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List of publications

The thesis is based on the following papers, which are referred to in the text by Roman numerals:

- I. Hindsén M, Christensen OB. Delayed hypersensitivity reactions following allergic and irritant inflammation. *Acta Derm Venereol* (Stockh) 1992; 73: 220–1.
- II. Hindsén M, Christensen OB, Möller H. Nickel levels in serum and urine in five different groups of eczema patients following oral ingestion of nickel. *Acta Derm Venereol* (Stockh) 1994; 74: 176–8.
- III. Hindsén M, Bruze M, Christensen OB. The significance of previous allergic contact dermatitis for elicitation of delayed hypersensitivity to nickel. *Contact Dermatitis* 1997; 37: 101–6.
- IV. Hindsén M, Bruze M. The significance of previous contact dermatitis for elicitation of contact allergy to nickel. *Acta Derm Venereol* (Stockh). (Accepted for publication).
- V. Hindsén M, Bruze M, Christensen OB. Flare-up reactions after oral challenge with nickel in relation to challenge dose, intensity and time of previous patch test reactions. (Submitted).
- VI. Hindsén M, Bruze M, Christensen OB. Individual variation in nickel patch test reactivity. (Submitted).
- VII. Hindsén M, Bruze M, Christensen OB, Schütz A. Nickel levels in urine and faeces following oral ingestion of nickel in atopics and non-atopics. (Submitted).

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Abbreviations

ICDRG International Contact Dermatitis Research Group
MEC Minimal eliciting concentration
SLS Sodium lauryl sulphate
STS Summarized test score
TIBC Total iron binding capacity
U-Ni Nickel in urine

Introduction

HISTORICAL BACKGROUND

Several hundred years ago the miners in Saxony found a red ore (1), which was thought to be copper. No copper could be extracted (2), however, so the ore was considered to be bewitched by the "mountain troll" and it was called "Kupfer-Nickelin" (1). In the middle of the eighteenth century the Swedish mineralogist Axel Fredrik Cronstedt identified nickel from the ore "Nickelin" (2). The expression 'nickel' originates from Nicholas (3). However, alloys with nickel were known in China hundreds of years before Cronstedt's investigation: they were known as *pai tung* (2).

At the beginning of the nineteenth century, nickel-copper-zinc alloys were sometimes used as a substitute for silver, because they were less expensive, and in 1857 nickel was used for the first time in coins (2). About twenty years later, nickel alloys were used in the steel industries (2), the first patent on nickel plating being issued in 1880 (4). Up until the end of the First World War, nickel was used mostly in military-related industries (2).

The first description of nickel dermatitis was given in Berlin in 1889 by Blaschko, who described it as "Das Galvaniseur-Ekzem" (5). Several similar reports from nickel platers followed from all over the world of a majority of workers in steel industries with the same kind of dermatitis (6-9). According to many reports (10-12), the first patch test with nickel sulphate was performed in 1925 by Schittenhelm and Stockinger in Kiel (6).

Until the 1930s, it had been thought that nickel dermatitis was an affliction peculiar to industry, but then came the first report of its occurrence as a result of handling consumer products (12). In 1930 Rothman described dermatitis from coins (13), in 1931 Lain saw three patients with dermatitis caused by spectacle frames made of "white gold" (14), and in 1933 Fox reported dermatitis from spectacles and a wrist watch (15). In his 1939 thesis, Bonnevie described many patients with nickel dermatitis from everyday products, mostly suspenders (16).

Of nickel dermatitis cases reported since the 1930s, the majority have been caused by everyday objects, e.g. jewellery, metal clothing, hairpins, keys and scissors. In the same period, occupational nickel dermatitis has declined (10).

NICKEL ALLERGY AND NICKEL DERMATITIS

Diagnostic tools

Patch testing. -

Since Jadassohn introduced the "Funktionelle Hautprüfung" in 1895 (17), epicutaneous testing has been used to diagnose contact allergy. As already mentioned, the first patch testing with nickel was performed in 1925 (6); this test was positive only on previously affected skin. Today, nickel is probably included in standard series all over the world, and usually as nickel sulphate 5% in petrolatum (18-20), although lower concentrations are used (20).

Different times for reading have been compared (21). A higher test concentration of nickel sulphate has been tried as well as another nickel preparation, nickel chloride (6, 22, 23). Occlusion time, also a factor of importance for patch test reactivity (24), has been shown to decrease if the concentration of nickel sulphate increases (25). The vehicle, too, is of significance for patch test reactivity (26, 27), as are regional variations (28, 29).

Intradermal testing. -

When a patch test reaction to nickel is doubtful, or there is a negative test reaction in a patient with a history of nickel dermatitis, intradermal testing has been recommended (30). In this test, 1 mM (0.016%) of nickel sulphate in saline is injected intradermally on the volar part of the forearm. Reading in this test is performed on Day 3. With regard to contact allergy to nickel, intradermal and patch testing give equivalent results, although the intradermal test is more sensitive (31, 32). Intradermal testing with nickel chloride has also been tried (33).

Results of patch testing. -

The number of nickel patch-test-positive patients is high and steadily increasing in the Western world, as disclosed in several reports of patch testing with a standard series (Table I). As indicated in Table I, which gives selected results from different decades, most patients with nickel allergy are female. The sex difference is small in Nigeria (38), and from Kuwait a ratio females:males of 1:3 has been reported (41). In the Marcussen (35) study, a probable relationship between

Table I. Selected results from nickel patch testing in consecutive dermatitis patients (references are given in parentheses)

Origin year	No. of patients			Percentage nickel positive		
	Females	Males	Total	Females	Males	Total
Denmark (34) 1934-April 1936	-	-	1223	8	1	5.2
Denmark (35)						
1936	-	-	-	-	-	7.4
1955	-	-	-	-	-	12.9
Europe (36) 1969	2786	2039	4825	10.2	1.8	6.7
Scotland (37) 1977	709	603	1312	25.4	4.9	16.0
Nigeria (38) 1985	223	230	453	12.4	11.0	11.7
Eastern Europe (39) 1987	1487	913	2400	10.5	2.1	7.3
Malmö, Sweden (40) 1997	205	85	290	29.8	5.9	22.7

The table is modified after Menné T, Christophersen J, Green A (12).

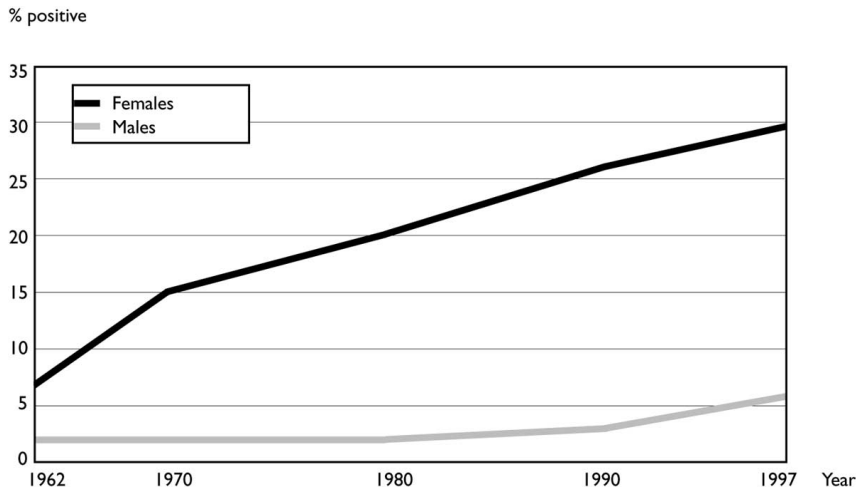


Fig. 1. Percentage of patch-tested patients with contact allergy to nickel at the Department of Dermatology in Malmö, 1962–1997.

the importation of nickel and nickel alloys on the one hand and the presence of nickel allergy on the other was seen in Denmark. With increasing use, more subjects were sensitized.

The increase in contact allergy to nickel, particularly in females in Malmö, Sweden, is demonstrated in Fig. 1.

Prevalence of nickel allergy in the general population. –

There are also a few studies of patch testing in the general population. With reservations about variation in the test technique, the prevalence of nickel allergy in females in the healthy population is around 10%. This is the case for the USA (42), Finland (43), Sweden (44) and Denmark (45). In Singapore, however, the results were higher 19.1% (46). In males, the prevalence is generally reported to be lower, 0.8–2.2% (43, 45), but in Singapore it is 9.3% (46).

Nickel exposure

Nickel is ubiquitous. About 0.008% of the earth's crust consists of nickel, and it occurs in soil, water, air and in the biosphere (47). Nickel occurs as a sulphide in the northern hemisphere and in tropical zones as an oxide (48). It is a common industrial metal (48) used mainly in the production of stainless steel and other nickel alloys with a high corrosion and temperature resistance (49). Nickel alloys and nickel platings are used industrially in the processing of machines, tools and electrical equipment (50, 51). It is also used in catalysts and pigments as well as in batteries (50, 51), but also in everyday products such as hair pins, jewellery, zippers, buttons, needles, pins, and spectacle frames (51). Because nickel is ubiquitous (47), exposure to it can occur through the skin by airways and the gastrointestinal tract.

Cutaneous nickel exposure. –

Although occupational nickel exposure used to be the main skin contact source (10, 52), major nickel exposure today is from everyday products and jewellery (10, 51, 52). The release of nickel from these products varies (53–56) depending on the corrosion resistance of these objects (52, 57). The mode of sensitization is through skin contact (52) and there is a clear

relationship between ear-piercing and nickel allergy (44, 45, 58–61). In 1994, the European Union issued a directive (62) on the release of nickel from metal objects in cutaneous contact. Occupational nickel contact has diminished (10, 52), but in some occupations exposure to nickel may still cause skin problems (52, 63–68).

Airway nickel exposure. –

Nickel is emitted into the air from both natural and man-made sources (52, 57). In certain industries, increased nickel exposure into the airways is seen (52) causing asthma and rhinitis (69–72) and there is a carcinogenic risk, too (52).

Oral nickel exposure. –

Nickel received daily through food varies in amount (52, 57, 73–76). The nickel content in different plants and animals varies (52, 57) and, with regard to plants, the highest levels are found in soya beans, nuts, oatmeal, cocoa and buckwheat (52, 57).

Nickel may also be present in food as a result of nickel-releasing utensils and of storage in nickel-releasing packages (52, 57). Several reports have been produced on the release of nickel from stainless steel (11, 77, 78).

Iatrogenic nickel exposure. –

Iatrogenic exposure to nickel means exposure to metallic implants, e.g. in orthopaedic surgery (screws, plates, prostheses, wires), but also as cardiac valve replacement, nickel dental prostheses, and release from intravenous fluids contaminated with nickel (52, 57, 79–81).

In reports on dermatitis said to be caused by the metallic implant (82, 83), retrospective (84) as well as prospective (85, 86) studies have shown the risk to be negligible.

Nickel detection. –

Individuals with contact allergy to nickel should minimize prolonged skin exposure to it. To detect nickel release in metal objects, the dimethylglyoxime spot test can be used.

First described by Feigl (87) and later modified (88, 89), one drop of 1% of alcoholic solution of dimethylglyoxime and one drop of 10% aqueous solution of ammonium hydroxide are applied on a cotton applicator and rubbed on the object; a pink colour is seen if nickel is released from the object. The test is only positive if more than 10 µg of nickel is released. New nickel detection methods are being prepared in accordance with the European Committee of Standardization (90, 91). Other methods used to detect nickel are atomic absorption spectrophotometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS).

Nickel dermatitis

Nickel dermatitis has been separated into primary and secondary eruptions (92–94). Primary eruptions are seen on direct metal contact sites. Secondary eruptions, seen in a high percentage by Calnan, Marcussen and Wagman (92–94), were mostly seen in the elbow flexures and eyelid, but also on the hands, arms and shoulders (92–94). These secondary eruptions were mostly eczematous, but erythema multiforme-like pictures and urticaria were also observed. In the discussion concerning the theory behind these secondary eruptions (51, 92), some researchers have suggested that they represent manifestations of systemic contact dermatitis (92). Others, however, doubt this (51).

Nickel allergy and hand eczema. –

The frequency of hand eczema is high among patients with nickel allergy, as indicated in Table II. The results of these studies may not be comparable, however, because neither the type of hand eczema nor its severity is always defined in the different studies.

With regard to localization of hand eczema in nickel allergy, pompholyx has been reported to be the most common variant, but any type may be encountered (11, 101). Patch test studies on pompholyx have shown nickel to be the most frequent allergen (102, 103). In females with hand eczema, nickel allergy is seen in about 12% (104).

Hand eczema usually has a multifactorial background, but occasionally a single nickel exposure may cause hand eczema. However, in nickel-allergic persons, hand eczema is often seen

Table II. *Hand eczema in patients with nickel allergy (references are given in parentheses) (Ni=Nickel)*

Study	Total no. of Ni-positive patients		% hand eczema in Ni-positive patients
	Females	Males	
Calnan 1956 (92)	400		20
Fisher, Shapiro 1956 (95)	36	4	40
Marcussen 1960 (10)	552	69	44
Cronin 1972 (96)	84		60
Christensen 1975 (11)	165		52
Menné 1982 (97)	279		43
Gawkrodger 1986 (98)	119	15	49
Moorty 1986 (99)	63	37	25

The table is modified after Wilkinson DS, Wilkinson JD (100).

in combination with wet work (105). Of several possible explanations, a simple one would be a straight summation of an irritant and an allergic contact dermatitis, or an enhanced nickel penetration due to skin irritation. A more complex interaction has been suggested in some experimental data. Earlier studies showed increased reactivity on a previous irritant dermatitis (106) and, when patch testing with nickel in combination with SLS an increased reactivity was seen (27). On the other hand, hyporeactivity has been observed when testing with SLS on sites where SLS had been applied daily for 3 weeks (107). This was seen after 6 and 9 weeks, but not after 3 weeks. It was unclear whether or not this hyporeactivity exists also when nickel is applied on previous irritant dermatitis sites.

The significance of a previous allergic contact dermatitis for elicitation of an allergic contact dermatitis on topical exposure to the same allergen or another allergen is also unclear. Obviously, further studies are needed to establish whether a previous dermatitis, caused by the same allergen or another one, or an irritant, is of any significance in the development and strength of an allergic contact dermatitis from nickel. As well as the exogenous factors mentioned above there could be others contributing to the hand eczema in nickel allergic individuals.

An association between nickel allergy and atopy has been reported by some authors (31, 39, 60, 99, 108, 109) but not by others (59, 92, 93, 110, 111). Patients with nickel allergy and hand eczema of the pompholyx type have been found to have a high frequency of atopy (11). In this latter study, the patients with nickel allergy, hand eczema of the pompholyx type, and atopy were found to have had a bad prognosis (11).

Systemic contact dermatitis. –

Over the past twenty years, several studies (Table III) have shown that oral challenge with nickel in nickel allergic individuals causes a flare-up at earlier or present eczema sites, including vesicular hand eczema and earlier patch test sites.

The flare-up reactions shown in these studies seem to be dose-dependent, but no clear dose-response is seen. The significance of the time interval between flare-up reactions after oral challenge and previous eczema has been unclear, as also has the importance of the intensity of the previous eczema for the flare-up reactions.

Nickel is ingested daily through food in widely varying quantities (52, 57, 73–76). The nickel challenge doses in nickel challenge studies were usually higher than the expected average intake in food. It remains unclear whether or not the daily intake of nickel in food is of any significance for allergic contact dermatitis to nickel.

AIMS OF THE STUDY

The purposes of the present investigation were to study:

- the variation in nickel patch test reactivity in nickel-hypersensitive females
- the development of nickel dermatitis after topical challenge with nickel on previous nickel dermatitis sites with regard to time between previous dermatitis and challenge
- the development of nickel dermatitis after topical challenge with nickel on previous irritant contact dermatitis sites

Table III. Some results of a single oral nickel challenge in patients with positive patch tests to nickel; the table shows the flare-up of eczema

Study	Method	Amount of nickel (mg)						No. of patients
		0.6	1.2	1.25	2.24	2.5	5.6	
Christensen Möller (11)	Double-blind placebo controlled						9/12	12
Kaaber et al. (112)	Single-blind placebo controlled	1/14	1/14			9/14		14
Cronin et al. (113)	Open	2/5		3/5		5/5		15
Sertoli et al. (114)	Double-blind placebo-controlled				13/20			20
Bedello et al. (115)	Placebo controlled				31/49			49
Roduner et al. (116)	Double-blind placebo- controlled					8/19		19

- the development of nickel dermatitis after topical challenge with nickel on a site with previous allergic contact dermatitis induced by another allergen
- flare-up reactions after oral challenge with nickel in relation to nickel dose, time after previous eczema, and intensity of previous eczema
- the urinary and fecal excretion of nickel after oral nickel administration with special reference to atopic subjects.

MATERIAL AND METHODS

Subjects

One-hundred-and-seventy-three females were enrolled in the seven studies (I–VII). As shown in Table IV, they belonged to different groups. The studies were approved by the Ethics Committee of Lund University Medical Faculty and informed consent was obtained from each patient.

Test substances

Nickel sulphate hexahydrate ($\text{NiSO}_4 \times 6\text{H}_2\text{O}$) 5% in petrolatum from the European standard series (Chemotechnique Diagnostics, Malmö, Sweden) was used in study I.

Nickel sulphate hexahydrate of high quality ($\text{NiSO}_4 \times 6\text{H}_2\text{O}$)

(Merck, Germany) was used to prepare aqueous test solutions (I, III, IV, V, VI, VII). In the first study (I), test solutions at 2.4%, 0.60% and 0.15% w/v were used. A stock solution of 12.5% w/v was made and further diluted with a factor of 2.5 down to 0.0013% w/v (III, IV, V, VI, VII) (Table V).

An aqueous stock solution of cobalt chloride hexahydrate, high quality ($\text{CoCl}_2 \times 6\text{H}_2\text{O}$) (Janssen Chimica, Belgium) at 7.8% was prepared and further diluted with a factor of 2.5 down to 0.20% w/v (IV) (Table V).

An aqueous solution of sodium lauryl sulphate (SLS) (Sigma Chemical, USA, 95% purity) at 2.0% was prepared and diluted with a factor of 2.0 down to 0.25% w/v (IV) (Table V). An aqueous solution at 3.0% SLS was prepared and used in one study (I).

Patch testing

Patch testing was performed in accordance with internationally accepted methods (117) using small Finn Chambers, diameter 8 mm (Epitest Ltd Oy, Finland), on Scanpor (Norgesplaster A/S, Norway) (I, III, IV, V, VI, VII). The tests were applied for 2 days and read 3 days after application (117). Tests with SLS (IV) were applied for 1 day (121) and reading was performed 3 days after application. In study VI, repeated patch testing (4 times) was performed during a 7-month period in females allergic to nickel. The design of study VI is shown in Fig. 2.

Table IV. No. of females in the 7 studies

Paper	No. of females	Nickel allergy*	Atopy**	Pompholyx***	Seborrheic dermatitis****	Cobalt allergy*	Controls*****
I	13	+					
II	12	–	+				
	11	–	+	+			
	10	+					
	10	+		+			
	9	–	–		+		
III	15	+	–	–			
	10	+	–	+			
IV	23	+				+	
V, VI	12	+	+	+			
	18	+	–	–			
VII	15	–	+				
	15	–	–				+

*Nickel and cobalt allergy was diagnosed according to ICDRG criteria (117).

**Atopic skin diathesis was diagnosed in studies III, V, VI and VII

according to the criteria of Svensson et al. (118). In study II, atopic skin diseases were defined as a previous or present lichenified, flexural dermatitis and/or head- neck- and shoulder dermatitis.

***Pompholyx was diagnosed as a recurrent vesicular palmar dermatitis (119).

****The diagnosis of seborrheic dermatitis was based on history and the criteria of Braun Falco et al. (120).

*****Mostly healthy hospital workers.

Table V. Aqueous solutions of nickel sulphate, cobalt chloride and SLS used for patch testing in various studies

	Concentration % w/v	Study					
		I	III	IV	V	VI	VII
Nickel sulphate	12.5		+	+	+	+	+
	5.0		+	+	+	+	+
	2.4	+					
	2.0		+	+	+	+	
	0.80		+	+	+	+	
	0.60	+					
	0.32		+	+	+	+	
	0.15	+					
	0.13		+	+	+	+	
	0.051		+	+	+	+	
	0.020		+	+	+	+	
	0.0081		+	+	+	+	
	0.0032		+	+	+	+	
0.0013				+	+		
Cobalt chloride	7.8			+			
	3.1			+			
	1.2			+			
	0.50			+			
	0.20			+			
SLS	2.0			+			
	1.0			+			
	0.50			+			
	0.25			+			

Evaluation of patch tests

Patch tests were scored according to ICDRG criteria: + = erythema, infiltration, possibly papules; ++ = erythema, infiltration, papules, possibly vesicles; +++ = intense erythema, infiltration, vesicles (I, III, IV, V, VI, VII) (123). In four of the studies (III, IV, V, VI), additional gradings were used, strong + and ++ reactions were graded + (+) and ++(+), respectively.

Reading of the patch test was done "blindly" in four studies (III, IV, V, VI), the reader of the tests not knowing the patient, the group she belonged to, or the result of previous dermatitis or of previous tests. At reading, the patients were covered with a cloth with only the back exposed.

Experimental contact dermatitis

Study I. -

In order to provoke allergic contact dermatitis, patch tests with 2.4% aqueous nickel sulphate on small Finn Chambers on Scanpor were used (I). To induce irritant contact dermatitis the skin was exposed to two different substances (I). An aqueous solution of SLS 3.0% was

applied on small Finn Chambers on Scanpor. The test units were removed after 48 h and the tests were read 24 h later. Dithranol cream 1.0% (Micanol, Hydropharma AB, Sweden) was tested simultaneously using Finn Chambers on Scanpor, but removed after only 1.5 h; reading was performed at the same time as SLS. The design of study I is shown in Fig. 3.

Studies III and IV. -

Using serial aqueous dilutions of nickel sulphate (III, IV) and cobalt chloride (IV) the lowest test concentration given a ++ reaction was defined. Of this threshold concentration, 1.0 ml of nickel sulphate and cobalt chloride, respectively, were micropipetted onto 6.0 × 7.0 cm filter papers and attached to 8.0 × 9.0 cm hydrocolloid dressings (Duoderm, Convatec, Denmark). The hydrocolloid dressing test was applied under an adhesive tape (Mefix, Mölnlycke, Sweden) to the lower back to induce the experimental allergic contact dermatitis. The hydrocolloid dressing was removed after 2 days and reading was performed 1 day later. The design of study III is shown in Fig. 4.

To induce irritant contact dermatitis the same procedure as with experimental allergic contact dermatitis was used (IV). The lowest test concentration in the preceding patch testing with SLS that gave a ++ reaction was used; 1.0 ml of the SLS solution was micropipetted onto 6.0 × 7.0 cm filter paper and attached to an 8.0 × 9.0 cm hydrocolloid dressing (Duoderm, Convatec, Denmark) and held with adhesive tape (Mefix, Mölnlycke, Sweden) attached to the lower back. The hydrocolloid dressing was removed after 1 day. Reading was performed 2 days later. The design of study IV is shown in Fig. 5. The numbers of patients provoked with nickel sulphate, cobalt chlorid, SLS and dithranol are given in Table VI.

Evaluation of experimentally induced allergic and irritant contact dermatitis

Nickel, cobalt and SLS dermatitis were evaluated 72 h after application to ensure that the dermatitis was as intended (III, IV).

Localization of previous test areas

To enable localization of previous experimental allergic and irritant dermatitis, as well as previous patch test sites (I, III, IV, V), we measured distances from different points of the back and in studies I and IV the patients also marked the areas with a skin marker.

Oral nickel challenge

Nickel absorption. -

To investigate the nickel levels in serum, urine and faeces, the patients ingested a capsule containing 4.48 mg nickel sulphate ($\text{NiSO}_4 \times 6\text{H}_2\text{O}$) in lactulose, the nickel content being 1.0 mg nickel (II, VII). The capsule was taken at 08.00 h. The patients were not allowed to eat or drink from midnight and were instructed not to eat or drink until 1 h after the nickel ingestion. The design of study II is shown in Fig. 6.

The patients also swallowed 1 Carminum capsule 0.2 g in lactulose 1 h before the nickel capsule and 24 h later (VII). The Carminum

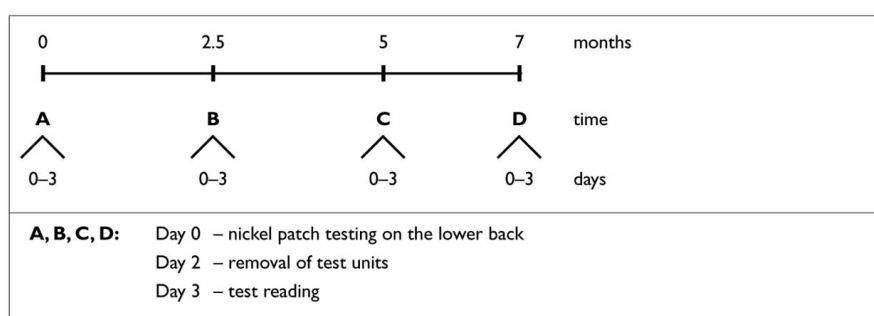


Fig. 2. Design of study VI. Individual variation in nickel patch test reactivity.

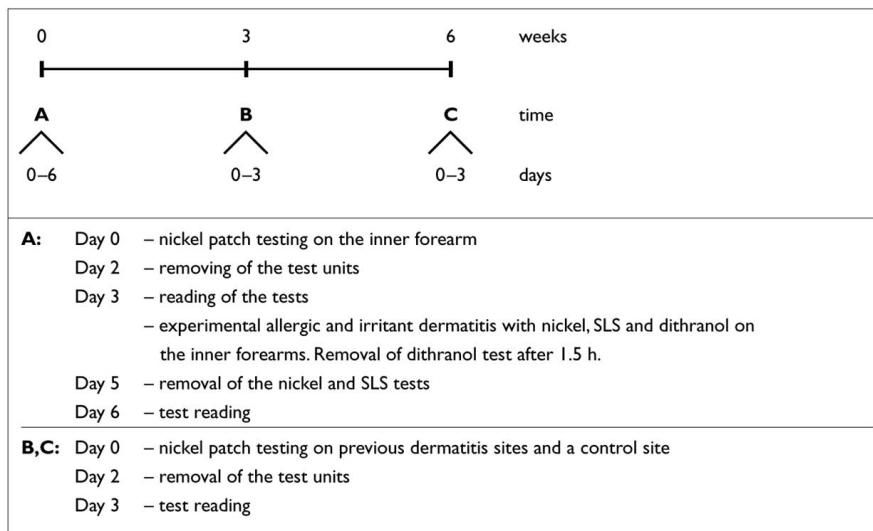


Fig. 3. Design study I. Delayed hypersensitivity reactions following allergic and irritant inflammation.

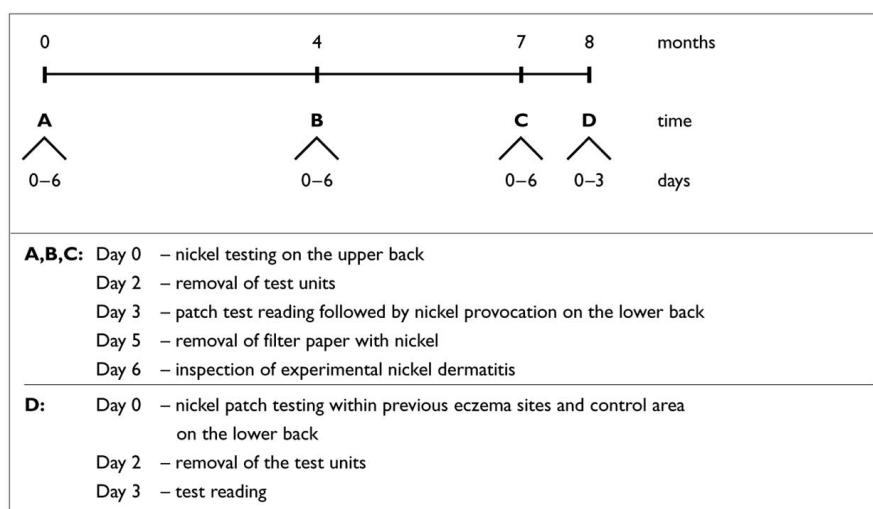


Fig. 4. Design of study III. The significance of previous allergic contact dermatitis for elicitation of delayed hypersensitivity to nickel.

capsule was taken to give the faeces a red colour to enable registration of the time course of the faeces passage. The design of study VII is shown in Fig. 7.

Flare-up reactions. –

To study the flare-up reactions in nickel-hypersensitive patients, a double-blind design was used (V). Ten patients were given 13.44 mg nickel sulphate ($\text{NiSO}_4 \times 6\text{H}_2\text{O}$) in lactulose with nickel content 3.0 mg; 10 patients were given 4.48 mg nickel sulphate ($\text{NiSO}_4 \times 6\text{H}_2\text{O}$) in lactulose with nickel content 1.0 mg; and 10 patients ingested a lactulose placebo capsule (V). The nickel capsule was taken at 08.00 h. The patients were not allowed to eat or drink from midnight and were instructed not to eat or drink until 1 h after the nickel ingestion.

Evaluation of flare-up reaction

The flare-up of previous patch test reactions was scored according to ICDRG criteria. The evaluation was done blindly, the patient covered with a cloth with only the back exposed. After this reading, other localized and/or systemic flare-up reactions were looked for. The design of study V is shown in Fig. 8.

Determination of nickel in blood, urine and faeces

Atomic absorption spectrophotometry. –

To identify and quantify the nickel content in serum and urine, an atomic absorption spectrophotometer (AAS) equipped with a graphite furnace was used (II).

Inductively coupled plasma mass spectrometry

Nickel in urine and faeces was determined by inductively coupled plasma mass spectrometry (ICP-MS). Prior to analysis, urine was diluted $\times 10$ with alkaline reagent, while faeces was at first digested using microwave equipment, and then diluted with deionized water (VII). The detection limit was 0.9 $\mu\text{g/l}$ for urine and 18 ng/g for faeces.

Statistical calculations

The scores were transformed to numerical values to enable statistical calculations – = 0, (+) = 0.5, + = 1, ++ = 1.5, +++ = 2, +++(+) = 2.5, ++++ = 3. Based on these numerical scores the challenge patch test reactivity was calculated in two ways: (i) the scores for all reactions representing one area were summed (summarized test scores = STS) and for the same area, (ii) the

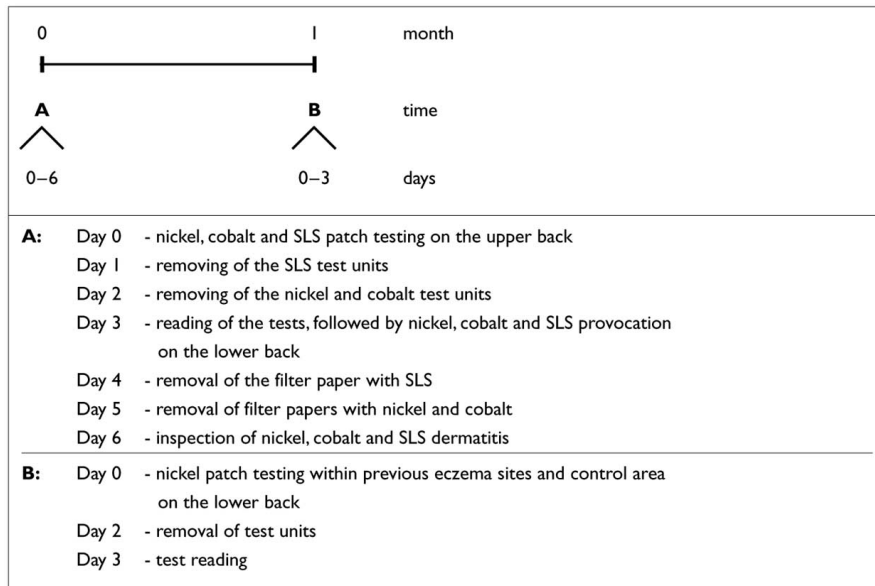


Fig. 5. Design of study IV. The significance of previous contact dermatitis for elicitation of contact allergy to nickel.

minimal eliciting concentration (MEC) (108), which was defined as the lowest concentration eliciting at least a + reaction was registered (III, IV, V, VI).

The positive test reactions were not always continuous. When negative and/or doubtful reactions were followed by the same number

or more positive reactions, the lowest positive concentration was registered as MEC. In all other situations the concentration above the first negative or doubtful reaction was registered as the MEC. For example, a patient could have positive reactions to 12.5, 2.0 and then 0.051%, meaning that 0.32% was negative or doubtful. In this case, 0.051% was registered as the MEC. In the seven studies the following statistical methods were used.

- Wilcoxon rank sum test (I)
- Multiple regression analysis (II)
- Page test and one-sided Wilcoxon signed-rank test (III)
- Friedman's test and two-sided Wilcoxon signed-rank test (IV)
- Kruskal-Wallis test, Mann-Whitney, Page's trend test, Wilcoxon signed-rank test as well as the Cochran Armitage trend test (V)
- Friedman's test, two-sided Mann-Whitney test, Spearman's rank correlation coefficient (rs) (VI)
- Mann-Whitney, Wilcoxon signed-rank test and Spearman's rank correlation (rs) (VII).

Table VI. No. of patients provoked with different concentrations of nickel sulphate, cobalt chloride, SLS and dithranol to induce an experimental contact dermatitis

	Concentration % w/v	Allergic study			Irritant study	
		I	III	IV	I	IV
Nickel sulphate	12.5		15	3		
	5.0		6	2		
	2.4	13				
	2.0		11	6		
	0.80		21	5		
	0.60					
	0.32		16	3		
	0.15					
	0.13		1			
	0.051		3			
0.020		2				
0.0081						
0.0032						
0.0013						
Cobalt chloride	7.8			6		
	3.1			3		
	1.2			4		
	0.50			1		
	0.20			5		
SLS	3.0				13	
	2.0					8
	1.0					5
	0.50					4
	0.25					2
Dithranol	1.0				13	

RESULTS

Repeated patch testing

In study VI, repeated patch testing was performed four times during a 7-month period. The MEC and STS for all patients are given in Table VII. The lowest eliciting concentration was 0.0032% in one patient, while two patients had completely negative test results on one test occasion. On comparing the four different test occasions nobody showed the same test reactivity. An individual variation of 250 times according to MEC was observed when comparing the two most divergent MECs in one patient.

There was no difference in test reactivity between the two groups atopy and non-atopy, nor was there any difference with regard to age. For the menstrual cycle there was a significant correlation on test occasions B and C, but not A and D. This correlation showed that MEC decreased, i.e. the sensitivity increased, with increasing number of days in the menstrual cycle. In the other study with repeated patch testing with a serial dilution with nickel sulphate (III) we also found

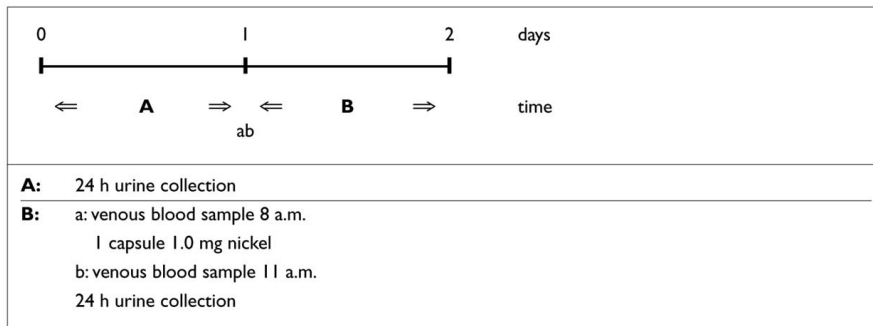


Fig. 6. Design of study II. Nickel levels in serum and urine in five different groups of eczema patients following oral ingesting of nickel.

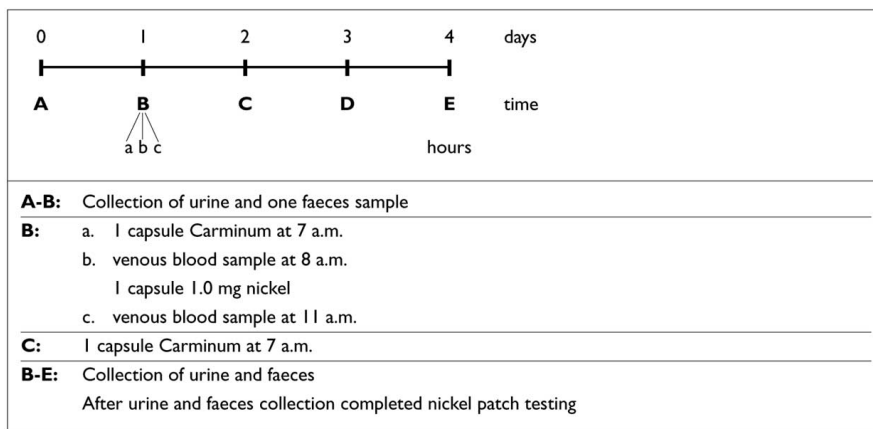


Fig. 7. Design of study VII. Nickel levels in urine and faeces following oral ingestion of nickel in atopics and non-atopics.

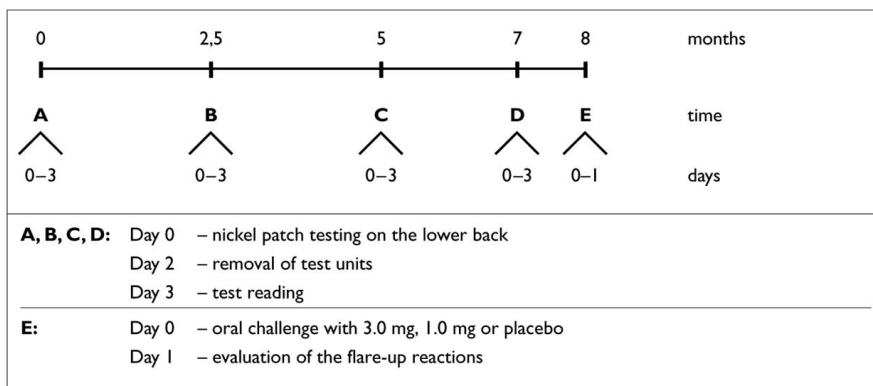


Fig. 8. Design of study V. Flare-up reactions after oral challenge with nickel in relation to challenge dose, intensity and time of previous patch test reactions.

a large variation in test reactivity, 100 times between the two most divergent MECs in one patient.

Nickel dermatitis on previous allergic and irritant contact dermatitis

A marked difference in test reactivity between nickel site and control site was illustrated by a difference in STS

after 3 weeks ($p=0.007$) and 6 weeks ($p<0.005$), respectively (I). There was no significant difference after 3 or 6 weeks between the control and dithranol or SLS sites (I).

When testing with a serial dilution of nickel sulphate on three earlier nickel eczema sites (III) where there had been nickel eczema 8, 4 and 1 month before as well as a control site, with Page test, a statistically significant tendency was found for both STS and MEC ($p=0.009$ for STS and

Table VII. Study VI. The minimal eliciting concentration (MEC) and summarized test score (STS) for all patients. (Patients 1–18 non-atopics, 19–30 atopics)

Patient	MEC:STS on test occasions A–D							
	A		B		C		D	
1	0.8	:4.5*	0.8	:10.5	0.32	:13	2.0	:8
2**	>5.0	:0*	>12.5	:0	>12.5	:0	>12.5	:0.5**
3	0.8	:8*	0.32	:14	0.8	:10	0.32	:12.5
4	2.0	:5.5*	0.32	:12.5	0.13	:16.0	0.008	:19
5	0.008	:19*	0.13	:12	0.02	:18.5	0.0032	:25
6	0.8	:6*	0.8	:12	0.32	:10	0.32	:11
7	5.0	:2*	12.5	:1	12.5	:0.5	12.5	:2
8	>5.0	:0*	0.8	:4	12.5	:1	5.0	:2
9	0.32	:7*	0.02	:15	0.05	:14	0.13	:12
10	0.8	:4*	5.0	:5.5	0.32	:8	2.0	:8
11	2.0	:1.5*	5.0	:3.5	5.0	:3	5.0	:3
12	>5.0	:0.5*	5.0	:3	12.5	:1	12.5	:1.5
13	2.0	:6	0.8	:11	0.32	:12.5	12.5	:3.5
14	2.0	:8	0.13	:11.5	2.0	:5.5	0.05	:11
15	0.8	:8.5	0.05	:12.5	0.13	:15.5	0.02	:16
16	5.0	:1.5	12.5	:1	5.0	:2.0	12.5	:1.5
17	0.8	:7	0.8	:6	12.5	:1.5	5.0	:3.5
18	2.0	:3.5	0.32	:8.5	>12.5	:0	2.0	:5
19	5.0	:3.5*	0.8	:11	0.32	:7	5.0	:3.5
20	0.02	:8.5*	0.13	:13	0.13	:6	5.0	:5
21	2.0	:2.5*	12.5	:1	5.0	:4	>12.5	:1
22	0.32	:7.5*	0.13	:9.5	5.0	:4	5.0	:5
23	0.02	:14.5*	0.05	:12.5	0.32	:12	0.13	:15
24	0.8	:1.5*	0.8	:7	0.8	:5.5	5.0	:6
25	0.8	:3*	2.0	:8	0.8	:6.5	2.0	:7
26	>5.0	:1*	>12.5	:0.5	12.5	:3	12.5	:2
27	5.0	:2*	0.8	:7	0.13	:13	0.32	:9
28	0.05	:5	0.32	:12	0.13	:14.5	2.0	:7.5
29	0.8	:4.5	2.0	:7	5.0	:5	0.32	:10
30	5.0	:2.5	0.13	:8	0.8	:6.5	5.0	:5

*=The highest test concentration of nickel sulphate was 5.0%.
 **=was excluded from the study.

p=0.006 for MEC). Furthermore, it was found that the shorter the time interval to the challenge the stronger the reaction. Statistical test results are given in Table VIII. Rank sums of STS and MEC for the nickel reactions on the lower back are presented in Figs 9 and 10.

When testing with a serial dilution of nickel sulphate on two earlier allergic contact dermatitis sites, nickel and cobalt,

and one earlier irritant contact dermatitis, SLS, and a control site (IV), a difference in reactivity for STS as well as for MEC was observed when considering the four test areas together (p<0.001). The highest reactivity for both STS and MEC was noticed for the nickel area followed by cobalt and the blank, and finally the SLS area with the lowest reactivity (Figs 11 and 12).

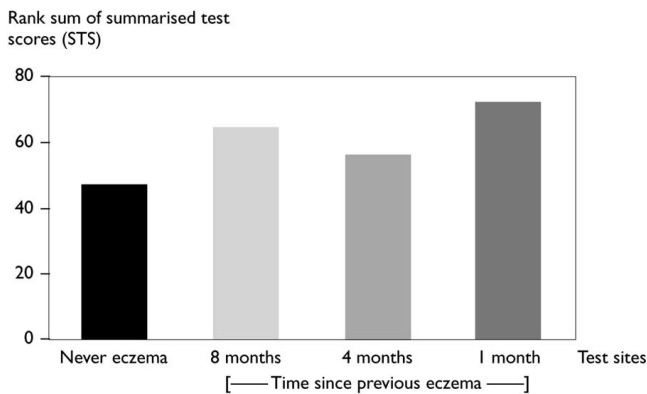


Fig. 9. Study III. Rank sum of summarized test scores (STS) indicating time interval since previous nickel dermatitis.

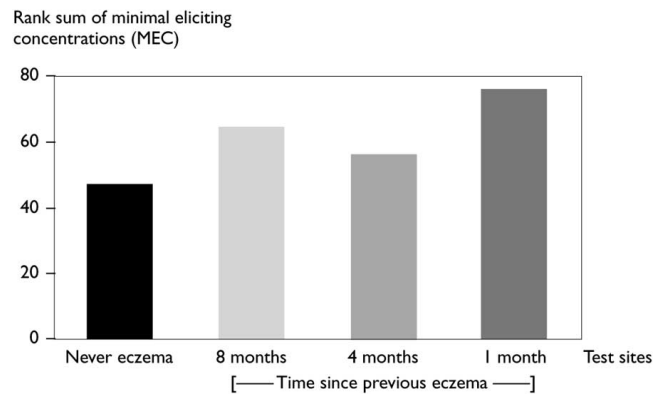


Fig. 10. Study III. Rank sum of minimal eliciting concentrations (MEC) indicating time interval since previous nickel dermatitis.

Table VIII. Study III. Statistical test results (*p*-values). Pair-wise comparisons*

Response	Page test	4 months* vs. 1 month	8 months* vs. 4 months	Never eczema* vs. 8 months
STS	0.009	0.04	0.5	0.03
MEC	0.006	0.12	0.3	0.03

One-sided Wilcoxon signed-rank test; *p*-values corrected for multiple comparisons according to the Bonferroni method.

Rank sum of minimal eliciting concentrations (MEC)

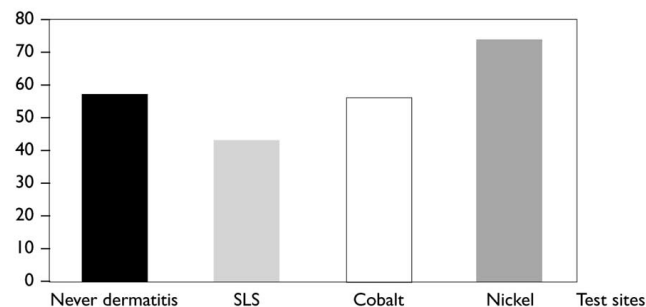


Fig. 11. Study IV. Rank sum of minimal eliciting concentrations (MEC) for the nickel reactions on four test sites on the lower back.

Rank sum of summarised test scores (STS)

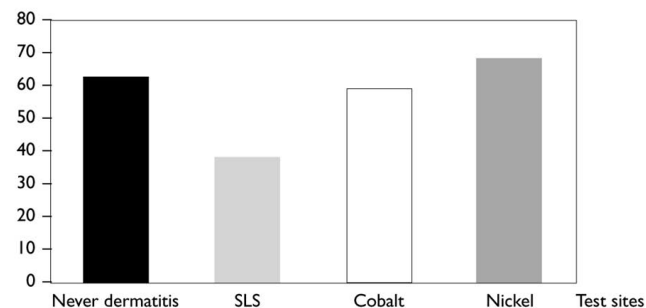


Fig. 12. Study IV. Rank sum of summarized test scores (STS) for the nickel reactions on four test sites on the lower back

Flare-up of previous positive patch tests after oral challenge with nickel

The flare-up reactions were found to be related to the nickel challenge dose (V) (Fig. 13). Flare-up reactions were related to time after previous patch tests. There were significantly more flare-up reactions at the youngest patch test sites (1 month old) than at the oldest (8 months old) test sites (Fig. 14). There was a statistically significant, positive correlation between the intensity of previous positive patch tests and the flare-up reactions (Fig. 15).

Nickel levels in serum and urine

Nickel concentration in urine was found to decrease with increasing age ($p < 0.01$) (II). When difference in age between

Positive reactions in %

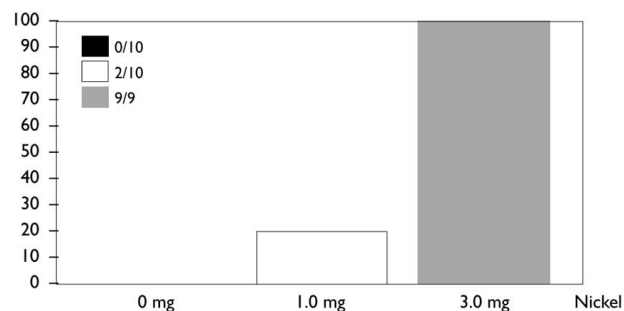


Fig. 13. Study V. The flare-up reactions were related to nickel challenge dose.

the eczema groups was taken into account, the levels of nickel in urine were significantly higher ($p < 0.005$) in the respective atopy groups compared to the control group. There was no significant difference in the mean value of nickel in serum between the five groups (II).

Nickel levels in urine and faeces

The urine nickel (U-Ni) during Days 2, 3 and 4, respectively, was numerically higher in atopics than in controls, but no significance was seen (Fig. 16). The cumulative excretion of faeces after oral nickel challenge was lower ($p = 0.075$) among the atopics than among the referents; median 1150 μg (range 240–1700) versus 1620 μg (460–3010) (VII).

Among atopics, a positive correlation between U-Ni Days 1–3 and TIBC was indicated (Day 1: $r_s = 0.66$, $p = 0.02$; Day 2: $r_s = 0.49$, $p = 0.09$; Day 3: $r_s = 0.56$, $p = 0.05$).

DISCUSSION

Variation in patch test reactivity

Since epicutaneous testing was introduced a hundred years ago (17), this has been an important tool for diagnosing contact allergy. In principle, contact allergy, once established, persists throughout life.

In study VI there was a large variation in patch test reactivity when testing with a serial dilution of nickel sulphate four times during 7 months. To my knowledge, repeated patch testing over such an extended period of time has not been performed previously. There have also been reports of variations in nickel patch test reactivity from one test occasion to another, with initial positive tests becoming negative at retesting (122–124). Sometimes these negative nickel reactions have been considered as being examples of disappearance of the contact allergy. Indeed, this may be the case, at least theoretically, but a negative test reaction could represent either a demonstration of the absence of contact allergy or a false negative reaction (125). To elicit a positive patch test reaction in a hypersensitive subject, the migration of a certain number of molecules of the sensitiser into the skin is required (125). Unfortunately, there is no biological response which with certainty will guarantee or exclude allergy, which means that the interpretation of a negative patch test reaction is never that there is no allergy, but that no allergy has been established (125).

In study VI, for a period of 1–10 years prior to the

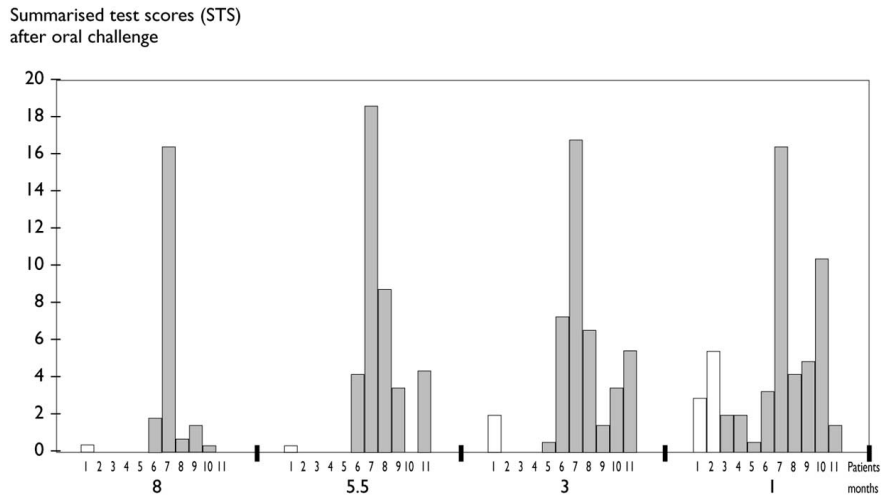


Fig. 14. Study V. Summarized test scores (STS) of flare-up reactions after oral challenge 1–8 months after nickel testing in 11 patients (□ = oral dose 1.0 mg, ■ = oral dose 3.0 mg).

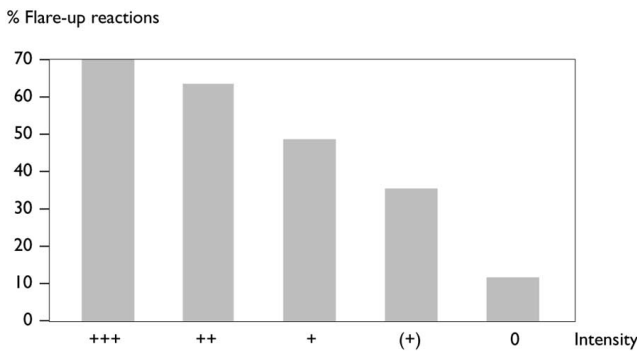


Fig. 15. Study V. The proportion of previous patch test reactions with flare-up after oral nickel challenge with regard to the intensity of previous test reactions.

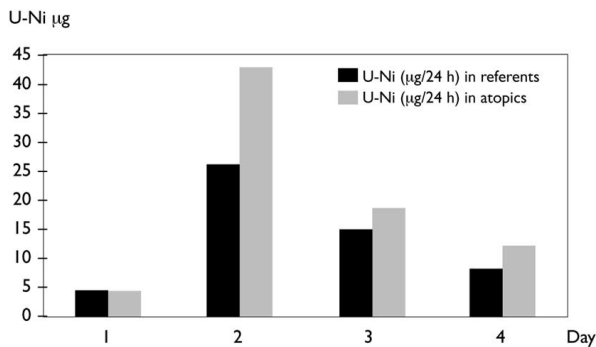


Fig. 16. Study VII. The daily amounts of nickel excretion in urine in atopics and referents (U-Ni = urine nickel).

investigation, all patients had been patch-tested with the ICDRG standard series (117), including nickel sulphate 5% and, on that occasion, had at least a ++ reaction. None of the patients showed the same patch test reactivity on all four occasions. In the present study the variation factor concerning the two most divergent MECs was 250. Two patients had completely negative test results on one test occasion, negative

to 12.5%, but a positive reaction to 0.32% on another test occasion (difference by a factor of 40).

There seem to be three major explanations for this large variation in patch test reactivity: technical, anatomical and immunological. We have tried to reduce the technical factors which might influence the patch test response. The same test system has been used. Even if it is possible to keep the occlusion time and dose constant, there will always be a variation in occlusion pressure. The variation in penetration of nickel into the skin from the patch test units can be investigated by cleansing the skin and extracting and analysing residual nickel in the patch units. We do not believe that variation due to technical factors could explain this big difference, however.

In study VI, the lowest positive concentration eliciting a positive reaction in one patient was 0.0032%. In a later study (III), where we tested the patients with the same serial dilution of nickel sulphate three times during 7 months, we found a variation in patch test reactivity up to 100 times. In this latter study, four patients reacted to 0.0032% and no patients were completely negative on any test occasion. The difference between the two studies was the localization of the tests. In study III, the tests were applied on the upper part of the back, in study VI on the lower back. In earlier studies, a variation in patch test reactivity due to anatomical region has been shown (28, 29) and in one study (28), a stronger patch test reactivity was found on the upper part of the back than on the lower. In order to exclude anatomical variations as a cause of varying patch test reactivity we eliminated this factor by using symmetrical parts on the lower back, and the skin areas have been patch tested in a randomized order.

Earlier studies have suggested that the patient's immunological status might be influenced by and vary according to stage in the menstrual cycle. Studies and case reports have shown increased test reactivity both to allergic and irritant reactions premenstrually (126–128). We found an increased premenstrual test reactivity on two test occasions, not, however, on the other two. Other authors have not seen any increased test reactivity before menstruation (129). Further studies are needed. There was no difference in patch test reactivity correlated to atopy.

When comparing interindividual test reactivity to serial dilutions of nickel sulphate we found about the same frequency of positive and negative reactions disappearing or appearing, respectively, from one test occasion to the next. We also found that the weaker the test reactivity the more prone it was to disappear, which concurs with the results of an earlier study (122).

Patch testing with serial dilutions of nickel sulphate enables calculation of the dose-response curves for the elicitation. Although we know that the patch test reactivity differs between nickel-allergic subjects and also intraindividually on different occasions, as shown in study VI, we usually think of nickel allergy as one and the same allergy in all subjects (130). However, at least theoretically there might be more than one type of nickel allergy, possibly depending on the formation of various nickel-protein complexes with different configurations and thus possibly different antigenic determinants (130). The sensitizing and elicitation capacity may differ between these complexes, and consequently the dose-response curves for elicitation will differ. Still, for one subject the dose-response curve for nickel may be expected to be the same for the various test occasions but shifted in parallel due to the variation in patch test reactivity. Indeed, when using the same statistical method as Andersen et al. (131) to calculate and compare the intra-individual dose-response curves for the four test occasions, the curves represented one principal dose-response relationship. Furthermore, the dose-response curves for all nickel-allergic females all had the same shape; there is thus no indication of more than one type of nickel allergy (130). The dose-response curve is illustrated in Fig. 17. Interestingly, this curve has the same shape as the dose-response curve for nickel reported by Andersen et al. (131).

Obviously, it is very important to bear in mind these results with a large variation in patch test reactivity when a patient has negative or doubtful test reactions but a history of allergic contact dermatitis. In this situation, the patient should be retested at a later date. The variation in nickel patch test reactivity may also be important clinically in those individuals in whom contact allergy to nickel has been established. To arrive at the diagnosis of allergic contact dermatitis from nickel there must also be a current exposure to nickel

explaining the dermatitis under investigation with regard to localization and course (132). In a nickel-hypersensitive person with the hands constantly and equivalently exposed to nickel, a chronic type of hand eczema is expected. This expectation assumes a stable nickel reactivity which, however, is not the case, at least in females, as shown in study VI. Whether this variation in nickel reactivity will also be reflected in the course of hand eczema from nickel is not known, but if it is the significance of the nickel exposure will readily be overlooked with possible individual consequences for diagnosis, treatment, rehabilitation and prevention of allergic contact dermatitis from nickel. This issue should be investigated with use tests with nickel and with concomitant correlations to present nickel patch test reactivity.

It should be pointed out that the present results and conclusions were obtained when testing with nickel in females. Whether this variation in test reactivity also exists in males or to other allergens is unclear.

Nickel eczema on previous dermatitis

Occasionally, a single nickel exposure may cause hand eczema which is then transient. Sometimes continuous nickel exposure is responsible for long-lasting hand eczema, which heals when the nickel exposure is eliminated. However, hand eczema usually has a multifactorial background with the combination of both endogenous and exogenous factors. Among the exogenous factors, we can identify both physical factors and chemicals which can be both irritants and sensitizers. The relative significance of the different contributing factors will differ in subjects with hand eczema (132), and, furthermore, may vary from time to time. Clinically, this means that the nickel exposure in an allergic person can cause, provoke and aggravate any preexisting dermatitis. If we are to gain a better understanding of the development of hand eczema, we need much more knowledge about the separate contributing factors regarding the significance of recurrent and continuous exposure, in particular to the interplay of the different factors (132). In studies I, III and IV the significance of a previous allergic or irritant contact

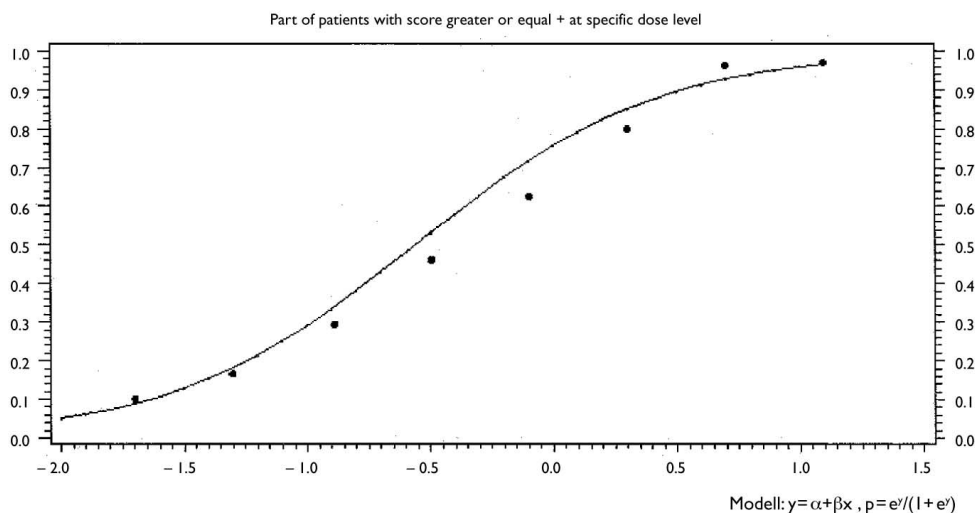


Fig. 17. A logistic model was used for the dose-response relation. Data from 20 patients were used.

dermatitis for the elicitation of allergic contact dermatitis from nickel on topical exposure was studied.

Significance of previous allergic contact dermatitis from nickel

We found increased nickel reactivity on previous nickel eczema sites compared to previous non-eczema sites (I, III, IV). We also found this re-test reactivity to be time-related (III). The shorter the time interval between previous eczema and topical challenge the stronger the reaction. Even after 8 months the test reactivity, compared to non-eczema sites, was increased.

Significance of previous allergic contact dermatitis from cobalt

No increased reactivity to nickel was observed on previous cobalt eczema sites compared to non-eczema sites (IV). The increased reactivity to nickel on a previous nickel dermatitis site therefore seems to be specific. Other studies also point in that direction. Thus oral challenge with nickel may cause a flare-up on previous nickel dermatitis sites, but no flare-up on sites of previous irritant dermatitis or at tuberculin test sites (133). In addition, in contact allergy to gold, systemic administration of gold induced flare-up of previous patch test reactions to gold but not at other patch test sites (134).

Significance of previous irritant contact dermatitis from SLS

An irritant contact dermatitis can be induced both physically and chemically. Unlike the allergic contact dermatitis, where the allergic inflammation is considered to be the same independently of the sensitiser, the first events in the inflammation resulting in the irritant contact dermatitis may differ due to the irritant involved through the target of the initial damage. Therefore, it is not obvious that the results obtained when one particular irritant has been used to induce contact dermatitis can be generalized to all irritants. In study I, dithranol and SLS were used to induce irritant contact dermatitis. These substances were chosen as we had had some experience with them at the department, and SLS is a commonly used irritant in experimental work. When nickel was challenged topically on skin with a previous irritant, contact dermatitis from dithranol and SLS, respectively, the nickel reactivity was unchanged 3 and 6 weeks after the induction of the experimental contact dermatitis. In this study (I), there was no testing with serial dilutions of SLS and dithranol prior to the provocation; i.e. no particular attention was paid to the intensity of the experimental contact dermatitis. Stronger reactions were therefore seen in some patients. In study V, the intensity of the experimentally induced contact dermatitis was significant for the subsequent flare-up reactions after oral nickel provocation. Because of this finding, efforts were made in study IV to get the experimentally induced contact dermatitis from nickel, cobalt and SLS with an intensity of the epidermal inflammation as equivalent as possible. With the design used (IV), a decreased nickel reactivity was demonstrated on topical challenge on a previous irritant contact dermatitis site. Hyporeactivity caused by SLS has earlier been observed in skin where SLS

has been applied once daily for 3 weeks and then followed by topical SLS challenge 3, 6 and 9 weeks later (107). The hyporeactivity was only demonstrated at SLS challenge after 6 and 9 weeks (107). The mechanism of this hyporeactivity is unclear (107). SLS-induced hyporeactivity from skin applications has also been reported in skin distant from the SLS exposure sites (135).

Significance of previous contact dermatitis

Nickel allergy is very common (45) and is often found in adults with hand eczema (11). Occasionally, single nickel exposure may cause hand eczema and with a further exposure the nickel hyperreactivity may be significant for the elicitation. However, most hand eczemas have a multifactorial background. Clinically, the combination of wet work and nickel allergy, in particular, has been considered to constitute a high risk for the development of hand eczema (105). This opinion is also supported by experimental data. An enhanced reactivity to nickel was observed some hours after damage of the skin barrier by SLS (106). Patch testing with SLS and nickel in combination has also resulted in increased nickel reactivity (27). However, most hand eczemas are chronic, which means that both previous and present factors of possible significance for the hand eczema have to be considered. In a nickel-hypersensitive patient with previous nickel dermatitis (III, IV) a current irritant exposure (106) may enhance the hand eczema while previous irritant dermatitis may impair (IV). The interaction of these factors is unclear and further investigation is needed.

Flare-up of previous patch test reactions after oral provocation

Several studies have shown a flare-up of previous eczema, particularly hand eczema of the pompholyx type, after oral administration of the allergen (11, 112–116, 136–138). These flare-up reactions seem to be dose-dependent (Table III), but no clear dose-response relationship has been found. In our double-blind, placebo-controlled study (V) we found these flare-up reactions to be clearly dose-related. Furthermore, they were found to be time-related (V), the shorter the time between patch test and nickel challenge the more and stronger the flare-up reactions. Time relations have not been described until now, but there are anecdotal reports on flare-ups of 10–12-year-old eczematous reactions (11, 139). These flare-up reactions are also correlated to the intensity of the previous patch test reactions (V). The stronger the reaction the more likely a flare will occur. Interestingly, we also noticed a flare-up at test dilution sites where no visible reaction was seen at the primary testing. This phenomenon has also been documented in gold allergy (134). Phenomenologically similar are the “eczematous” histopathological findings in negative patch test reactions in hypersensitive individuals patch-tested with a serial dilution of the sensitiser giving both positive and negative reactions (140, 141). Kligman has called these negative patch test reactions “non-visible allergic reactions” (141).

The flare-up reactions described above were seen in experimental nickel challenges. The amount of nickel required was usually much higher than the average in the daily food intake (52, 57, 73–76). The reactivity to oral nickel challenge

varies, which may indicate an individual sensibility to nickel. Some of the patients did not react at all, some had flare-up reactions at earlier eczema sites, including previous patch tests, or even eczema at new sites. In many cases we have observed lesions after oral nickel provocation similar to "secondary eruptions" (92–94). It is not known whether or not there is an intraindividual variation in nickel reactivity after systemic administration of nickel, as in repeated patch testing. This question is very interesting in light of the possible relationship between hand eczema of the pompholyx type and nickel allergy. In patients in which systemic nickel is considered to be the incriminated agent for pompholyx, the hand eczema is often characterized by acute exacerbations. These are frequently considered to be explained by increased nickel ingestion due to nickel-rich food and/or by nickel released from cooking utensils (11, 77, 78). However, if there is a variation in the intraindividual nickel reactivity after systemic administration a constant systemic nickel exposure may still result in a pompholyx with exacerbations. This issue should be elucidated by a repeated procedure including patch testing followed by oral challenge with nickel.

It is presently unclear whether or not accidental ingestion of nickel contained in food stuffs has any significance for flare-ups or chronicity of nickel dermatitis. The experimental data are based on an inorganic nickel compound (nickel sulphate) and the patients have been challenged when fasting. We probably do not ingest any, or only minimal, amounts of inorganic nickel, and the nature of the organic nickel present in foodstuffs is not fully known. However, some clinical data indicate that dietary nickel may be significant for hand eczema, but it has to be kept in mind that this is a controversial topic.

A high-nickel diet has been shown to impair patients with nickel dermatitis (142). A low nickel diet has been tried (143, 144) with positive results in the majority of the patients, but no controlled studies have been performed. Urinary nickel excretion seems to increase in patients with a flaring nickel dermatitis (145). Consequently, blood and urinary nickel levels decrease when the nickel dermatitis clears after a low-nickel diet (146).

The importance of endogenous nickel has to be elucidated in studies with organic nickel in foodstuffs and not when the patients are fasting, as in these experimental nickel provocation studies.

Nickel in serum, urine and faeces

Experimental challenge with nickel orally in persons hypersensitive to nickel has frequently resulted in exacerbation of hand eczema of the pompholyx type. Nickel is ingested daily through food, but whether this is of any significance for nickel eczema is unclear, even though some clinical data indicate that it is. Earlier reports (11, 101) have shown that hand eczema is a frequent complication among patients with nickel allergy and the predominant type of hand eczema is pompholyx. Patients with the combination pompholyx, nickel allergy and atopy have been shown to have had a bad prognosis (11). Theoretically, there are three major possible explanations for the bad prognosis of the combination of nickel allergy, hand eczema of the pompholyx type and atopy (i). Nickel-allergic atopics have a stronger allergy to nickel than non-atopics, i.e. although atopics and non-

atopics most likely ingest the same amount of nickel, a lower number of nickel ions is required to be absorbed in atopics to cause systemic contact dermatitis from nickel. However, we did not find any difference in patch test reactivity between atopics and non-atopics (VI) (ii). The reactivity to nickel and the degree of absorption to nickel may be the same for atopics and non-atopics, but eating habits may differ with ingestion of more nickel-rich foodstuffs in atopics. However, although this question has not been directly addressed in our studies we do not have any indication of different nickel ingestion (iii). With the same reactivity and ingestion of nickel, an increased absorption of nickel in atopics could explain the combination of pompholyx, nickel allergy and atopy.

Studies II and VII were performed to investigate whether patients with atopy have increased absorption of urinary nickel. We found significantly increased nickel in urine (II) in atopics after oral challenge with nickel, which may indicate an increased absorption of nickel. To further investigate this possibility we also assayed nickel in faeces (VII) after oral nickel challenge. According to Sunderman (147), the intestinal absorption of nickel is best correlated to nickel excretion in urine provided the oral intake of nickel or amount of nickel excreted in faeces is known.

In study VII we collected urine during 3 days, and also faeces. The urinary nickel excretion in atopics increased (Fig. 16), albeit statistically not significant. In atopics we also found less nickel in faeces. As atopics seem to absorb more nickel than non-atopics, the decrease in faecal nickel in atopics seems logical. However, the decrease was too large to be explained by the increased nickel absorption as indicated by the urinary nickel excretion. Whether there is any depot in the body where nickel can be stored has to be investigated further. Other items to be elucidated in additional studies are the significance of organic nickel in foodstuffs when the participants are not fasting.

Earlier studies in rats have shown increased intestinal absorption of nickel in iron-deficient rats (148). Also, atopics have been reported to have significantly lower serum ferritin (149). We found significantly higher TIBC in atopics, and this was correlated to increased nickel in urine. This might indicate that the iron status of atopic females is of importance for the nickel absorption. These results may suggest interesting therapeutic possibilities interfering with the intestinal absorption of nickel in nickel-allergic atopics with hand eczema of the pompholyx type.

GENERAL SUMMARY AND CONCLUSION

In the present studies on nickel allergy I found a large inter- and intraindividual variation in nickel patch test reactivity when testing with a serial dilution of nickel sulphate four times during a period of 7 months. None of the females had the same test reactivity on the four test occasions. No convincing correlation to menstrual cycle was found. Therefore a negative test, despite a positive history of nickel allergy, may be an indication for a later retest.

There was also an increased nickel test reactivity at earlier nickel eczema sites compared to non-eczema sites. The test reactivity was stronger the more recently there had been an eczema before topical nickel challenge. Even at 8-month-old previous eczema sites the nickel test reactivity was increased compared to previous non-eczema sites. The increased nickel

reactivity was also found to be specific. The test reactivity increased only on earlier nickel eczema sites compared to earlier cobalt or SLS dermatitis sites. On the other hand, hyporeactivity was demonstrated when nickel was tested on a previous irritant (SLS) dermatitis site.

With topical exposure to nickel, earlier events on the skin area in question – specific or non-specific dermatitis – are crucial for the results of re-exposure. Time factors are most important.

In the study on oral provocation with nickel, the flare-up reactions were found to be clearly related to nickel dose. There were also significantly more flare-up reactions at the youngest patch test sites (1 month old) than at the oldest test sites (8 months old). There was a statistically significant positive correlation between the intensity of previous positive patch tests and the number of flare-up reactions. Interestingly, clinically negative patch test reactions (at the lowest concentration of serial dilutions) also showed flare-up reactions. With oral exposure to nickel, nickel dose, time between previous nickel eczema and oral provocation and intensity of previous test reactions are of vital importance for flare-up reactions.

I have also studied nickel in serum and urine in different eczema groups and found significantly more urinary nickel in young atopics than in controls after oral nickel provocation. In the last study when nickel in faeces and urine were analysed, an increased amount was demonstrated in urine in atopics, while a lower amount was found in faeces. There was also a significant positive correlation between urinary nickel and TIBC in atopics.

Orally administered nickel results in increased urinary and less increased faecal nickel excretion in atopics than in controls. This may be interpreted as increased intestinal nickel absorption and may explain the stronger clinical reactions seen in atopic subjects. The iron status of atopic females may have significance for nickel absorption, suggesting an interesting therapeutic possibility interfering with the nickel absorption.

The skin of a patient with contact allergy to nickel, although clinically normal, may retain a "memory" of earlier events, leading to stronger or weaker test reactions than expected. My studies on test reactivity of post-eczematous skin have thus demonstrated several factors of significance for the development of allergic contact dermatitis from nickel, with possible consequences for the diagnosis, treatment, rehabilitation and prevention of this disease.

COMPREHENSIVE SUMMARY IN SWEDISH

Kliniska och experimentella studier av nickelallergi

Nickelallergi är vanligt, särskilt hos kvinnor. Nickelallergi leder ofta till periodiskt eller kroniskt eksem, främst på händerna. Dessa eksem kan vara svårläkta och leda till långa sjukskrivningsperioder. Nickel finns överallt i omgivningen och det går inte att helt undvika exposition för denna metall.

Syftet med dessa studier har varit att få bättre kunskap om vilka faktorer som kan vara av betydelse för nickelallergiskt kontakteksem.

Hos patienter med känd nickelallergi noterades en förvånansvärt stor variation i reaktiviteten hos en och samma individ som allergitestades med nickel vid upprepade tillfällen.

Ingen patient uppvisade samma testreaktivitet vid alla testtillfällen. Även ett helt negativt testresultat noterades hos ett par patienter (delarbete 6).

I tre av delarbetena (delarbete 1, 3 och 4) studerades vad ett tidigare, men läkt eksem har för betydelse när huden åter exponeras för nickel. Ett tidigare nickelallergiskt eksem gav ökad reaktivitet och eksembenägenhet. Dessutom observerades en tidsrelation till det tidigare eksemet: ju kortare tid mellan tidigare eksem och förnyad nickelkontakt, desto kraftigare eksem. Även med det längsta tidsintervallet, 8 månader, fanns dock en viss ökad reaktivitet. Däremot registrerades en minskad reaktivitet och eksembenägenhet i ett hudområde där det en månad tidigare funnits ett icke-allergiskt kontakteksem orsakat av det hudirriterande ämnet natriumlaurylsulfat.

I delarbete 5 studerades uppflamningen av tidigare nickeleksem efter peroralt nickelintag. Vi fann att denna uppflamningsreaktion var relaterad till nickeldos och intensiteten av det tidigare nickeleksemet: ju kraftigare eksem, desto större benägenhet för uppflamning. Förvånansvärt nog påvisades även uppflamning av kliniskt negativa testreaktioner, d.v.s. hudområden som varit i kontakt med nickel utan att synligt eksem uppkommit. Tidsintervallet mellan tidigare eksem och nickelprovokationen visades även vara av betydelse: ju kortare tid mellan tidigare nickeleksem och nickelprovokation, desto större benägenhet för eksemet att flamma upp.

Om nickel tas in via magtarmkanalen ger detta ökad utsöndring av nickel i urinen hos främst atopiker (patienter med ärftlig benägenhet att utveckla eksem), liksom en mindre ökning av utsöndringen med avföringen. Sammantaget tyder detta på ökat upptag av nickel från magtarmkanalen hos atopiker. Handeksem av pomfolyxtyp har experimentellt provocerats med peroral tillförsel av nickel. Därför kan ökat nickelupptag hos atopiker eventuellt bidra till att handeksem av pomfolyxtyp hos atopiker med nickelallergi har speciellt dålig prognos. Dessutom påvisades hos patienter med atopi en korrelation mellan ökade nivåer av nickel i urinen och ett ämne i blodet (TIBC) som är involverat i järnomsättningen. Detta kan tyda på att järnstatus kan ha betydelse för nickelupptaget via magtarmkanalen med eventuella framtida möjligheter att behandlingsmässigt påverka nickelabsorptionen (delarbete 2 och 7).

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