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MICROMORPHOLOGY AND SPECIFICITY OF ORALLY INDUCED FLARE-UP REACTIONS IN NICKEL-SENSITIVE PATIENTS

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Abstract. Micromorphology and specificity of orally induced flare-up reactions of 4–7 weeks old nickel patch test sites, compared with control test sites with tuberculin and benzalkonium, were investigated in nickel-sensitive patients who had pompholyx. Of the test sites, only that treated with nickel flared after ingestion of nickel, as also did the hand eczema. The clinical picture and light microscopical findings agreed and were both eczematous.

Key words: Nickel allergy; Hand eczema; Flare-up reactions; Micromorphology

Ingested nickel activates hand dermatitis in nickel-sensitive patients (4, 5, 9, 10). Flare-up reactions at earlier patch test or contact dermatitis sites, along with a maculo-papular rash on the abdomen, elbow flexures and sides of neck, have also been described in nickel-sensitive patients following ingestion of nickel (4, 5).

From a pathogenetic point of view, flare-up reactions are interesting, but the nature of these reactions has not hitherto been investigated in man. The study described here was undertaken to investigate the micromorphology and specificity of flare-up reactions in nickel-sensitive women. The study was carried out by specific and non-specific patch testing as well as on the hand eczema proper.

MATERIAL AND METHODS

Patients

The study was performed on 5 female patients, 27–50 years of age. All were hypersensitive to nickel, proved by previous patch testing and they had a history of metal sensitivity of several years' duration. The patients were periodically affected by hand eczema of the pompholyx type (3).

Tests

Patch testing (Finn chambers) was carried out on the buttocks (Fig. 1). Nickel sulphate 5% in petrolatum was applied to duplicate sites. A single intradermal test with tuberculin 2 TU (purified protein derivate, PPD, Statens Seruminstitut, Copenhagen, Denmark) and duplicate patch tests with benzalkonium chloride 1.0% in aqueous solution served as controls. The test patches were removed by the patients after 48 hours—or earlier in the case of itch or pain. All tests were read 72 hours after application, at which time the margins of the test reactions were carefully marked with a fuchsin–silver nitrate ink. In one patient the concentration of tuberculin and benzalkonium chloride had to be increased to 5 TU and 2.0%, respectively, because of negative reactions; in this case even 5 TU tuberculin proved negative. In another patient the nickel test was negative on the buttocks, but subsequent patch tests on the upper arms were clearly positive. With these exceptions, all tests resulted in obvious inflammatory reactions. During the following weeks until provocation the patients carried out the marking themselves. They were not allowed to use any topicals on the test areas.

Provocation

The patients were admitted to the ward 4–7 weeks after testing. At clinical examination the infiltration of all tuber-

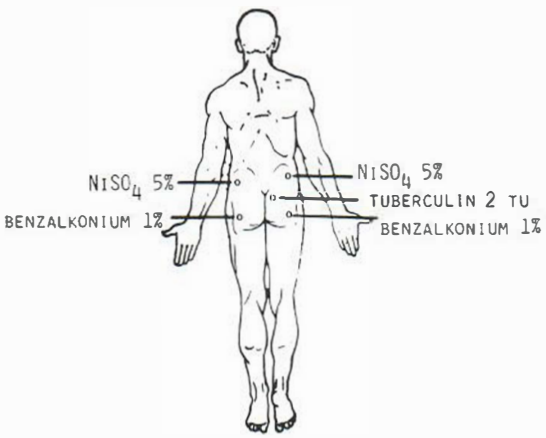


Fig. 1. Localization on the buttocks, of patch tests with nickel sulphate and benzalkonium chloride, and intradermal test with tuberculin, 4–7 weeks before oral nickel provocation.

culin and benzalkonium reactions had disappeared. In 2 patients—those examined 4 weeks after testing—a slight erythema and infiltration remained in the nickel test areas but no residues were observed in the other 3 patients. The pompholyx was more or less active in 4 patients, but inactive in one.

Punch biopsies, 2 mm diameter, for light microscopy and for direct immunofluorescence (IF) were taken from the centre of the marked test areas (from the nickel and benzalkonium sites of the left buttock). Biopsies for the same purposes were obtained from the left hypothenar region, an area of usual vesicular activity, but not, however, at the time of examination.

The patients were given a capsule containing 25 mg nickel sulphate ($NiSO_4 \cdot 6H_2O$) with a nickel content of 5.6 mg. During the subsequent 24 hours the patients were examined repeatedly. At the end of the 24 hours a new set of punch biopsies were taken from contralateral but comparable skin areas on the buttocks and palms; for the tuberculin reaction, one area only was used.

Histopathological examination

The biopsy material was fixed immediately in 10% buffered formalin solution. After histotechnical preparations, the 3–4 μm thick sections were stained according to the following methods: haematoxylin-eosin, Giemsa, toluidine blue, McManus, and Weigerts elastin stain.

Direct immunofluorescence

The specimens were quickly frozen or preserved in a transport medium described by Michel (10) for a few hours before being frozen in a mixture of propane–butane to the temperature of liquid nitrogen. Cryostat sections were incubated with fluorescein-labelled antisera from rabbit anti-human IgG, IgA, IgM, complement 3 and fibrinogen (purchased from DAKO Immunoglobulins Ltd., Copenhagen) and examined according to the technique detailed by Beutner et al. (2).

RESULTS

Clinical reactions

In all 5 patients the nickel patch tests flared after oral provocation. The patients noticed itching of the test areas 4–10 hours after nickel ingestion and at 10 hours, erythema and some infiltration could be observed. At 24 hours there was a marked eczematous reaction, with erythema, infiltration, papules and/or vesicles on an area 2–3 times larger than the original patch test. One patient patch tested with nickel on her upper left arm 4 months before challenge reacted with an intense eczematous flare on that site as well as on the patch tests on her buttock. The intensity of the flare reactions was not related to the time interval between patch test and provocation. No clinical reaction whatsoever was observed in the former tuberculin and benzalkonium test sites.

Four patients noticed itch in their palms 8–10 hours after nickel ingestion and at 24 hours there was an increased vesicular response in the palms. The fifth patient had palmar itch only but no visible change. In addition, all patients flared on a few sites of earlier contact dermatitis such as earlobes or left wrist. A maculo-papular eruption, mainly on neck and abdomen, occurred in 3 of the 5 patients. It appeared already after 3–4 hours and intensified during the next 20 hours.

Histopathological examination

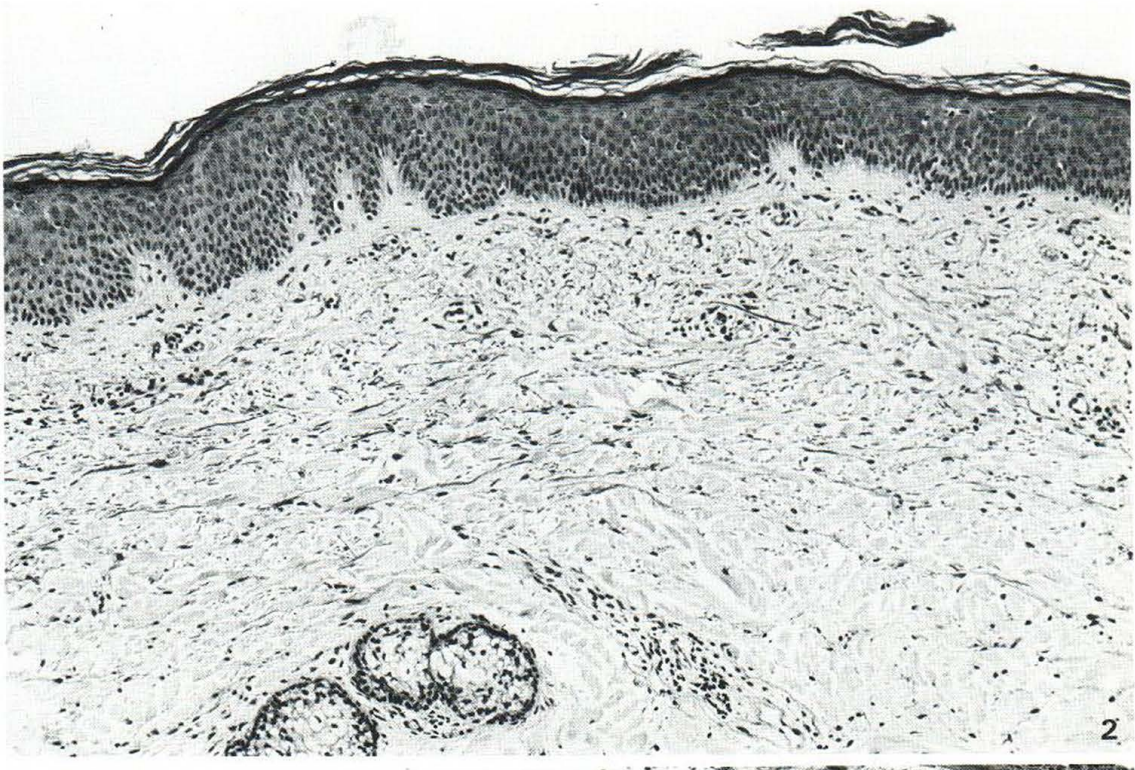
In the preprovocational test biopsies there was no or only a slight inflammatory reaction, with slight dermal oedema and a few lymphocytes. No difference was observed between the three different tests (nickel, tuberculin, benzalkonium chloride) and the palm in this respect (Figs. 2 and 4). In the surroundings of the superficial dermal vessels, scattered mast cells filled with metachromate granula were observed. There was no epidermal spongiosis or vesicular formation.

In the biopsies taken from the benzalkonium chloride and tuberculin tests 24 hours after oral nickel provocation, no difference was observed in comparison with biopsies taken the day before.

In most biopsies taken from the nickel tests and the palm 24 hours after provocation, striking histological changes were found, compared with preprovocational biopsies. There was a marked dermal oedema and a strong lymphocytic reaction around the superficial dermal vessels. Only a few polymorphonuclears were observed and no difference between pre- and postprovocational biopsies was found in this respect. There was also a marked epidermal reaction, with spongiosis and in some cases a pronounced formation of vesicles and/or bullae (Figs. 3 and 5). Some metachromatic granules were observed outside mast cells.

Fig. 2. Skin from left buttock immediately before oral nickel provocation. Almost normal histological appearance of the epidermis and the dermis. There are a few lymphocytes in the perivascular zones of the dermis. No spongiosis or spongiotic vesicles in the epidermis. Htx-eos. ×110.

Fig. 3. Skin from same patient and same but contralateral localization as in Fig. 2, 24 hours after oral nickel provocation. There is a marked spongiosis, with spongiotic vesicles in epidermis and some coagulated exudate on epidermal surface. In the upper dermis there is perivascular oedema and cellular exudate consisting mainly of lymphocytes. Near the basal epidermis there are lymphocytes, some of which are beginning to infiltrate the epidermis. Htx-eos. ×110.



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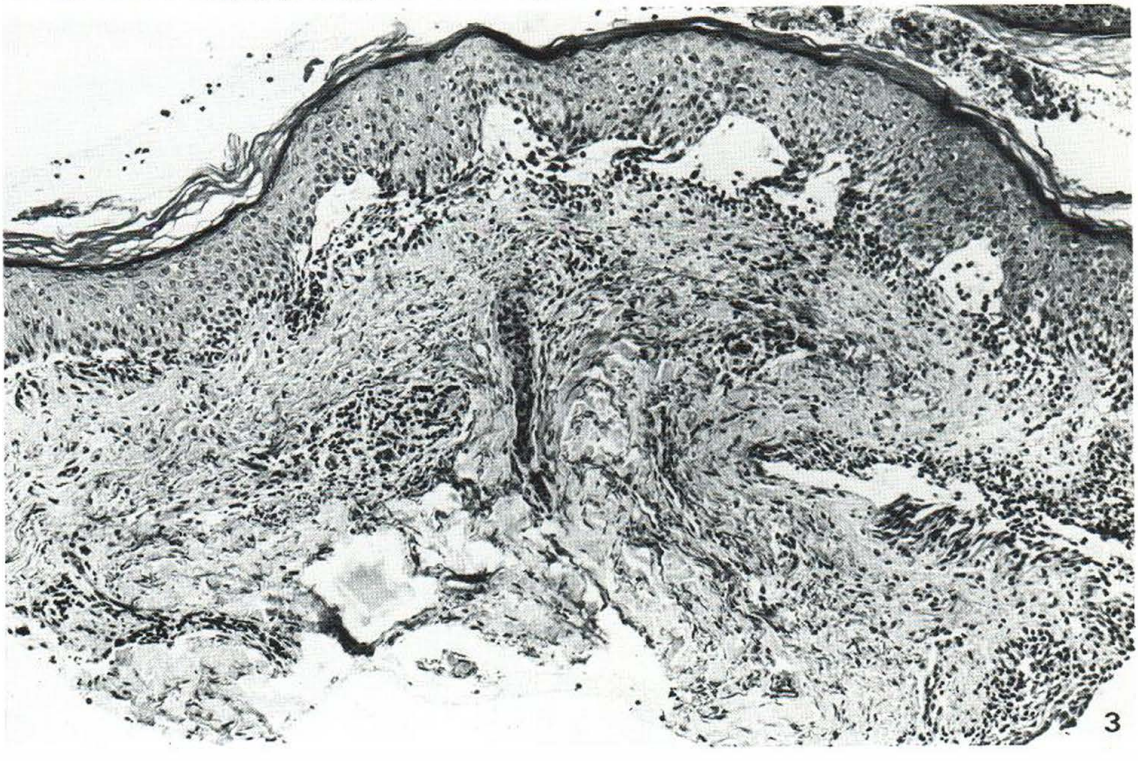

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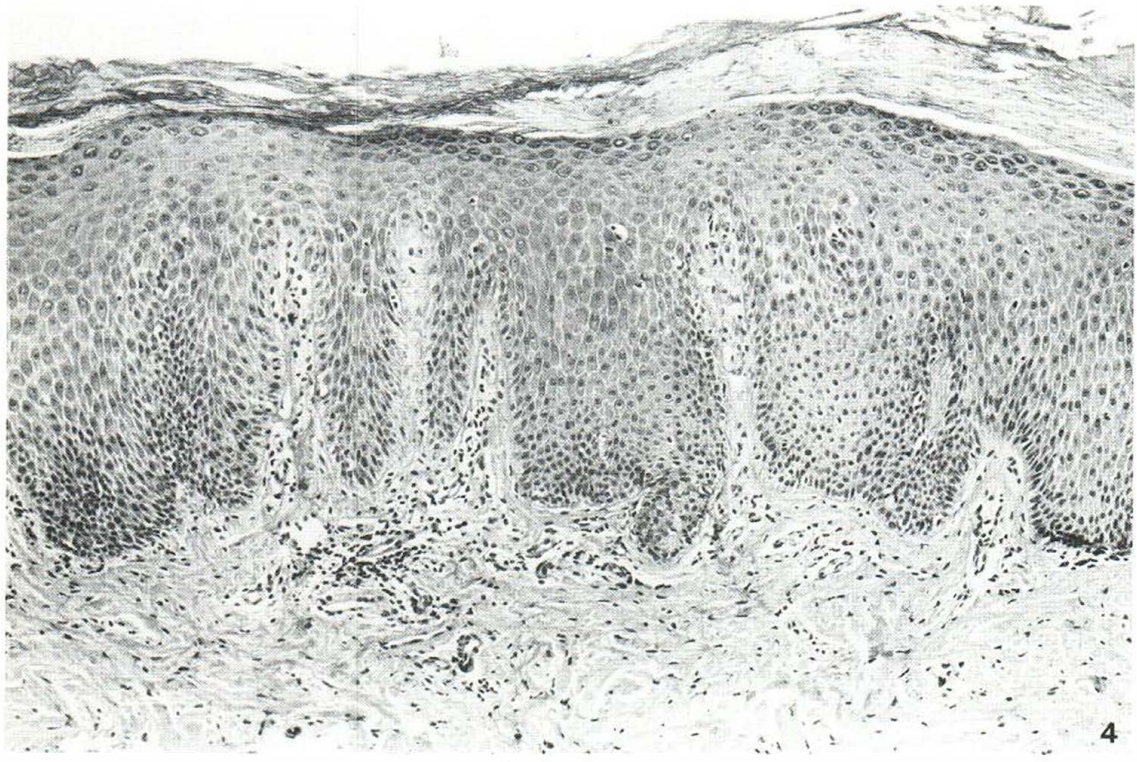

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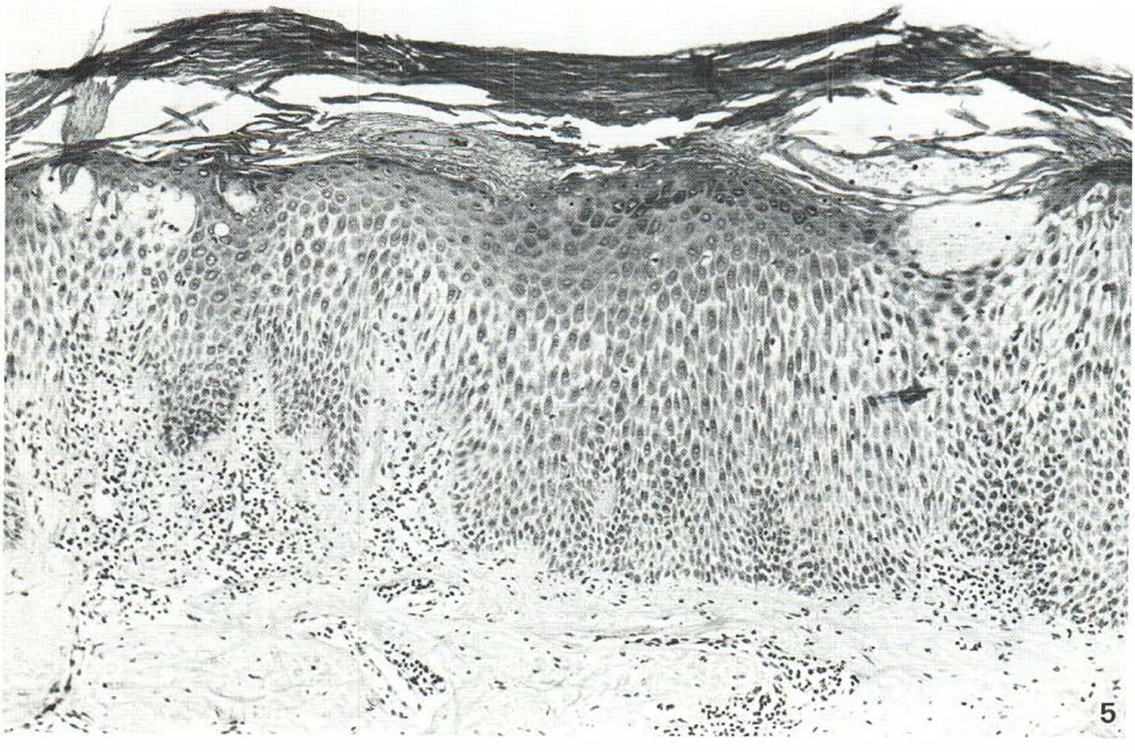

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Immunofluorescence examination

No significant findings of IgG, IgA, IgM, complement 3 or fibrinogen were observed when the specimens taken before and after provocation with nickel were compared using the direct immunofluorescence technique.

DISCUSSION

Clinically, more or less pronounced eczematous reactions with erythema, infiltration, papules and/or vesicles were observed in 4–7 weeks old nickel test patches in all 5 patients, 24 hours after oral provocation with nickel. Besides flare-up on the nickel patch test sites, the patients also flared on sites of earlier contact dermatitis. Three patients were certain that they had had no dermatitis for the past 2–4 years in these areas. Judging by the varying intervals between patch testing or previous contact dermatitis reactions and oral nickel provocation, we can conclude that flare-up reactions on such sites can be provoked from 4 weeks up to 4 years later. The interval between the original eczema and the flare-up reaction in humans can thus be longer than that in guinea pigs. In this animal, flare-up reactions were consistently provoked 2 weeks after patch testing, but not after 3 months (12).

The benzalkonium chloride and tuberculin tests were chosen as examples of a toxic dermatitis and of a delayed-type hyper-sensitivity reaction, respectively. Neither was reactivated, thus indicating that the oral antigen had no effect on the inflammatory process *per se*, nor on any type IV reaction other than nickel allergy. No patient with nickel allergy and pompholyx plus another non-related contact allergy was available for study. Apart from this, our provocation study speaks in favour of a specific activation by the antigen in question.

The result of the light microscopic examination agrees with the reactions observed clinically. The histopathological reactions both in the palms and on the nickel patch test sites, with dermal oedema, perivascular lymphocytic infiltration and epidermal spongiosis/vesicles observed after oral nickel provocation were clearly eczematous. The absence of any particular histopathological changes at the tuberculin and benzalkonium test sites also agrees with the absence of clinical reactions.

The histological findings in flare-up reactions in guinea pigs, showing a dense infiltrate of polymorphonuclear leukocytes as described by Polák & Turk (12) do not agree with the findings in the present study. In fact, few polymorphonuclear leukocytes were found in the flare-up reactions of the nickel-sensitive women. Moreover, the dermal picture resembled that described during the spontaneous flare-up in man following DNCB sensitization (14).

One may suspect that the mast cells play some part in the flare-up reactions investigated. Metachromatic granula were observed outside the mast cells and a degranulation seemed to have occurred. Dvorak et al. (8) have studied in detail the delayed hypersensitivity reactions in humans and found mast cells to be present in increased numbers compared with controls. Mast cell degranulation was suspected upon observation by light microscopy, but was more precisely demonstrated by electron microscopy. Even if the flare-up reactions studied by us are induced in some other way than regular contact dermatitis (patch test reaction) and can therefore hardly be compared, it is noteworthy that mast cell degranulation was also observed. One can only speculate on the role of mast cells in human delayed hypersensitivity and flare-up reactions. Specific, sensitized lymphocytes or their lymphokines may attract and degranulate mast cells (7) causing dermal oedema, erythema and increased cellular inflammatory exudate. The fact that the flare-up reactions were more intense and oedematous than the original patch test reactions appears to confirm that mast cell degranulation plays an active part in this reaction.

Basophil leukocytes have been observed in increased numbers in delayed hypersensitivity reactions in man (1, 6, 7, 15). However, the histopathological method used in the present study did not reveal any basophil leukocytes.

Neither immunoglobulins of the IgG, IgA and IgM class, nor complement 3 and fibrinogen were found in the flare-up reactions. Polák et al. (13)

Fig. 4. Palmar skin from another nickel-allergic patient immediately before oral provocation. There is some parakeratosis and only slight perivascular lymphocytic infiltration, but no spongiosis or spongiotic vesicles. Htx-eos. ×110.

Fig. 5. Palmar skin (contralateral) from same patient as in Fig. 4, 24 hours after oral nickel provocation. Histological picture of an acute eczematous dermatitis with marked epidermal spongiosis and spongiotic vesiculation, as well as perivascular infiltration of lymphocytes which are also seen at the basal epidermis. Htx-eos. ×110.

believe that flare-up reactions in guinea pigs are mediated by humoral antibodies formed and/or released by sensitized lymphocytes in the patch test site. In the present study, samples were taken at one time interval only, which could have given a false-negative result. With this reservation, our results speak against a pathogenetic role of humoral antibodies in the type of flare-up reactions examined. Possibly, such antibodies or immune complexes might be of importance for the appearance of the maculo-papular eruptions observed in connection with oral nickel provocation (4, 5).

Intense flare-up reactions at patch test sites or earlier contact dermatitis reactions are usually not presented by nickel-sensitive patients at examination. We have now shown that the pompholyx type of hand eczema, so common in females with nickel allergy, reacts in a similar way to oral provocation, both clinically and histopathologically, as do the flare-up reactions mentioned. They are all true secondary eruptions and further studies on their micromorphologic and immunopathogenic dynamics are certainly warranted.

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