to BA in different concentrations but did decrease the number of strong oedema reactions. This effect could be due to vasoconstriction caused by a potent corticosteroid.

The flare produced by the intradermal injection of histamine is known to be dependent upon normal innervation of the skin. Prior injection of a local anaesthetic, procaine hydrochloride 2 %, prevents this axon flare (57). However, as Kligman (57) reported, this pretreatment only partly abolished the DMSO flare. He also suggested that the DMSO flare involves more than the axon reflex mechanism and that DMSO can elicit erythema directly, independent of its histamine liberating properties. Maibach & Johnson (67) failed to abolish contact urticaria induced by diethyltoluamide with 0.1 ml of a 1 % lidocaine solution injected intradermally before the test.

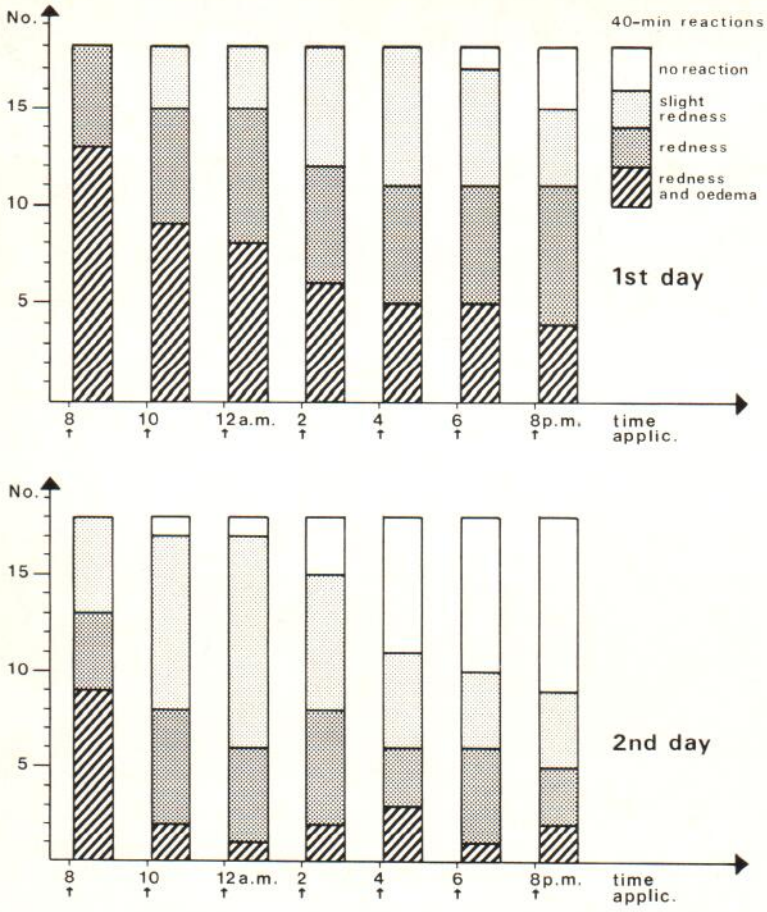


*Fig. 12.* Contact urticarial reactions to benzoic acid 5.0 % in petrolatum when the open test was repeated on the forearm skin every two hours on two subsequent days. Results recorded 20 min after the test substance was removed.

The results with lidocaine and BA obtained in the present study varied from total disappearance of the contact urticarial reaction to no effect. However, the diminishing influence of lidocaine was almost significant ($p < 0.05$), suggesting that the cholinergic mechanism (67) may be involved in the production of contact urticaria from BA.

**Repeating the test on the same test site**

The strength of the reactions to BA decreased significantly during the first day ($p < 0.01$, counted from the numbers of oedema reactions at 8.00 a.m. and 8.00 p.m.) (Fig. 12). The skin regained part of its reactivity during the night, but this reactivity again decreased during further applications. The total number of reactions decreased more on the second than on the first day ($p < 0.05$).

After the disappearance of the response to BA, the histamine scratch test was carried out on two persons on the test site and on the control site of the other forearm. There was no difference in the reactivity to histamine between the two skin sites.

Strehler (111) noticed that skin lost the whealing response when treated with repeated applications of Trafuril®. However, the skin no longer reacting to Trafuril® was fully capable of producing a dermographia elevata reaction after mechanical stimulation. He suggested that the site of the decreasing reactivity was not in the capillaries but per-

Table V. *Frequencies of objective and subjective symptoms in the peroral challenge tests with benzoic acid, sorbic acid, cinnamic acid, and sodium benzoate in 106 patients*

| | Benzcic acid No. | Sorbic acid No. | Cinnamic acid No. | Sodium benzoate No. |
|---|---|---|---|---|
| *Objective symptoms* | | | | |
| redness of the skin | 4 | 2 | 2 | 3 |
| oedema of the lips or tongue | 4 | 1 | 0 | 0 |
| oedema of the fingers | 0 | 0 | 0 | 1 |
| urticaria | 3 | 1 | 1 | 2 |
| rhinitis | 1 | 1 | 0 | 1 |
| asthma | 0 | 1 | 0 | 0 |
| fever | 0 | 0 | 0 | 1 |
| *Subjective symptoms* | | | | |
| tingling or itching | 14 | 19 | 9 | 13 |
| headache | 3 | 4 | 4 | 7 |
| pain in the stomach | 0 | 2 | 1 | 2 |
| nausea | 2 | 0 | 1 | 0 |
| diarrhoea | 1 | 1 | 1 | 1 |
| sweating | 0 | 1 | 3 | 1 |
| vertigo | 0 | 0 | 0 | 2 |

haps in some cells of the skin which liberated active substances, and that the mechanisms of whealing after chemical and mechanical stimulation are different. Later, Kligman (57) was able to demonstrate almost complete degranulation of mast cells after several applications of DMSO to the same skin site.

The reduction in the amount of the mediator, whatever it might be, is a more obvious reason for the decreasing contact urticarial reactivity of the skin after repeated applications of BA than the decreased reactivity of dermal vessels.

## THE CORRELATION BETWEEN PERORAL CHALLENGE TEST AND SKIN TEST RESULTS

Objective and subjective symptoms in the peroral challenge test are listed in Table V. Objective symptoms were seen in 15 % and only subjective symptoms in 33 % of the patients tested (Table VI). In the atopy group, 21 patients had objective or subjective symptoms in the challenge test from one substance, 12 patients from two, three or four substances; 18 patients had no symptoms. In the non-atopic group, the corresponding figures were 13, five, and 37 patients. As a whole, objective or subjective symptoms were more frequent in the atopic than in the non-atopic group ($p < 0.001$). They were recorded more frequently in the atopics than in the non-atopics from BA ($p < 0.05$), SA ($p < 0.05$), and SB ($p < 0.05$), but the difference with respect to CA was not significant (Table VI). BA elicited objective symptoms more often in the atopic than in the non-atopic group ($p < 0.05$), but no such difference was noticed in the challenge tests with other substances. SA gave subjective symptoms significantly ($p < 0.01$) and SB almost significantly ($p < 0.05$) more often in the atopic than in the non-atopic patients.

No correlation was seen between the anamnestic hypersensitivity to salicylates and reactivity in the challenge test to BA, SA, CA, or SB (Table VI).

There was no difference between older ($\geq 36$) and younger ($\leq 35$) patients in the frequency of objective or subjective symptoms either in the atopic or in the non-atopic patient group.

Table VI. *Challenge test results in 106 patients and anamnestic hypersensitivity to salicylate (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ versus the non-atopic patient group, 2 × 2 contingency table and Fisher's exact probability test)*

| Test substance | Dose mg | Atopics (51 patients) Objective symptoms No. (%) | Only subjective symptoms No. (%) | Total No. (%) | Non-atopics (55 patients) Objective symptoms No. (%) | Only subjective symptoms No. (%) | Total No. (%) |
|---|---|---|---|---|---|---|---|
| Benzoic acid | 200 | 8 (16)* | 7 (14) | 15 (29)* | 2 (4) | 4 (7) | 6 (11) |
| Sorbic acid | 500 | 2 (4) | 15 (29)** | 17 (33)* | 2 (4) | 5 (9) | 7 (13) |
| Cinnamic acid | 200 | 1 (2) | 7 (14) | 8 (16) | 2 (4) | 2 (4) | 4 (7) |
| Sodium benzoate | 500 | 3 (6) | 14 (28)* | 17 (33)* | 3 (6) | 6 (11) | 9 (16) |
| Any substance | | 9 (18) | 24 (51)** | 33 (65)*** | 7 (13) | 11 (20) | 18 (33) |
| Anamnestic hypersensitivity to salicylate | | 2 (4) | 1 (2) | 8 (16) | 2 (4) | 1 (2) | 4 (7) |

All the patients with objective symptoms in the challenge test were women. Subjective symptoms alone were seen in 40 % of all women and in 23 % of all men challenged. This difference was not statistically significant.

Objective symptoms appeared 30 min to seven hours (mean 2.6 hours) after the ingestion of the test substance (Table VII). Symptoms appeared somewhat earlier in the atopic than in the non-atopic group, but the difference was not significant. The duration of objective symptoms was longer ($p < 0.05$) in the atopic group than in the non-atopic group (Table VIII). The time of appearance and the duration of subjective symptoms were statistically similar in atopic and non-atopic patients (Tables VII and VIII).

Table VII. *Appearance of symptoms in the challenge tests*

| Test substance | Objective symptoms Atopics mean (range) h | Objective symptoms Non-atopics mean (range) h | Only subjective symptoms Atopics mean (range) h | Only subjective symptoms Non-atopics mean (range) h |
|---|---|---|---|---|
| Benzoic acid | 2.1 (1—5) | 3.5 (2—5) | 4.1 (0.5—12) | 6.8 (3—12) |
| Sorbic acid | 2.0 | 3.3 (0.5—6) | 5.4 (0.5—12) | 4.2 (2—6) |
| Cinnamic acid | 2.0 | 2.5 (2—3) | 4.6 (1—12) | 5.5 (2—9) |
| Sodium benzoate | 1.5 (0.5—2) | 4.3 (1—7) | 3.8 (0.5—11) | 3.5 (1—6) |
| Any substance | 2.0 (0.5—5) | 3.5 (0.5—7) | 4.5 (0.5—12) | 4.7 (1—12) |

Table VIII. *Duration of symptoms in the challenge tests (* = $p < 0.05$ versus objective symptoms in the non-atopics, Student's t-test of two means)*

| Test substance | Objective symptoms Atopics mean (range) h | Objective symptoms Non-atopics mean (range) h | Only subjective symptoms Atopics mean (range) h | Only subjective symptoms Non-atopics mean (range) h |
|---|---|---|---|---|
| Benzoic acid | 4.1 (1—10) | 1.5 (1—2) | 1.4 (1— 4) | 1.0 |
| Sorbic acid | 8.5 (7—10) | 2.0 (1—3) | 2.1 (1—10) | 1.8 (1—4) |
| Cinnamic acid | 12.0 | 3.0 (1—5) | 1.7 (1— 5) | 4.0 (1—7) |
| Sodium benzoate | 4.3 (1— 8) | 2.7 (1—4) | 2.3 (1— 8) | 2.2 (1—5) |
| Any substance | 5.4 (1—12)* | 2.3 (1—5) | 2.0 (1—10) | 2.0 (1—7) |

Table IX. *Repetition of the challenge tests in eight female patients*

| Patient | Age | Test substance | First challenge | Interval | Second challenge |
|---|---|---|---|---|---|
| 1. | 64 | benzoic acid | redness of the skin | 4 months | itching |
| | | sorbic acid | itching | 4 months | negative |
| | | sodium benzoate | redness of the skin | 4 months | headache |
| 2. | 58 | benzoic acid | urticaria | 16 days | headache |
| | | sorbic acid | redness of the skin | 19 days | redness of the skin |
| | | cinnamic acid | headache, sweating | 14 days | negative |
| | | sodium benzoate | headache | 4 days | negative |
| 3. | 50 | sodium benzoate | itching | 6 days | itching |
| 4. | 50 | sodium benzoate | tingling and itching | 4 days | negative |
| 5. | 49 | sorbic acid | oedema of the lips | 5 days | oedema of the lips |
| 6. | 43 | sodium benzoate | oedema of the fingers and fever | 5 months | fever |
| 7. | 29 | cinnamic acid | redness of the skin | 2 days | negative |
| 8. | 26 | benzoic acid | redness of the skin | 9 days | tingling and itching |
| | | sorbic acid | tingling and itching | 9 days | negative |

Table X. *Results of the challenge tests compared with the 30-min reactions in the skin tests in 106 patients (++ = redness and oedema, + = redness, — = negative)*

| | | Results of the skin tests | | | | | |
|---|---|---|---|---|---|---|---|
| | | Chamber method | | | Open method | | |
| Test substance | Results of the challenge tests | ++ No. | + No. | — No. | ++ No. | + No. | — No. |
| Benzoic acid | objective | 4 | 3 | 3 | 7 | 0 | 3 |
| | subjective | 6 | 2 | 3 | 8 | 1 | 2 |
| | negative | 40 | 27 | 18 | 58 | 15 | 12 |
| Sorbic acid | objective | 0 | 1 | 3 | 0 | 1 | 3 |
| | subjective | 4 | 3 | 13 | 8 | 4 | 8 |
| | negative | 25 | 15 | 42 | 33 | 15 | 34 |
| Cinnamic acid | objective | 2 | 0 | 0 | 3 | 0 | 0 |
| | subjective | 3 | 2 | 4 | 4 | 1 | 4 |
| | negative | 33 | 31 | 31 | 58 | 19 | 17 |
| Sodium benzoate | objective | 0 | 0 | 6 | 0 | 0 | 6 |
| | subjective | 0 | 1 | 19 | 0 | 1 | 19 |
| | negative | 0 | 4 | 76 | 3 | 7 | 70 |

The peroral challenge test was repeated in eight patients with objective or subjective symptoms in the first challenge. The results are shown in Table IX. In the second challenge test, objective symptoms were produced again in three out of eight cases. Usually, patients with subjective symptoms only had a negative result in the second challenge test.

There was no correlation between the positive skin test result and the occurrence of objective or subjective symptoms in the challenge test (Table X).

Peroral challenge tests with food preservatives have mainly been performed with derivatives of BA, but not with SA and CA used in the present study. Common objective symptoms from BA or SB in the challenge tests are urticaria, asthma, angio-oedema, nasal congestion, sneezing, rhinitis, hoarseness, cough, erythema of the skin, redness of

Table XI. *Selected reports of frequencies of positive results in peroral challenge tests with sodium benzoate*

| Author and year of publication | No. of patients | Diagnoses of patients | Positive No. | (%) |
|---|---|---|---|---|
| Juhlin et al. (1972) | 8 | hypersensitivity to aspirin | 3 | (38) |
| Michaëlsson & Juhlin (1973) | 52 | recurrent urticaria or angio-oedema | 24 | (46) |
| Michaëlsson et al. (1974) | 7 | allergic vascular purpura | 2 | (29) |
| Thune & Granholt (1975) | 41 | recurrent urticaria | 4 | (10) |
| Doeglas (1975) | 22 | urticaria and hypersensitivity to aspirin | 5 | (23) |
| Warin & Smith (1976) | 111 | chronic urticaria | 12 | (11) |
| Freedman (1977) | 14 | asthma | 4 | (29) |

the eyes, increased tear secretion, sweating, fever, and purpura (17, 29, 54, 75, 76, 90, 91, 92, 114). Subjective symptoms are itching, headache, palpitations, breathing difficulties, irritability, gastric pain, hot flushes, and sensations of fatigue, drowsiness, thirst, swelling, stinging in the lips and throat, pressure across the forehead, and heaviness in the head (17, 75, 90, 114).

In the present study, the most frequent objective symptom was erythema of the skin. As a whole, both objective and subjective symptoms in the challenge tests were quite similar to those reported earlier from derivatives of BA.

The frequencies of positive challenge test results with SB have varied considerably, as seen in Table XI. There is no simple explanation for this variation, but the criteria for positive reactions may vary. In addition, the patient series are seldom if ever comparable. Most of the patients of previous authors had urticaria or hypersensitivity to aspirin, but most of the patients in the present study had AD, infectious eczema, chronic urticaria, or psoriasis. In the present study, objective symptoms from SB were seen in 6 % of the patients; this figure is below the frequencies reported earlier (Table XI).

Cross-reactivity between acetosalicylic acid and benzoates in peroral challenge tests has been reported to be common. Juhlin et al. (54) reported that three of their seven patients with asthma sensitive to aspirin also had symptoms from SB. Ros et al. (90) found that 35 of the 44 patients sensitive to benzoates also reacted to aspirin.

Symptoms in the challenge tests with food additives, including benzoates, have usually been reported to appear within the first few hours, though sometimes they have not appeared until 12 hours after the administration of the substance (17, 75, 114, 121). The clinical course of symptoms in the challenge tests of the present study was quite comparable to the courses reported by the authors mentioned above.

There were some findings in this part of the study which are not easy to explain. Firstly, all the patients with objective symptoms were women. Secondly, the responses of some patients varied greatly in the rechallenge tests, which might suggest that at least some, if not all, of the reactions from ingested BA, SA, CA, and SB were non-specific placebo reactions. The same phenomenon of varying responses at different times was also noticed by Rosenhall & Zetterström (91) when patients sensitive to acetosalicylic acid were challenged. In addition, anamnestic salicylate

hypersensitivity showed no correlation with positive challenge test results to BA, SA, CA, and SB. Moreover, the skin test results did not correlate with the peroral challenge test results, indicating that the skin test cannot be used as a predictive test in the search for substances possibly causing adverse reactions when taken perorally.

## GENERAL DISCUSSION

The present investigation was undertaken when it was found that the preservatives in preparations for topical use and in foodstuffs frequently cause NICU. The test methods, the frequency of such reactions and their natural course, and the mechanisms underlying the reaction were the main topics of this investigation.

*Test methods*

The most common test for NICU is the open test in which a small amount of test substance is applied to the volar side of the forearm and the result is recorded after 30 min (82). In this study, the open test was compared with the chamber test. The former was more sensitive than the latter, which was, however, quite suitable for screening purposes because only a small area was needed for testing. The ideal time for recording the results was 40 to 45 min after the application of the test substance both in the open test and in the chamber test with an occlusion time of 20 min.

The strength of the reactions to BA varied in different skin areas. In this study, the most sensitive areas were the back, chest, dorsal sides of the forearm and upper arm, and thighs. The response was weaker on the volar side of the forearm, hand and leg; the soles were unreactive. Thus, the recommended test sites are the back and extensor sides of the upper arm and forearm.

*Mechanisms of NICU*

NICU can be caused e.g. by releasing histamine or other vasoactive substances from mast cells in the skin, or by the direct influence of urticariagenic substances on dermal vessels. Before the urticarial response can occur, the substance must penetrate the epidermal barrier, which mainly consists of the corneal layer (68). The thickness of the corneal layer and the permeability of the skin vary in different skin areas. Feldman & Maibach (21) found that the absorption of hydrocortisone through the epidermis was at its highest in the skin of the scrotum and then, in decreasing order, on the forehead, scalp, back, and forearm. Absorption was lowest on the palms and soles. These authors concluded that the absorption of hydrocortisone was greater in areas where hair follicles were large and numerous and the stratum corneum thin.

The properties of the molecule also influence the absorption. Usually, substances are absorbed through the epidermis of the palms and soles with great difficulty because the corneal layer is thick and hair follicles are absent. These areas are quite insensitive to Trafuril® (35, 111). On the other hand, the penetration of small molecules such as water through plantar and palmar skin may exceed the penetration through most other skin sites (100).

The variations in the reactions to BA in different skin areas noticed in the present study were hardly due to the varying thickness of the corneal layer because the removal of the corneal barrier by scratching or stripping the skin did not enhance the response.

The vehicle of the test substance also in-

fluences the strength of the contact urticarial reaction. BA and SA produced reactions at lower concentrations in water than in cream and ointment bases and in petrolatum. The reason for this remained unresolved because there was no correlation between the water content of the vehicle and the strength of the reactions.

The density of tissue mast cells and differences in the reactivity of dermal vessels can also play a part in the variation of the contact urticarial response in different skin areas. Binazzi & Rampichini (2) found mast cells to be most numerous in the skin of the scrotum and present in the smallest numbers in the legs. Abdel-Aal et al. (1) found the largest number in the forearm skin and the smallest number, in decreasing order, in the skin of the back, abdomen, and legs. On the other hand, Mikhail & Miller-Milinska (77) found no differences in mast cell counts in the normal skin of the neck, hand, forearm, upper arm, chest, back, abdomen, thigh, and leg. Eady et al. (19) noticed great variations in mast cell counts in different sections of the same biopsy sample. The histamine content of the skin also varied markedly between biopsy samples from skin sites only 2 cm apart. These findings may partly explain the variations in the reactivity of different skin sites to urticariagenic agents. However, more information on mast cells, their distribution and function is needed before this question can be clarified.

Also, the last link in the chain of events leading to the urticarial response, namely the reactivity of dermal blood vessels, can vary in different skin areas. Histamine injected intradermally produces a much smaller wheal and flare response on the legs than on the arms and back (107). However, strong allergic urticarial reactions can appear on the hands and feet, parts of the body which do not seem to produce NICU.

Some agents eliciting NICU seem to act through the liberation of histamine while others do not. Reactions to Trafuril® cannot be blocked by a locally or systemically applied antihistaminic agent (35, 111). It has been suggested that the mechanism of the reaction is not histamine liberation but direct influence on dermal blood vessels (109). On the other hand, reactions to DMSO (57) and to cobalt chloride (107) can be blocked both with local antihistamine and by emptying the histamine storage of mast cells with compound 48/80.

According to the results of the present study, contact urticaria to BA seems to be elicited either via direct influence on skin blood vessels or via vasoactive substances (bradykinin, S-RSA, etc.) other than histamine because the reaction could not be blocked with peroral antihistamine or compound 48/80. Direct influence on blood vessels is not, however, likely because the histamine reaction did not diminish after repeated applications of BA. Questions which must still be clarified are the role of the innervation of the skin in the reaction and why local anaesthesia blocked the contact urticarial response to BA in some patients.

The chemical properties of substances and their ability to produce NICU have been investigated very little. In this study, acids were more potent than corresponding alcohols and salts. On the other hand, strong contact urticariagenic agents, DMSO and cobalt chloride, are not acids, and e.g. salicylic acid, acetosalicylic acid, lactic acid, and citric acid are not able to produce wheal and flare reactions.

More specific methods are needed for the investigation of the mechanisms of contact urticarial reactions. The development of a standardized laboratory model would be especially valuable. Such a model would be of great benefit in the study of the contact urticariagenic properties of new substances.

*Peroral challenge*

The interpretation of symptoms in challenge

tests is often difficult, and results must be evaluated with caution. All four substances challenged produced quite similar symptoms, which may indicate the same basic mechanism in the production of the reaction. This similarity between symptoms has previously been noticed by other authors investigating reactions to aspirin, azo dyes, and derivatives of BA (17, 54, 75, 92).

The absorption of the test substance from the gastrointestinal tract, the substance's metabolism, and the reactivity of target organs can vary from one individual to another. Genetic factors, state of nutrition, and, perhaps, many unknown factors may also influence the challenge test results at different times. The placebo effect must also be kept in mind, because both objective and especially subjective symptoms can be nonspecific placebo reactions.

Because the reactivity varies greatly, a negative result in the challenge test does not rule out the possibility that symptoms will be elicited by the same substance another time. Nor does a positive result always mean that a person must follow a strict, special diet free of this substance.

No correlation was seen between strong skin test reactions and objective symptoms in the challenge test. Thus the skin test could not be used to predict sensitivity to preservatives taken perorally.

*NICU and atopy*

It may be difficult to decide whether a person is atopic or not because borderline cases are numerous and there is no internationally accepted definition of atopy. An atopic person has or has had eczema localized in the face and extensor sides of the extremities in infancy, shows flexural lichenification in adulthood, and exhibits a tendency towards a chronically relapsing course. The atopic skin is usually dry and pruritic and shows white dermographism (38). Other signs and symptoms of atopy are allergic rhinitis, asthma, and immediate allergic skin test reactivity. Close relatives of an atopic person often have the same atopic signs and symptoms.

Many clinicians have noticed that atopic persons often complain of itching and redness of the skin after using creams and ointments. According to the results of the present study, this irritation may be caused by preservatives, but no significant differences were found in the frequency or strength of the NICU reactions between atopics and nonatopics. Some other physicochemical reactions in dry atopic skin may underlie the reaction, appearing as tingling or itching sensations.

It is important that NICU be kept in mind as a possible cause of symptoms and that optimal methods be selected for a study of the patients. It is probable that the number of substances causing NICU is much greater than is now known. The search for new substances should therefore be systematically continued.

## SUMMARY AND CONCLUSIONS

The term contact urticaria is given to an immediate wheal or wheal and flare reaction which appears when certain agents make external contact with the skin. It is said to be a rare phenomenon in dermatological praxis.

The aim of this study was to investigate NICU in man, especially that caused by substances naturally present or commonly used as additives in foods and as preservatives in topical preparations. The main topics of this study were substances causing NICU, a comparison of the open and chamber methods for testing NICU, the natural course of the reaction, the role of atopy in reactivity, the effect of the vehicle and concentration of the test substance on the reaction, the mechanisms of NICU, the ability of substances causing NICU to produce symptoms in peroral challenge tests and the correlation between results in the skin test and in the peroral test.

The main results of this investigation can be summarized in the following conclusions:

1. The contact urticariagenity of the following substances was studied: BA, SB, SA, CA, cinnamic chloride, acetic acid, sodium acetate, ethyl alcohol, butyric acid, butyl alcohol, lactic acid, sodium lactate, citric acid, sodium citrate, methyl-, ethyl-, and propyl-para-oxy-benzoates, tartrazin, salicylic acid, acetosalicylic acid, perfume mixture, balsam of Peru, polymyxin B sulfate, and propylene glycol. Immediate reactions were seen to BA, SB, SA, CA, balsam of Peru, acetic acid, ethyl alcohol, butyric acid, and butyl alcohol.

2. BA, SA, and CA were used in comparing the open and chamber test methods. BA and CA but not SA elicited wheal and flare reactions more frequently in the open test than in the chamber test. The open test was more sensitive than the chamber test but the latter method was suitable for screening purposes if many substances are to be tested simultaneously because only a small test area is needed.

3. Most of the skin reactions in the open test to BA 5.0 %, SA 2.5 %, and CA 5.0 % in petrolatum appeared within 45 min and disappeared within two hours. The optimum for recording the results was 40 to 45 min after the application of the test substance both in the open test and in the chamber test with 20 minutes' occlusion.

4. Atopic persons were no more liable to get NICU from substances used in this study than non-atopics.

5. The effect of the vehicle and concentration of the contact urticariagenic agent was studied with the chamber test using BA in water and in petrolatum, and SA in water, petrolatum, O/W emulsion, and W/O emulsion. BA and SA elicited reactions most easily in water and, in decreasing order, in W/O emulsion, petrolatum, and O/W emulsion. The lowest concentrations of BA eliciting wheal and flare reactions were 0.050 % in water and 0.10 % in petrolatum, and those of SA 0.050 % in water, 0.10 % in W/O emulsion, 0.25 % in petrolatum, and 0.50 % in O/W emulsion. BA and SA were even contact urticariagenic below 0.2 %, a concentration at which they are usually used as pre-

servatives in topical preparations and food products.

— Scratching or stripping the skin did not strengthen the urticarial response to BA in petrolatum in the chamber test.

— The most sensitive skin sites were the back and the dorsal sides of the upper and lower arm, while the hands showed few reactions and the soles did not react at all.

— Repeated applications of BA in petrolatum to the same skin site diminished the whealing gradually and finally abolished it in most cases. After the disappearance of reactivity to BA, the skin was fully capable of reacting to histamine (in the scratch test). This indicates that the decreasing reactivity to BA in repeated applications was due to the emptying of the storage of mediator(s) in the skin rather than to fatigue of the dermal vessels and thus a failure to react.

6. NICU from BA is probably mediated by vasoactive substances other than histamine because the reaction was not inhibited using antihistamine (hydroxyzine) perorally prior to the test or by emptying the histamine storage in the skin with compound 48/80.

7. In peroral challenge tests with BA, SA, CA, and SB, objective symptoms were seen in 15 % and subjective symptoms in 33 % of the 106 patients tested. Most of the reactions might have been non-specific and comparable to placebo reactions. No correlation was seen between the reactivity in the skin test and that in the peroral test.

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Oulu, April 1980

Arto Lahti

## APPENDIX

The compositions of Novalan® (O/W emulsion), Hydran® (W/O emulsion), and Ambilan® used as vehicles in this study:

| | | | |
|---|---|---|---|
| Novalan® | Emulsifier | | 32.0 |
| | Petrolatum | | 8.0 |
| | Methyl-para-oxy-benzoate | | 0.1 |
| | Water | ad | 100.0 |
| Hydran® | Emulsifier | | 6.0 |
| | Petrolatum | | 54.0 |
| | Methyl-para-oxy-benzoate | | 0.07 |
| | Propyl-para-oxy-benzoate | | 0.03 |
| | Water | ad | 100.0 |
| Ambilan® | Emulsifier | | 20.0 |
| | Petrolatum | | 20.0 |
| | Paraffin liquid | | 10.0 |
| | Sorbic acid | | 0.2 |
| | Water | ad | 100.0 |

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