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HUMAN LEUKOCYTE MOBILIZATION AND MORPHOLOGY IN NICKEL CONTACT ALLERGY USING A SKIN CHAMBER TECHNIQUE

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Abstract. An improved skin chamber technique has been devised and used for quantitative evaluation of the leukocyte mobilization rate (LMR). The method was applied in 10 nickel-hypersensitive patients exposed to nickel sulphate. Each patient served as his own control and for additional control purposes, 5 healthy individuals without nickel hypersensitivity were studied. The kinetics of the mobilized leukocytes were followed over a 48-hour period. After an initial lag phase of 2–4 hours, maximum migration was observed from the 24th to the 48th hour, with a wide interindividual variability in the number of mobilized cells at the time of maximum LMR response. The median cumulative leukocyte count was 1.412×10^6 leukocytes/cm^2/48 h. In the same period a statistically significant increase in the basophils for all the nickel allergic patients was observed. In 8 out of 10 patients a statistically significant effect upon the lymphocytes could be demonstrated. Six of these 8 patients had an increased lymphocyte mobilization. Throughout the period the neutrophil granulocytes were the dominant cell type, although the number decreased as the number of basophils and lymphocytes increased. The chamber technique is a valuable means for quantitative evaluation of leukocyte mobilization and morphology in skin exudates during exposure to an allergen in delayed hypersensitivity reactions.

Key words: Skin chamber; Nickel hypersensitivity; Leukocyte mobilization and morphology

Patch testing is a basic in vivo test for the evaluation of cell-mediated immunity. A positive skin reaction provides evidence of lymphocyte sensitization to the antigen used. Many in vitro models have been introduced to quantitate the cell-mediated immunity in the individual subject. One of the most promising has been the lymphocyte transformation test, especially for groups of patients with nickel contact dermatitis (11, 15, 17, 25).

Another attempt to quantitatively evaluate this problem has been to assess the histological changes in contact hypersensitivity (9, 10), but the classification of cells by light microscopy of histological sections is intricate. None of these techniques, however, has permitted proper evaluation or correlation between the lymphocyte reactivity and the intensity of the patch tests or the severity of the dermatitis.

The skin chamber technique has proved to be a useful method for the study of the cytology of the inflammatory exudate both in healthy individuals (21, 22) and in contact hypersensitivity (27), but the original methods did not permit quantitation of the cellular migration, and it can be questioned whether the migrated cells that adhere to the coverslips placed on the epidermal abrasions are representative. The coverslip itself and changing it may introduce artefacts.

The skin window technique has been improved to obtain a more quantitative assessment by using a glass or perspex chamber to cover the abrasion (2, 12, 20). This technique has successfully demonstrated a reduced leukocyte mobilization in patients with psoriasis (3, 19). Furthermore, this technique permits the study of various mediators released from the abraded skin window site to the chamber medium (5). In the present study we have used an improved skin window technique to investigate the mobilization of leukocytes in nickel-hypersensitive patients, with and without exposure to nickel.

The primary objectives of the study were (*a*) to determine how migrating cells were influenced by nickel, (*b*) to determine how the cell composition of the inflammatory exudate due to nickel was related to the time sequence, (*c*) to compare the mobilization pattern of the different leukocytes in the exudates of control subjects and nickel-hypersensitive patients.

PATIENTS AND CONTROLS

Fifteen volunteers of both sexes, divided into two groups, comprised the study material.

Group 1. Included 10 patients (nos. 1–10) (8 females, 2 males) with a mean age of 37 years (age range 20–65), all suffering from allergic contact dermatitis to nickel, veri-

fied clinically by the history and patch tests. Patch testing was performed according to the recommendations of The International Contact Dermatitis Research Group with 5.0% nickel sulphate in petrolatum. The nickel patch tests were in all cases strongly positive (erythema, oedema and vesicles). At the time of the investigation the eczema was inactive or only mildly active. The forearm was uninvolved in each patient, and the eczema never exceeded 5% of the skin surface.

Group II. Five volunteers (5 females) with a mean age of 26 years (age range 23–32) with no eczema or history of allergic contact dermatitis, participated as controls. Patch tests for nickel sulphate, potassium dichromate and cobalt chloride proved negative in these patients.

Neither group was undergoing medical treatment at the time of the investigation and no systemic glucocorticoids had been given for at least 6 months and no other anti-inflammatory drugs (including topical glucocorticoids) for at least 3 days. None of the patients had any signs or history of atopic disease. Patient 9 had psoriasis. Patient 14 suffered from seborrhoeic dermatitis and multiple sclerosis. All patients showed normal blood leukocyte and differential counts.

Informed consent, in accordance with the principles of the Helsinki Declaration, was obtained from all subjects.

Skin chamber technique

A dermabrasion was performed with surgical scalpels (nos. 10, 11, 20 and 22 Swann-Morton) after thorough cleansing of the skin with 70% isopropanol and 0.7% rodalon, on the inner surface of both forearms of the patients with nickel hypersensitivity and the right forearm of the controls.

The procedure of abrading was superficial and stopped when the area appeared uniformly glossy and the capillary network of the corium was visible as vividly red pin-points. The denuded area was variously sized between 2.5 and 6 cm^2. The abraded area was calculated by a metric method combined with weighing. The areas were determined in duplicate. The lesions were clinically evaluated at the termination of the experiment and 7 days later.

The skin lesion was covered with a sterile chamber of epoxy material as described by Wandall (26) with tubes and stopcocks to allow filling and emptying. The chamber was fixed with glue (Ducocement; Dupont de Nemours SA, Belgium) and hypoallergenic tape. Autologous serum was chosen as chamber medium, because it mimics physiological conditions (12, 13). Autologous serum was obtained in sterile tubes 24 hours prior to the experiment and stored at 4°C.

The nickel sulphate was mixed with the autologous serum just before the first injection into the chamber. The collection of chamber medium was routinely performed at 3, 6, 9, 12, 15, 17, 22, 24, 27, 30, 33, 36, 39, 41, 46, and 48 hours after abrasion. When not processed immediately, the aspirates were stored at 4°C. The chambers on the right arm of the eczema patients and the chambers of the controls were immediately refilled with 0.5 ml autologous serum containing 10×10^{-3} M nickel sulphate. The leukocytes were harvested from the chamber medium by centrifugation, resuspended, and counted in a modified Neubauer haemocytometer. The counts never showed more than 20 erythrocytes per 100 leukocytes. The cytology of the exudate was assessed by counting 100 cells on May-Grünwald-Giemsa stained coverslips.

Abbreviations

LMR-L: Leukocyte Mobilization Rate in the Left chamber, i.e. the number of leukocytes accumulating in the left chamber per hour per cm^2 abraded epidermis plotted mid-way between two collections.

LMR-R: Leukocyte Mobilization Rate in the Right chamber, i.e. the number of leukocytes accumulating in the right chamber per hour per cm^2 abraded epidermis plotted mid-way between two collections.

CLM: Cumulated Leukocyte Mobilization, i.e. the total number of leukocytes mobilized into the chamber per cm^2 abraded epidermis during a given period.

Statistical analysis

Analysis of variance in a linear normal model, applying logarithmic transformation of the LMR ratios and logarithmic transformation of the observed percentages of basophils and lymphocytes.

RESULTS

Clinical response evoked by exposure of nickel sulphate in chamber

After removal of the chambers at the end of the experiment all the nickel-allergic patients except nos. 4 and 7 showed erythema, infiltration, and papules or vesicles in the left skin window exposed to nickel sulphate. Patients 4 and 7 had erythema, but no vesicles. During the investigation period 5 patients (nos. 3, 6, 8, 9, and 10) complained of itching in the left skin window. Patient 8 had a particularly strong reaction at 24 hours, with oedema, infiltration and erythema extending 4 cm in all directions from the excoriation. This reaction lasted for about 10 hours. In this patient the increased leukocyte mobilization occurred very rapidly, with differential counts showing PMN (70–80%), lymphocytes (10–20%), basophils (5–10%), and monocytes (5%).

When the right chambers were removed, none of the patients, except no. 5, showed signs of eczematous reaction. Patient 5 reacted with vesicles, erythema and infiltration even in the right skin window. A subsequent patch test with the chamber material proved negative. No signs of eczema were present in the control group.

Chamber cell counts

The kinetics of the mobilized leukocytes showed no statistically significant difference ($p > 0.05$) throughout the investigation period either for the controls or for the right chamber without nickel in the nickel-

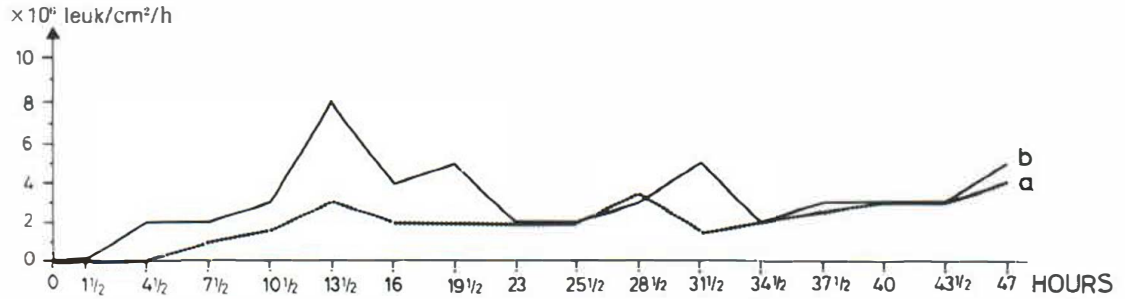

Fig. 1. a: Median values for the leukocyte migration rate in the right chamber (LMR-R) without nickel, from the nickel hypersensitivity patients. *b*: Median values for the LMR in the right chamber (LMR-R) without nickel, from the normal volunteers.

hypersensitive patients (see Table 1 and Fig. 1). The effect of nickel exposure upon the LMR is expressed as the difference between the LMR in the left chamber (LMR-L) containing nickel and the corresponding LMR in the right chamber (LMR-R) without nickel as a percentage of the LMR-R. The random intra-individual fluctuation of leukocyte mobilization in the left and right chamber is estimated during the first 15 hours. During this period an analysis of variance showed no statistically significantly difference ($p>0.05$) between the LMR-L and LMR-R. From the 15th hour up to the end of the experiment, 6 patients (nos. 1, 2, 3, 4, 7, and 8) had an increased leukocyte mobilization ($p<0.05$). Four patients (nos. 5, 6, 9, and 10) showed no statistically increased LMR values ($p>0.05$). These results are summarized in Fig. 2.

Cytology of the chamber exudates

The chamber cell population from the control group consisted throughout the collection period of 76 to 100% polymorphonuclear neutrophils (PMN), 0–19% lymphocytes and 0–9% monocytes and macrophages. The eosinophils and basophils never exceeded 3%.

The cell populations of the nickel-hypersensitive patients were identical for both chambers until the 15th hour sample. From the 15th hour up to the end of the experiment the differental counts for the chamber without nickel consisted of 75–96% PMN, 1–23% lymphocytes, 1–6% monocytes and macrophages, and 1–6% basophils. The eosinophils never exceeded 2%.

Differential counting in the chamber with nickel revealed 55–100% PMN, 0–35% lymphocytes, 1–9% monocytes and macrophages, 1–13% basophils, and 1–22% eosinophils.

The differences between the percentage appearance of basophils in the left chamber containing nickel and the corresponding percentage of basophils in the right chamber without nickel are depicted in Fig. 3. During the first 15 hours no statistically significant difference ($p>0.05$) between the basophils in the two chambers was observed, but during the second period, from the 15th to the 48th hour, all patients had a statistically significant increased basophil mobilization ($p<0.01$) in the nickel-exposed chamber.

The corresponding curves for the differences of the percentage appearance of lymphocytes in the

Table 1.

Diagnosis	No. of subjects	Leukocytes per chamber per cm² × 10⁶, medium (range) Time of collection 15 hours	24 hours	48 hours
Normal	5	115 (71–363)	265 (125–640)	645 (368–1 193)
Nickel-allergic without nickel exposure	10	188 (57–406)	307 (200–1 950)	700 (375–6 165)
Nickel-allergic with nickel exposure	10	290 (84–445)	588 (275–2 450)	1 412 (800–7 725)

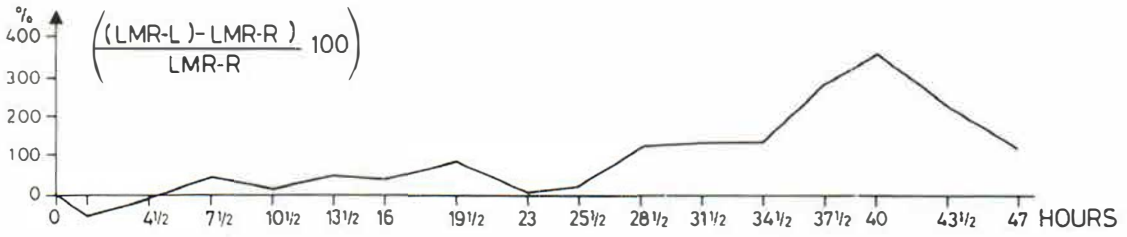

Fig. 2. Median values for the difference between LMR in the left chamber (LMR-L) containing nickel and the corresponding LMR in the right chamber (LMR-R) without nickel, expressed as a percentage of the LMR-R, from the nickel hypersensitive patients.

two chambers are shown in Fig. 4. During the second period the picture is much more diffuse. Patients 1, 3, 6, 8, 9, and 10 had a statistically significantly ($p<0.01$) increased percentage of lymphocytes in the chamber with nickel.

In 2 subjects, nos. 4 and 7, the increase in lymphocytes first appeared at the end of the second period, whereas at the beginning of that period they showed decreased values. No difference was observed in patients 2 and 5 ($p>0.05$). The overall effect of nickel in the chamber upon the cell composition in the exudate is illustrated in Fig. 5. During the first 15 hours no difference between their cell compositions was noticeable, but subsequently a continuous increase in lymphocytes and basophils was observed. This increase occurred concomitantly with a decrease in PMN. The monocytes remained constant throughout the period.

DISCUSSION

Leukocyte mobilization in healthy persons has been described previously in studies using the skin window chamber technique. The methods and practical details of producing skin windows and the chamber media have varied. This might account for discrepancies in the values of mobilized leukocytes in different laboratories, even when almost identical methods were used, thus underlining the importance of using relative values and proper controls. Thus, Wandall (26) found a median cumulative mobilization of 74×10^6 leukocytes/cm²/24 hours and 200×10^6 leukocytes/cm²/48 hours, compared with 265×10^6 leukocytes/cm²/24 hours and 645×10^6 leukocytes/cm²/48 hours in the present study.

Much attention has been devoted during recent years to the cells participating in the delayed hyper-

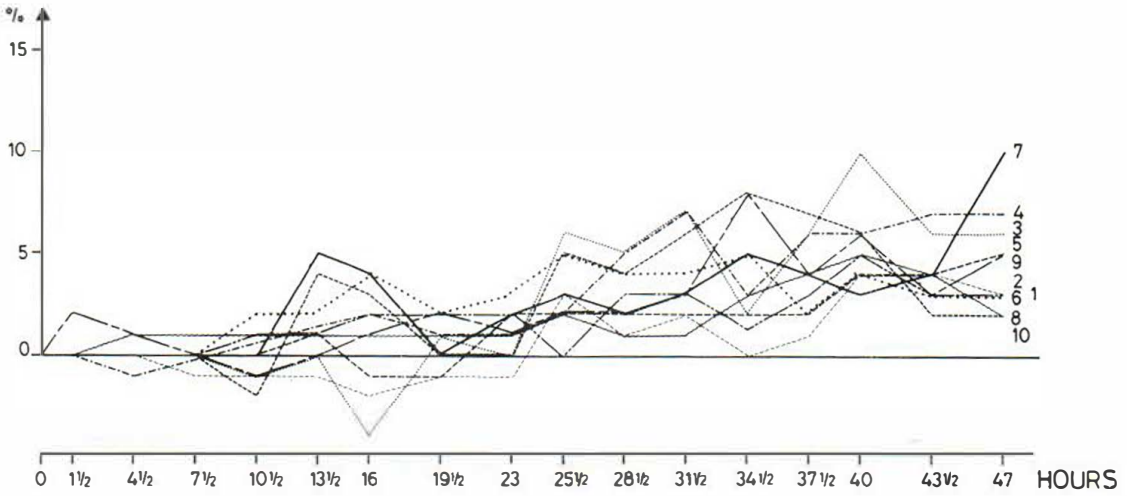

Fig. 3. Differences between the percentage appearance of basophil leukocytes in the left chamber containing nickel and the corresponding percentage of basophil leukocytes in the right chamber without nickel, from the nickel-hypersensitive patients.

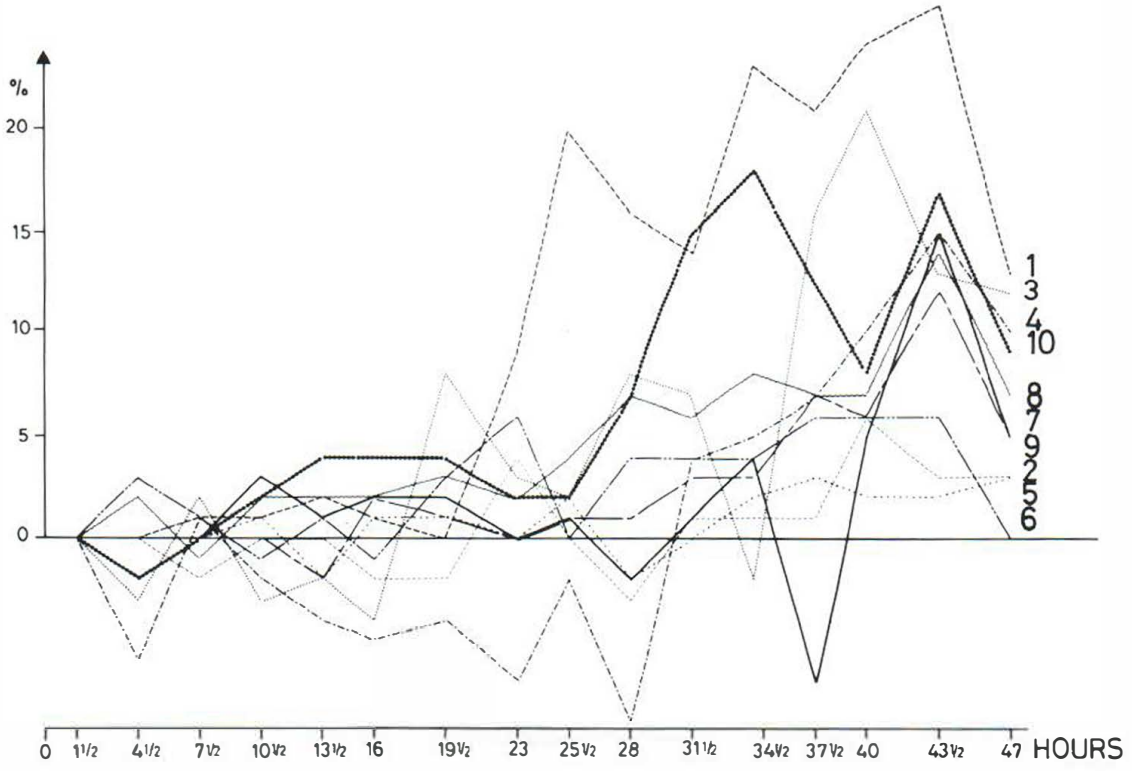

Fig. 4. Differences between the percentage appearance of lymphocytes in the left chamber containing nickel and the corresponding percentage of lymphocytes in the right chamber without nickel, from the nickel-hypersensitive patients.

sensitivity reactions. Particular attention has been attached to the role of the basophil leukocyte in allergic contact dermatitis. An increase in basophils in the exudate was found in allergic reactions after exposure to dinitrochlorobenzene (DNCB) by Wolf-Jürgensen (27) using the Rebuck skin window method. This observation has been confirmed by others, using the cantharidin blister technique (1, 16) and histopathological evaluation (7, 13). Aspegren et al. (1) reported a marked increase in basophils in exudates from positive allergic patch tests. No increase was found in toxic reactions. In an extensive histological study, Dvorak & Mihm (6) investigated the participation of basophil leukocytes in the lesions of allergic contact dermatitis. The initial lesion consisted of perivascular accumulations of lymphocytes, followed by an influx of basophils (max. between 24 and 48 hours) and, subsequently of eosinophils. Such reactions parallel the reaction of cutaneous basophil hypersensitivity as described in experimental animals (7).

All the nickel allergic patients reacted with a statistically significant elevated basophil mobilization from the 15th to the 48th hour and a unique migration pattern (see Fig. 4), compared with the other cell types in the exudate.

Whereas the basophil cell has been a characteristic finding, the remaining cell composition differs substantially in these studies. The coverslip technique used by Wolf-Jürgensen (27) gave a predominance of macrophages at 6 to 36 hours. With the chamber technique there was a predominance of PMNs during the 48-hour investigation period, although the PMN response declined after 20 hours with a parallel increased mobilization of basophils and lymphocytes. The macrophage response seen with the coverslip technique may represent a foreign body reaction (14, 19) against the glass coverslip or an artefact due to procedure.

The lymphocytes displayed a greater individual fluctuation in the second period from 15 to 48 hours than any other cell type (see Fig. 5). The factors responsible for this phenomenon are not known. Accumulated metabolic products, or generation

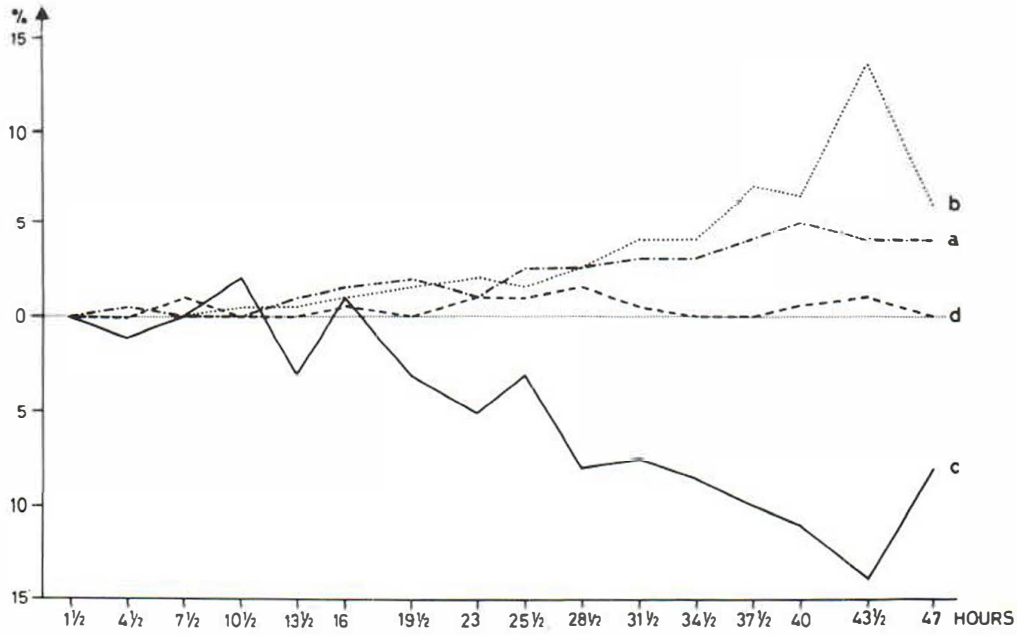

Fig. 5. All the curves are from the nickel-hypersensitive patients, plotted at the collection time. *a*: Median values for the differences between the percentage appearance of basophil leukocytes in the left chamber (containing nickel) and the corresponding right chamber (without nickel). *b*: Median values for the differences between the percentage appearance of lymphocytes in the left chamber (containing nickel) and the corresponding lymphocytes in the right chamber (without nickel). *c*: Median values for the differences between the percentage appearance of polymorphonuclear neutrophil granulocytes (PMN) in the left chamber (containing nickel) and the corresponding PMN in the right chamber (without nickel). *d*: Median values for the differences between the percentage appearance of monocytes in the left chamber (containing nickel) and the corresponding monocytes in the right chamber (without nickel).

and/or release of lymphokines, especially lymphotoxin (18), may be one reason. In the in vitro lymphocyte transformation test (25), not all the nickel-allergic patients had an increased lymphocyte transformation with nickel sulphate. The mechanism behind the unexpected, lower lymphocyte response could be due to the same unknown factors as observed in our study.

The nickel exposure had no apparent influence on the percentage content of eosinophils, monocytes and macrophages in the chamber exudate, compared with the corresponding chamber without nickel.

The improved skin window technique used by us in nickel-hypersensitive patients allows the appropriate allergen to remain in continuous contact with the denuded skin over a long period. We were surprised to find 4 patients (nos. 5, 6, 9, and 10) with a non-significant increase in LMR in the nickel-containing chamber. Patient 9 had psoriasis, besides the nickel allergy, and this may explain the slow LMR (3, 19). Patient 5 had a positive skin reaction on both the left and right arm, suggesting an abnormal general reactibility. However, this was not followed by an increased LMR in either the left or right chamber. The maximum LMR occurred between 24 and 48 hours after the initial nickel exposure in all patients, but showed a great individual variation with regard to time of maximum response. This observation may parallel the individual difference in reaction time after exposure of an allergen when patch testing (8).

Our studies have not provided evidence of any difference in the leukocyte mobilization or differential counts of the chamber exudate in the non-exposed skin window from the nickel-allergic patients, compared with the healthy individuals. The present results suggest that the nickel concentration used in this study merely alters the LMR and the cell composition and causes increased basophil mobilization at the application site. By the skin window chamber technique a relative quantitative study of the cyto-

logy of the exudate under the evolution of an eczematous reaction can be made. Furthermore this technique makes possible investigation of biochemical changes of viable 'target' cells in delayed hypersensitivity reactions.

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