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Nickel–Epidermal Interactions: Diffusion and Binding[1]

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To elucidate some of the variables associated with nickel contact allergy, the diffusion of nickel through the epidermis and the binding of nickel to the epidermis were investigated. Diffusion of ^{63}Ni through the epidermis from solutions of surfactants was found to be slight. Considerable amounts of nickel, however, were bound to the epidermis. An epidermis powder was prepared, and selected functional groups were inactivated by chemical means. The nickel uptake of these modified epidermis powders as compared to the untreated material indicates that carboxyl groups are involved in the binding of nickel to a greater extent than are amino groups.

It has been reasonably well established that, for simple chemicals to elicit contact sensitivity, it is necessary that the eliciting compound be applied to the surface of the skin, penetrate the horny layer, and combine with a body protein. The body reacts to this conjugated protein. Nickel sensitivity is among the most common allergic contact dermatitides encountered. However, little experimental data on the interactions of nickel with skin and their possible relationship to nickel sensitivity have been reported.

The objective of this work was to study both the diffusion of nickel through the epidermis and the binding of nickel to the epidermis. The effects of sweat and detergents on the diffusion of nickel, and the effects of chemical modifications of the epidermis on the binding of nickel were included in our study.

Wells (1956) showed that Ni^{2+} penetrates at sweat-duct and hair-follicle ostia, and has a special affinity for keratin. Kolpakov (1963) used cadaver skin as an experimental model to study the permeability of nickel compounds. On the basis of qualitative chemical tests and histological observations, he reported that nickel does not penetrate the skin. Dugard and Scheuplein (1963) demonstrated that the permeability of the stratum corneum towards "polar entities" (i.e., ions) is increased by the presence of surfactants. Samitz and Pomerantz (1958) previously proposed that sweat and/or detergents may be involved in nickel sensitivity. In the case of nickel sensitivity, however, transepidermal diffusion of the hapten may not be necessary to initiate the allergic response. One of two hypotheses suggested by Soubrier *et al.* (1966) involves the modification of cutaneous proteins by interaction with nickel. Grasso (1970) attributes the persistence of nickel reactions to the absorption of small quantities of the "reactive factor" followed by its continuous action over an extended period of time.

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Diffusion Experiments

The diffusion of nickel through the epidermis was studied by essentially the same techniques we used previously (Samitz *et al.*, 1967). Epidermis was separated from autopsy skin by gentle scraping after a brief immersion in boiling water (Kligman and Christophers, 1963). Microscopic examination of the separated material confirmed that the separated material was epidermis. The epidermis was cut into pieces approximately 25.4 × 25.4 mm and secured in our diffusion cells with the outer surface facing upwards (Fig. 1). The lower reservoir was filled with 5 ml of pss.

Nickel sulfate solutions at concentrations of 1×10^{-1} M, 1×10^{-2} M, and 1×10^{-3} M were prepared in pss, and each was treated with sufficient ^{63}Ni to give specific activities of 1 μCi/ml. Two ml of carrier–tracer solution was introduced to the upper reservoir of each diffusion cell.

Periodically, 1 ml aliquots were removed from the lower reservoirs and transferred to counting vials. Ten ml of 'Aquasol' were added to each vial, and the radioactivity was measured in a liquid scintillation counter. Aliquots of the original carrier–tracer solution were similarly measured, and the percentage of the nickel sulfate diffusing through the epidermis was calculated. The results of these calculations are presented in Table 1.

One ml of carrier–tracer nickel solution (2×10^{-3} M $NiSO_4$ containing 0.2 μCi ^{63}Ni/ml) was mixed with 1 ml of either pss, sweat, or 2% surfactant in pss and introduced to the upper reservoir. One ml aliquots were removed from the lower reservoir periodically, and their nickel contents were measured by liquid scintillation counting. The results of these diffusion studies are summarized in Table 2. These data reflect the average of five separate experiments.

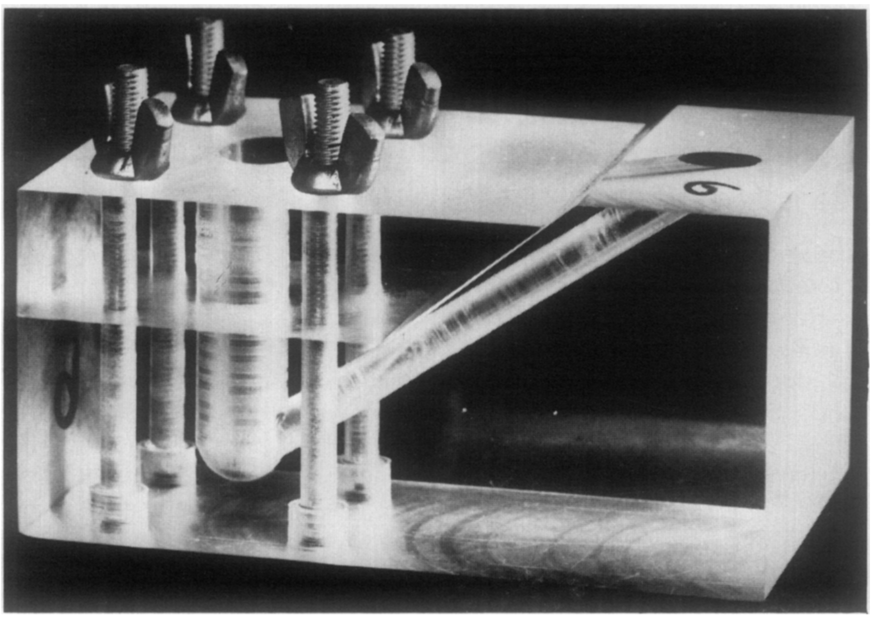

FIGURE 1.

TABLE 1
EFFECT OF NICKEL CONCENTRATION ON THE DIFFUSION OF NICKEL

Initial nickel concentration in upper reservoir	Percentage of nickel diffused after		
	17 hours	24 hours	90 hours
1.00×10^{-1} M	0.000	0.024	0.000
1.00×10^{-1} M	0.018	0.026	0.031
1.00×10^{-2} M	0.000	0.000	0.000
1.00×10^{-2} M	0.000	0.007	0.025
1.00×10^{-3} M	0.000	0.009	0.066
1.00×10^{-3} M	0.000	0.000	0.000

TABLE 2
PERCENTAGE OF NICKEL DIFFUSED THROUGH HUMAN EPIDERMAL TISSUE[a] MEDIUM ORIGINALLY CONTAINING NICKEL[b]

Time (hr)	pss	X-100	EOB	915	LF-6	LR-74	Sweat
1	none	0.003	none	0.02	0.003	0.001	0.003
2	none	none	none	none	none	0.001	0.003
3	none	0.02	none	0.04	0.08	0.001	0.003
4	none		none	0.01		0.001	0.001
5	none	none		0.030		0.001	0.001
48		0.02		0.05	0.06		
192		0.07		2.8	0.7		

[a] From pss, sweat, or 2% surfactant containing 2×10^{-3} M $^{63}NiSO_4$.

[b] Media originally containing nickel: pss—physiological saline solution; X-100—TRITON X-100, alkylphenoxypolyethoxyethanol; EOB—KYRO EOB, ethoxylated ethanol; 915—SWIFT #915, N, N-hydroxyalkyl-(n-alkyl)amide; LF-6—alaphatic alcohol/ethylene oxide adduct; LR-74—*n*-alkoxypolyethoxyethanols; sweat—human sweat collected from volunteers.

Binding Experiments

At the conclusion of the diffusion measurements, four of the epidermis samples were rinsed in water for 30 minutes and radioassayed for bound nickel. These epidermis specimens showed high radioactivity, indicating the binding of nickel.

The binding of nickel by skin was investigated by both the indirect approach suggested by Mali *et al.* (1964) and by the direct approach employed by Anderson (1960) in his studies on chromium.

An epidermis powder was prepared from autopsy skin. Epidermis was removed as described previously, washed with ether and then pss, and dried in a vacuum desiccator. The dry material was pulverized in a Wiley mill, and the material passing through the 30 mesh screen was collected. This epidermis powder was divided into six 300 mg portions. One portion was retained as control epidermis powder, and the others were modified by chemical means to block carboxyl groups by methylation (Lillie, 1954) and benzoylation (Pearse, 1953), to inactivate amino groups by acetylation (Fraenkel-Conrat *et al.*, 1949) and deamination (Peters and Van Slyke, 1932) and to block sulfhydryl groups (Wells, 1956).

For the indirect approach to the binding of nickel by epidermis, nine 25 mg samples of the control epidermis powder and nine 25 mg samples of each of the modified epidermis powders were weighed into separate test tubes. Exactly 5 ml of carrier-tracer nickel solution (1.00×10^{-4} M $NiSO_4$ containing 5×10^{-3} μCi ^{63}Ni/ml) was added to each tube. Eighteeen test tubes containing no epidermis powder were also treated with 5 ml of the carrier–tracer nickel solution. All tubes were incubated at 0°C for 2 weeks. The tubes were vortexed briefly every third day during the incubation period. At the end of the incubation period, 1 ml aliquots of the clear supernates were removed from each tube, and their nickel contents were determined by liquid scintillation counting.

The aliquots from the 18 tubes containing only the carrier–tracer nickel solution had a mean activity of 9511 ± 133 cpm/ml. When the aliquots of the supernates from the tubes containing the epidermis powders showed activities significantly less than 9511 ± 133 cpm/ml, binding of nickel was assumed. The difference between 9511 cpm/ml and the activity of the aliquot was taken as a quantitative measure of the amount of nickel bound to the epidermis powder.

For the direct method, the epidermis powders were separated from the carrier–tracer nickel solutions by filtration. The recovered epidermis powders were washed with three 10 ml portions of pss and dried in a vacuum desiccator. Each of the washed and dried recovered epidermis powders were weighed into separate Kjeldahl flasks and digested with sulfuric and nitric acids. The digests were neutralized with ammonia and diluted to exactly 10 ml with water. One ml aliquots of the resulting solutions were counted in the liquid scintillation counter, and the count rates were converted to micrograms nickel by reference to a calibration curve prepared with dilutions of the original 1.00×10^{-4} M carrier–tracer nickel solution.

The binding experiments both by direct and indirect methods, were repeated using a second set of epidermis powders. The above procedures were followed exactly with the exceptions that only five samples of each epidermis powder were used and the radioactivity of the blank was 10,240 ± 152 cpm/ml.

Comparisons of the amounts of nickel bound per gram of epidermis powder showed no significant differences between the first and second series of measurements. Consequently, mean values of the nickel binding for each of the six epidermis powders were calculated on the basis of 14 measurements. These values with their standard deviations are tabulated in Table 3. These values are subject to larger uncertainties than indicated by the standard deviation, due to the uncertainties in the radioactivity of the blanks. Those values marked by an asterisk in Table 3 are effected to the greatest extent.

DISCUSSION

The data in Table 1 show that diffusion of nickel through the epidermis from solutions of 1×10^{-1} M to 1×10^{-3} M is slight at best.

The data in Table 2 show that the diffusion of nickel through the epidermis from physiologic saline solution does not take place within 5 hours. Sweat or detergents do little to enhance the diffusion of nickel. Even under the most severe conditions—48 hours—less than 0.1% of the nickel diffused through the epidermis.

Although the two approaches to the evaluation of nickel binding summarized in Table 3 do not show agreement, there is little doubt that the nickel is primarily

TABLE 3
BINDING OF NICKEL BY EPIDERMIS

Chemical modification	Nickel bound, μg/g[a] Indirect method	Direct method
None: control epidermis powder	448 ± 58	148 ± 16
Methylation	14 ± 6[b]	54 ± 12
Benzoylation	11 ± 6[b]	26 ± 14
Deamination	1062 ± 258	424 ± 66
Acetylation	237 ± 29	121 ± 19
Sulfhydryl blocked	51 ± 20	55 ± 10

[a] When 25 mg samples of epidermis powder were treated with solutions containing 1.00×10^{-4} M $^{63}NiSO_4$ and incubated at 0°C for 2 weeks.
[b] See text.

bound by free carboxyl groups. Coddington and Perkins (1961) have reached a similar conclusion from their studies with human serum albumin. Rao (1962), however, has reported that the imidazole groups of bovine serum albumin are the primary binding sites, with carboxyl groups competing to some extent. Cotton (1964), on the other hand, has demonstrated that methylation of the carboxyl groups of bovine serum albumin abolishes nickel binding. He has also shown that the binding is weak. Spruit *et al.* (1965) have reported that the binding of nickel to skin is reversible, and they proposed that the skin could serve as a reservoir of nickel. That the binding of nickel is weak and reversible may explain the differences observed by our direct and indirect methods. The washing procedures employed in the recovery of the epidermis powders could be responsible for the removal of weakly bound nickel. Hence, the values obtained by the direct method are lower than those obtained by the indirect method.

The data in Table 3 show that amino groups may be involved to a lesser extent, as indicated by the apparent decrease in binding upon acetylation. Like Cotton (1964), we attribute the increase in nickel binding upon deamination to denaturation of the protein. Sulfhydryl groups also are involved in the binding of nickel to epidermis.

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