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INVESTIGATIVE REPORT

Human Stratum Corneum Penetration by Nickel

In vivo Study of Depth Distribution after Occlusive Application of the Metal as Powder

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Sequential tape stripping was implemented on three healthy volunteers to examine the surface distribution of nickel through human stratum corneum *in vivo* following occlusive application of the metal as powder on the volar forearm. Exposure sites were stripped 20 times at intervals from 5 min to 96 h post-dosing and the strips analyzed for metal content by Inductively Coupled Plasma-Mass Spectroscopy with a detection limit for nickel of 0.5 ppb. The gradients of nickel distribution profiles increased proportionally with occlusion time, but after the 10th strip to the 20th strip continued at constant levels. Total nickel removed with 20 stratum corneum strips to the level of the glistening layer after maximum occlusion of 96 h was 41.6 μg/cm^2 ($\pm$ 12.2; average $n = 3$). In order to normalize the nickel depth distribution profiles, stratum corneum removed by stripping of untreated skin after occlusion was determined by weighing. Following application of nickel dust over 24 h, analysis of the 20th strip still indicated nickel present at 1.42 μg/cm^2 ($\pm$ 0.68; average $n = 3$). These data indicate that, in contact with skin, nickel metal is oxidized to form soluble, stratum corneum-diffusible compounds which may penetrate the intact stratum corneum, presumably by the *intercellular* route, and have the potential to elicit allergic reactions. ***Key words: adsorption; allergy; contact dermatitis; human; inductively coupled plasma, mass spectroscopy; in vivo; metal; nickel; shunt diffusion; stratum corneum; tape stripping.***

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As the most frequent allergen in industrialized countries, nickel can cause delayed as well as immediate type allergy following iatrogenic, respiratory, and gastrointestinal as well as dermal exposure (1–10). Sustained exposure to nickel metal dust in the industrial setting can induce various forms of dermatitis, collectively described as "plater's itch" or "nickel rash": papules, erythema, and vesicles progressing to weeping eczema (11). These effects in the epidermal tissues are an indication of the formation of a skin-diffusible form of the metal apt to penetrate beyond the stratum corneum (SC). Analysis of workroom air in processes such as welding and machining (12–14), of hard-metal grinding and nickel-refining (15–18), or mining operations (19) identified fine airborne metal dusts of a mean 3 µm particle size. Such dust settling on the skin surface presents a surface area optimal for chemical corrosion to occur rapidly, forming a variety of hydrophilic and lipophilic salts with the potential for diffusion.

In the present context, the term "stratum corneum penetration" is used to indicate exogenous material found in the SC, in contrast to the term "dermal absorption", which denotes permeant that potentially becomes available systemically. Despite many investigations into the behavior of nickel in contact with the skin, questions relative to its mode of action remain unanswered. One paradox in the behavior of nickel became apparent when it was investigated for skin diffusivity. In *in vitro* tests with human skin, permeability coefficients for water-soluble inorganic nickel salts were measured in the range 10^{-6} to 10^{-4} cm/h, with lag times of up to 90 h preceding the appearance of the permeant in the acceptor phase (20–24). This is difficult to reconcile with the phenomenon that even contact of intact skin with nickel-releasing metallic objects may elicit dermatitis in those allergic to the metal (25–28). Oxidation of nickel in sweat has been investigated repeatedly (29–34) and appears to be an important factor in the pathogenesis of nickel allergy. Released with sweat are chloride ions, butyric, pyruvic and lactic acid (35–37). Furthermore, the acid mantle of the skin contains urocanic and pyrrolidone carboxylic acid (38, 39), as well as fatty acids, primarily oleic and linoleic (40–43). These compounds are likely to solubilize metal in amounts apparently sufficient to elicit an allergic reaction when a nickel-alloy coin is taped to the skin of highly sensitive individuals (44–47).

Epidemiology suggests a steady increase in the occurrence of nickel allergic hypersensitivity in the general population. It is reasonable to assume that such reactions stem from frequent skin contact with nickel-containing metal objects in normal, everyday activities, and are not due to exposure to preformed, inorganic nickel salts such as sulfate or chloride as occur in industrial processes such as electrowinning and electroplating.

The purpose of the present investigation into the fate of metallic nickel brought into intimate contact with the skin was (i) to obtain evidence of nickel complexes which diffuse through the SC, (ii) to trace the path and kinetics of diffusion through the SC, and (iii) to confirm adsorption and reservoir formation by penetrating metal in the SC.

Investigation of the kinetics and penetration depth of permeants by tracing the concentration profiles in SC has been rendered facile by using the virtually non-invasive method of SC stripping with adhesive tape (48–52) and by the possibility of detecting minute quantities of nickel using ICP-MS analysis. It thus becomes possible to analyze for the presence of elements such as nickel in skin and other biological materials, with detection limits in the order of 0.5 ppb (53), making the use of radioisotopes unnecessary.

Thickness of the SC on the volar forearm has been measured in several volunteers, indicating a mean of 12.3 µm (54) and

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12.9 μm (55). Investigated by Schwindt et al., on average each tape strip removes one layer of corneocytes, approximately 0.5 μm thickness, and after the first two to three strips, for a given skin site and test subject each subsequent tape strip removes the same amount of SC down to approximately the 20th strip ($r^2 = 0.99$) (55). Although furrows in the SC account for the uncertainty in single-layer stripping (56), by progressive SC removal with 20 strips it nevertheless becomes possible to estimate actual nickel concentration profiles to the level of the living epidermal tissue.

MATERIALS AND METHODS

Subjects

Three healthy Caucasian volunteers of the Fitzpatrick skin type III (2 men, 1 woman; 31–62 years, mean 41) without anamnesis of nickel intolerance participated after giving informed consent. The study, conducted over a period of 2 years, was approved by the University of California Committee on Human Research. The skin on the volar forearm of the volunteers was not pretreated prior to dosing to avoid altering the natural state of the skin, e.g., by shaving or cleansing with water or detergent; informed consent was obtained from all subjects. Replicate experiments for statistical purposes were conducted on the same volunteer to minimize experimental variability. Dosing followed by stripping was avoided earlier than 30 days after a previous experiment.

Materials and chemicals

Nickel metal powder (99.7%; particle size 3 μm) was a commercially available grade purchased from the Aldrich Chemical Co. (Milwaukee, WI), and concentrated nitric acid from EM Science (Gibbstown, NJ). High purity deionized (DI) water was prepared by passing it through a bank of Milli Q (Millipore, USA) filters. The occlusive application system consisted of a plastic chamber (Hill-Top Res., Inc., Cincinnati) and transparent dressing (Tegaderm, 3M Health Care, St. Paul, MN). Polypropylene tape with a backing of pressure-sensitive acrylate adhesive (Transpore, 3M Health Care, St. Paul, MN) of 2.54 cm width was used for sequential SC removal by stripping. The term "tape" is used forthwith as a synonym for Transpore. A 5% solution of Extran 300 (EM Science, Gibbstown, NJ) in DI water was used for skin decontamination prior to stripping. A Gyrotory water bath (model G76; Edison, NJ, USA) was used for agitation of scintillation vials containing tape strips in acid. Tapes were weighed on a high-precision balance sensitive to 10 μg (Mettler AT 20).

Occlusion and stripping of untreated skin. For the purpose of normalizing nickel values, the weight of SC removed with sequential strips was determined on untreated skin, with 6 iterations for each occlusion period, on the volar forearm of one of the 3 participating volunteers. For occlusion of the SC, 12-mm plastic chambers (1.15 cm^2) were placed on the pre-marked area of the flexor surface on the arm, the chamber covered with transparent dressing and left for the predetermined length of time (5 min, 30 min, 3 h, 24 h, and 96 h). At the end of the occlusion period, the chamber was removed and the site covered with circles of tape (pre-cut with a 12-mm paper punch). Constant and uniform pressure (100 g/cm^2) was applied on the tape for 5 sec by resting an appropriate weight on the area, and the tape then gradually removed from the skin in one draw. The tape circles were weighed immediately before application and after removal to determine the stripped weight of SC. Room temperature and humidity were controlled at 18–20°C and a relative humidity between 50% and 55%. Stripping was repeated sequentially 20 times on each site of occlusion.

Nickel application, occlusion, decontamination and stripping

Nickel powder application and stripping after increasing intervals of occlusion involved three volunteers following an identical protocol. Prior to application, skin sites were cleansed with DI water and dried using cotton swabs. Nickel powder was applied on the volar forearm between wrist and antecubital fossa. Stripping was conducted during the months of October through March, so the exposed skin of the volunteers exhibited no noticeable tanning. Twenty-five milligrams of nickel powder was placed on a 12-mm plastic chamber (1.15 cm^2) and the chamber placed on the pre-marked area of the flexor surface of the arm of the volunteer, covered with transparent dressing and left undisturbed for the predetermined length of time (5 min, 30 min, 3 h, 24 h, and 96 h). Treated areas were covered with a rigid, perforated plastic shield open at both ends to ensure free air circulation but at the same time prevent mechanical abrasion. The shield was held in place by taping it to the skin, the tape strips being applied transversally across the shield in such a way as not to cover the air vents. At the end of the exposure period, the occlusive materials were removed, the site carefully washed with a metal-complexing detergent containing EDTA (Extran 300) using cotton swabs to remove all traces of metal left on the skin surface, and rinsed with DI water. The skin was then dried by dabbing with cotton balls and finally by passing an airstream over the surface for 1 min. The area of application was then stripped sequentially 20 times after covering the 1.15 cm^2 treated area with a 1 sq. inch (6.45 cm^2) of the pre-cut adhesive tape strips, thus abundantly exceeding the perimeter of treated skin. Constant and uniform pressure (100 g/cm^2) was applied on the tape for 5 sec, which was then gradually removed in one draw. The 1-inch portions of the tape with adhering SC were cut off and placed individually in scintillation vials; 5 ml of concentrated nitric acid (70%) was added and the vials agitated vigorously for 3 h on the rotary shaker. The acid solution was then diluted 20-fold with DI water.

Analytical method

Quantitative nickel analyses were performed at Lawrence Berkeley National Laboratory. All nickel concentrations were determined on VG Elemental Plasma Quad III Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) (Parsons, 1983). The results of the analysis were reported as μg/L nickel. The detection limit for nickel by the ICP-MS method was 0.5 ppb.

Controls

All reagents and materials, including skin strips taken from the unexposed general area of application and the tape material, were analyzed for nickel content. Values were below nickel detection limits (< 0.5 ppb) for reagents and materials. Negative-control skin strip values for nickel were corrected where appropriate (> 2 ppb). Mean nickel control values lay between 1 ppb and 0.5 ppb from strip numbers 1 to 20.

Statistical analyses

Analyses of the data were performed using ANOVA on Sigma Stat and Microcal ORIGIN. The probability value $p < 0.05$ was considered significant. The correlation between the cumulative amounts of SC removed and tape strip number were determined for each exposure time by regression analyses using Microcal ORIGIN. Individual profiles of normalized cumulative nickel amounts per unit area were plotted against cumulative SC amounts per unit area. For normalized nickel values, area under each curve (AUC) to the end point of measurement (cumulative nickel weight in to 20 strips) was calculated using Microcal ORIGIN.

RESULTS

Fig. 1 compares nickel localization within SC layers in terms of amount of metal per unit area of skin according to time of occlusion, plotted as function of strip number. Nickel values decrease from the superficial to the deeper layers of SC, with gradients increasing commensurate to occlusion time, but remain fairly constant beyond the 10th to the 20th strip, at levels from 0.5 μg/cm^2 to 2 μg/cm^2 after occlusion for 96 h. ICP-MS analysis of tape strips to trace the nickel depth profile was performed on strip numbers 1, 3, 5, 10, 15, and 20.

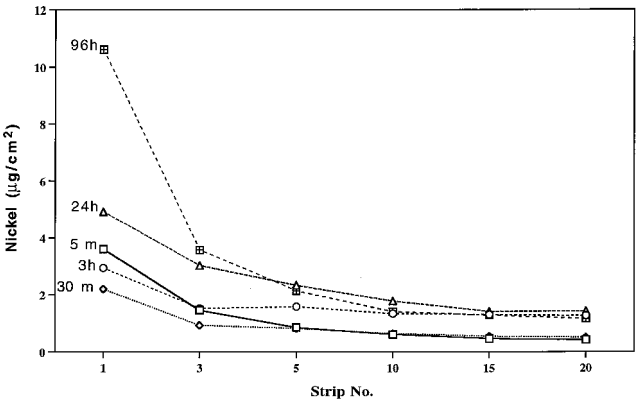

Fig. 1. Plots of average values for nickel removed by sequential tape stripping on the ventral forearm of three human volunteers after increasing periods of occlusion, as assessed by ICP-MS. Data points correspond to strip nos. 1, 3, 5, 10, 15, and 20.

Removal of SC following occlusion of untreated skin was recorded over periods of 5 min to 24 h (Fig. 2). Regression analysis of tape strip number as a function of SC weight removed ($n = 6$) produced highly linear fits to the 20th strip with minimal SD ($r = 0.95$–0.99; Fig. 2; error bars not visible). Total SC removed decreased insignificantly from 5 min and 30 min to 3 h of occlusion, but increased significantly from 3 h to 24 h (Table I).

In Fig. 3, cumulative nickel amounts per cm^2, transformed to yield a plot of normalized values, i.e. corrected for weight

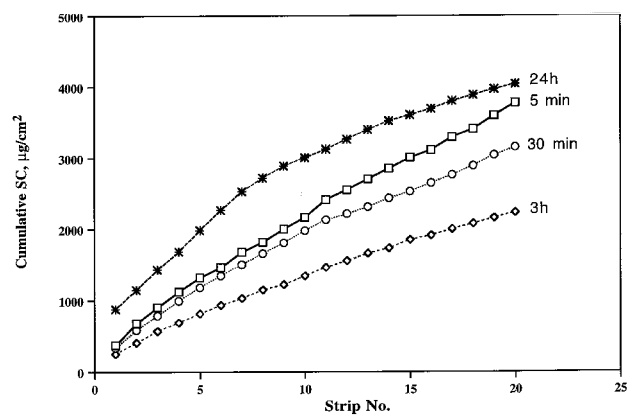

Fig. 2. Cumulative curves of stratum corneum removed by 20 sequential tape strips ($n = 6$) on the ventral forearm of one human volunteer after increasing periods of occlusion, as assessed gravimetrically.

Table I. *Stratum corneum mass removed by* in vivo *stripping of untreated human forearm skin in function of occlusion time (*n = *6)*

Occlusion time	Average totals (µg)	*r*-values[a]
5 min	3777	0.99
30 min	3154	0.98
3 h	2235	0.99
24 h	4041	0.95

[a] Pearson's *r*.
Only significant (< 0.05) *p*-value is stratum corneum removed after 3 h versus 24 h.

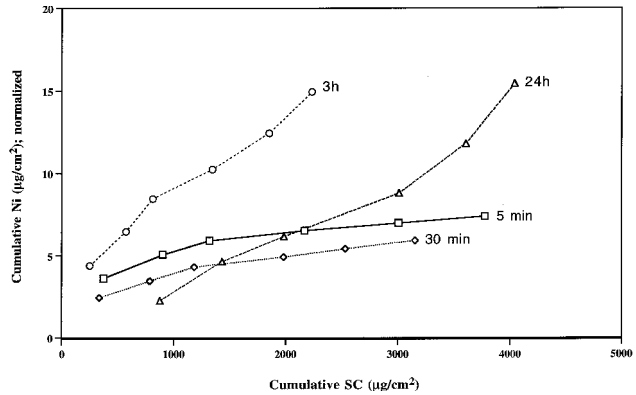

Fig. 3. Profiles showing average cumulative values for nickel removed with stratum corneum from three human volunteers, as assessed by ICP-MS. Nickel values are plotted as the dependent variables, following increasing periods of occlusion. They are normalized for average stratum corneum removed with sequential strips from the arm of one human volunteer, over six iterations. The independent variables represent cumulative weight of stratum corneum removed. Data points correspond to strip nos. 1, 3, 5, 10, 15, and 20.

of SC removed, are plotted versus total SC stripped, normalized by the tape area. The total amounts of nickel stripped after 3 h and 24 h are significantly larger than those after 5 min and 30 min. It can be seen that the slopes for the two extended occlusion periods (3 h and 24 h) increase with increasing depth of SC stripped, reflecting continuing levels of nickel present at that depth. For 96 h, nickel uptake into the SC could not be normalized because SC removed for that period of occlusion was not determined.

Total nickel amount (area under the curve, AUC) determined in the SC removed by stripping after 96 h occlusion is 41.6 µg/cm^2 (Table II).

DISCUSSION

While superficial appendageal penetration by nickel via the pilosebaceous unit and eccrine gland was visualized by others (57–59), the present observations indicate that also deep-reaching intercellular penetration can occur. After occlusion times as short as 30 min, the SC is hydrated to a degree that, before reaching the 20th strip, the glistening layer of the epidermis becomes apparent, signaling exposure of the stratum granulosum (48, 60, 61). In some cases stripping resulted in the appearance of traces of blood as a result of this trauma.

Table II. *Nickel removed with 20 strips from human volar lower arm after occlusion with 21.7 mg/cm^2 nickel powder*

Occlusion time	AUC[a] (µg/cm^2)	Significant ($p < 0.05$) differences
5 min	15.8	vs. 24 h and 96 h
30 min	14.1	vs. 24 h and 96 h
3 h	27.8	
24 h	38.7	
96 h	41.6	

[a] Average area under the curve of triplicate stripping experiments on three volunteers based on measured values after prior decontamination of the surface.
Only significant differences (*p*-values < 0.05) are listed

Because nickel is still present at the level of the 20th strip (0.4 to 1.4 μg/cm^2), this is taken as an indication that the metal has penetrated to the deepest layers of the SC, and is thus likely to have reached viable epidermis.

The plots of nickel versus strip number (Fig. 1) indicate continued, measurable diffusion of the metal down to the 20th strip, with levels trending higher with increasing length of occlusion. As was the case with water soluble, inorganic nickel salts (Hostynek et al., Acta Derm Venereol Suppl), an increasing amount of nickel is found adsorbed by the SC with increasing time of occlusion, the gradients decreasing monotonally with increasing SC depth. Beyond the 10th strip, for all time intervals the slope levels off and continues at constant values of 0.5 to 1 μg/cm^2 to the 20th strip.

The results obtained from gravimetric analysis of SC tape strips taken from untreated skin for the purpose of normalization show that, depending on the time of occlusion, the amount of SC removed by consecutive strips varies as reflected by the varying slope of the cumulative curves (Fig. 2). Within the same occlusive period, that amount remains fairly constant. Earlier investigators have shown that surface tape stripping of normal (non-occluded) skin also removes near constant amounts of tissue up to 20 strips ($r = 0.95$–0.99) (48, 54, 62–65). The slope of cumulative graphs of SC stripped off in the function of occlusion, and the total amounts removed with 20 strips were unexpected. Over the first 3 occlusive periods of 5 min, 30 min, and 3 h, the slopes decrease with time. While the decrease from 5 min to 30 min is not significant, the trend becomes more pronounced going to 3 h, and may be attributed to decreasing adhesivity of the SC to tape due to an increasing moisture build-up on the SC surface. At 24 h, however, the trend is the reverse. Apparently loss of cohesion between cell layers due to hydration more than makes up for the lack of adhesion to the tape, and consequently more SC weight is removed with each strip (Table I).

From the partial data obtained for SC characteristics after occlusion on one subject, certain provisional conclusions can be drawn: the cumulative tracings of nickel mass versus SC weight (Fig. 3) point to two distinctly different patterns of diffusion as a function of occlusion time. Early-stage, limited diffusion, which in the experiment was interrupted at 5 min and 30 min, tapered off beyond the 5th strip (after the first 3 data points). Longer contact lasting 3 h and 24 h resulted in curves which indicate a continuing increase in nickel content with each additional strip.

We hypothesize that the two patterns suggest two distinct routes of diffusion for two types of ion pairs of differing polarity: The level of nickel detected in the SC after brief occlusion may be due to relatively rapid, "short-circuit" penetration through shunts by relatively polar permeants (66). It has also been observed by others that penetration by xenobiotics through sweat ducts could occur within 1–5 min following exposure, with no comparable transport occurring through the SC in that time span (67–69). Such penetration of sweat glands and follicles as an early-stage event is presumably due to nickel ion paired with chloride, or low-molecular weight acids in sweat, an effect that may vary in function of sweating rate. The nickel detected in tape strips is presumed to be metal which has remained adsorbed to the poral epithelium by forming stable protein complexes upon penetration.

Those early-stage values appear to be overlaid by nickel values stemming from the more important, slow diffusion detected over 3 and 24 h of lipophilic nickel salts formed with fatty acids encountered on the skin surface. The second type of diffusion proceeds via the intercellular "mortar", or the lipid bilayer, whereby nickel-depth values are detected at lower SC levels than those measured for water-soluble salts. Occlusion was shown to accentuate such differences in the diffusion process (70).

The protocol followed here allows exclusion of the potential occurrence of artefacts, in the form of metal particles lodged in the SC layers, which could elude decontamination prior to stripping. Following application of nickel metal patches, the treated areas were left undisturbed, and in the case of occlusion times beyond 30 min were protected by rigid plastic shields. Although extremely fine particles may penetrate the loose, superficial SC layers, we thus would not expect them to migrate further beyond the initial resting place. The literature on human skin penetration by particulates indicates that follicles will be penetrated up to a size of 50 μm, and the SC by an optimal diameter of 5 μm, migrating to a depth of ca. 10 tape strips (71, 72). However, in those studies the intent was to promote depth penetration of a xenobiotic, such as unguents, sunscreens, etc., and such penetration was achieved by massaging the treated skin area.

Clinical observations

In spite of occluded, protracted and repeated application on the skin of finely distributed nickel metal with evidence of penetration, none of the three volunteers developed clinical signs of allergy or inflammatory response beyond the immediate trauma which results from stripping.

CONCLUSIONS

The present observations serve to confirm the function of the intercellular lipid matrix of the SC as an alternative pathway for the diffusion of lipophilic nickel salts generated *in situ*. The role of skin as a toxicologically important route of exposure to xenobiotics in general and metal compounds in particular is thereby underscored, as altogether three routes of entry present themselves to potentially toxic agents.

Immunogenic nickel ion is likely to be formed *in situ* in reactions with skin exudates, to form small, hydrophilic complexes or salts, such as the chloride, pyruvate or lactate, of facile diffusion through the aqueous environment of sweat ducts. Lipophilic salts instead will preferentially penetrate via the intercellular lipid bilayer (73), as we had hypothesized for the relatively lipophilic nickel nitrate (reported separately).

Formation of skin-diffusible, lipophilic salts by nickel on contact with the skin may thus be a contributing factor to the significantly increased risk of more general, adverse health effects observed among nickel refinery workers (74).

Use of the virtually non-invasive method of tape stripping, combined with the highly sensitive analytical method of ICP-MS with nickel detection limits on the order of 0.5 ppb, can provide a predictive and diagnostic tool for the assessment of hazardous exposure to airborne, readily oxidized metals. This would be relevant particularly in respect to dusts other than nickel, such as cobalt, chromium or lead, generated in certain industries, which harbor the potential for serious health effects in consequence of skin penetration and thus systemic absorption.

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