Nickel Concentrations in Nasal Mucosa, Plasma, and Urine in Active and Retired Nickel Workers*

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ABSTRACT

The objective of the present investigation was to study the influence of the occupational nickel exposure on the concentration of nickel in nasal mucosa, plasma and urine. Plasma, urine and biopsy specimens of nasal mucosa from 318 nickel workers, 15 retired nickel workers and 57 non-exposed controls were analyzed for nickel by atomic absorption spectrometry. The results showed that nickel exposure led significantly to raised nickel concentration in nasal mucosa, plasma and urine both in active and retired nickel workers. The average nickel concentration in the nasal mucosa was highest in workers exposed to the highest atmospheric nickel concentration, inhaled as nickel subsulphide and oxide dust. Workers exposed to aerosols of nickel chloride and sulphate at a lower atmospheric nickel concentration had, on the other hand, the highest mean nickel concentration in plasma and urine. The mucosal, plasma and urine nickel concentration were significantly correlated to duration of nickel exposure. The accumulated nickel in the nasal mucosa was retained for years after termination of the nickel exposure, and slowly released with an estimated half-life of 3.5 years.

Introduction

Epidemiological investigations suggest that nickel or nickel compounds induce cancer in the respiratory tract, and a definitely increased risk for development of nasal carcinoma in nickel refinery workers has been noted.\textsuperscript{5,6,12,17,18,22,24,28,29,30} An increased incidence of nasal cancer at a Norwegian nickel refinery was reported by Pedersen et al in 1973.\textsuperscript{17} Torjussen and Solberg\textsuperscript{28,29} made histological examinations of nasal biopsies from nickel workers at the same refinery and found an increased incidence of carcinoma and dysplasia of the surface epithelium, particularly in process workers exposed to high atmospheric nickel concentration.

As a local carcinogenic effect is suspected in nasal carcinoma in nickel exp-

The investigation has been supported by grants from: Norsk Forening til Kreftens Bekjempelse, Norway, Landsforeningen mot Kreft, Norway and Falconbridge Nikkelverk A/S, Norway.
posed workers, it seemed relevant to study to what extent the nickel content in the nasal mucosa is reflected by the nickel concentrations in blood and urine. Accordingly, the primary objective of the present investigation was to procure quantitative data from active and retired nickel workers and from controls on the nickel content in nasal mucosa, plasma and urine. Moreover, the authors wanted to study how duration of exposure and variation in nickel concentrations and nickel compounds in the working atmosphere influenced the nickel content in the nasal mucosa, blood and urine. As the nickel-related nasal carcinoma frequently appears several years after the end of exposure, our final aim was to study to what extent accumulated nickel was retained in the nasal mucosa.

Materials and Methods

The Nickel Refining Processes

The raw material at the refinery is converter matte (about 50 percent nickel, 30 percent copper, 20 percent sulphur and some trace metals) which is refined through crushing, roasting, smelting and electrolysis. People working with the three first mentioned processes, the roasting/smelting category, are mainly exposed to dry dust, containing nickel subsulphide and oxide, corresponding to an average atmospheric concentration of about 0.5 mg Ni per m$^3$ (range 0.1 to 1.0 mg per m$^3$). The electrolytic workers are mainly exposed to aerosols of nickel sulphate and chloride, with an average air concentration of about 0.2 mg Ni per m$^3$ (range 0.1 to 0.5 mg per m$^3$). Non-process workers are exposed to miscellaneous nickel composites corresponding to an average air concentration of 0.1 mg Ni per m$^3$ (range 0.01 to 0.5 mg per m$^3$). The nickel concentrations in the various working atmospheres were registered by the Department for Occupational Hygiene and Safety at the plant from atomic absorption analyses of air samples collected by stationary and portable apparatus.

The nickel exposed group. Subjects employed at least eight years at the plant and working with crushing, roasting, smelting or electrolysis on the first of October 1976 were all selected for the investigation. Twenty percent of the remaining non-process workers employed at least eight years were selected at random by a computer. This primary selected material comprised 370 individuals of whom 318 consented to attend the investigation (316 men, 2 women). In table I are shown the mean age and length of nickel exposure, allocated to three different working categories.

The retired nickel workers. Fifteen male pensioners with at least eight years previous employment as process workers were chosen by the medical officer to participate in the investigation (table I). Ten persons had been working with crushing, roasting or smelting and five with electrolysis during their employment, which had ended from six months to 10 years before the investigation.

The control group. This group consisted of 57 male volunteer patients (table I), admitted to the County Hospital at Kristiansand for a scheduled standard surgical operation. The subjects were selected consecutively to match the nickel exposed group by age. Persons with former or present employment in the nickel industry, patients presenting obvious nasal or paranasal disease and patients with general systemic diseases were excluded.

Collection of relevant informations. Work history, occupational nickel exposure and smoking habits were evaluated from a questionnaire and an interview.

Collection of samples. The samples were collected in the morning before breakfast during the last three months of the year 1976 from active and retired nickel workers and during the last three months of 1977 from the controls.
1. **Blood.** Samples were drawn into heparinized Vacutainer tubes.* The blood from active and retired nickel workers were centrifuged immediately and the plasma analyzed within two to three hours. Samples from the controls were collected during general anesthesia before the start of a surgical operation. The blood was centrifuged immediately, the plasma decanted into a second Vacutainer tube and stored at −35°C until analyzed.

2. **Urine.** Samples were collected in acid-washed urine glasses and decanted into Vacutainer tubes.† The urine from the active and retired nickel workers was analyzed within two to three hours after being collected. Samples from the controls were stored at −35°C until analyzed.

3. **Biopsy.** Specimens were taken from the anterior curvature of the middle nasal turbinate with a biopsy forceps $ either from the nasal cavity with the best air-flow passage, or from the side showing pathological changes. In active and retired nickel workers the nasal cavities were thoroughly rinsed with saline, followed by application of Tetracaine/Adrenaline (2 g of tetracaine and 0.1 g of Adrenaline in 100 ml aqueous solution) to the mucosal surface. The specimens were collected in small stoppered plastic tubes and kept for one to two hours in a refrigerator until weighing and analysis were performed. Specimens from the controls were taken during general anesthesia, collected in tared stoppered plastic tubes, immediately weighed, and stored at −35°C until analyzed.

All the plasma, urine and tissue samples from the control group were analyzed during a five day period.

**Sample analysis.** Measurements of nickel concentrations in plasma, urine and nasal mucosa were made with an atomic absorption spectrophotometer (Model 603)$ equipped with a graphite furnace (Model HGA-76; Auto Sampling-System, AS-I)$ and a recorder (Model 56)$ according to previously described methods.1,2,5

**Statistical methods.** The Student t-test was applied to calculate the significance level for testing differences between means.

Simple correlation coefficients were calculated between the nickel concentrations in nasal mucosa, plasma or urine and the number of years from the first employment at the nickel refinery. Corresponding correlations were calculated between the nickel concentrations and the three separate working categories by evaluating the employment as follows: If the subject was employed in a given working category, 1, otherwise 0. The significance of the correlation coefficients was tested by Student t-test. P-values less than five percent were regarded as statistically significant.

**Results**

**NICKEL CONCENTRATIONS IN NASAL MUCOSA, PLASMA AND URINE**

**The control group.** In figure 1 are shown the individual measurements for

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* Becton-Dickinson, 07070-320 KA.
† Becton-Dickinson, 07070-320 OU.
§ Wolff No. 8150.00.
nickel concentrations in plasma and urine. The mean nickel concentrations for plasma and urine are 1.9 µg per liter and 4.9 µg per liter, respectively (table II). The correlation between the plasma and urine nickel concentrations is not statistically significant ($r = 0.09, P > 0.10$) (table III). Plasma nickel concentration of 4.5 µg per liter and a urine nickel concentration of 10 µg per liter have been chosen as the upper normal limits, as 55 out of a total of 57 measurements (96.4 percent) were below these values.

In figure 2 is shown the individual relation between the nickel concentration of the nasal mucosa and the plasma. The mean nickel concentration for nasal mucosa is 12.9 µg per 100 g wet weight (table II). The correlation between the nickel concentrations for plasma and nasal mucosa is not statistically significant ($r = 0.01, P > 0.10$) (table III). In 54 controls (94.7 percent) mucosal nickel concentrations were below 53 µg per 100 g wet weight, taken to represent the normal upper limit.

The nickel exposed group. In figure 3 are given the individual results for the nickel concentration in plasma and urine. There is a statistically significant correlation between these two parameters ($r = 0.69, P < 0.0001$). The mean nickel concentrations for plasma and urine are 6.3 µg per liter and 49.1 µg per liter, respectively (table II). The highest nickel concentration in plasma was 36 µg per liter, and in urine 600 µg per liter. Two hundred and eighty-three workers (89 percent) had nickel values for either plasma or urine above normal. In 178 subjects (56.0 percent) plasma nickel concentration was above normal limit, as compared to 276 subjects (86.8 percent) with raised urine nickel.

In figure 4 is shown the individual relation between the nickel concentration of nasal mucosa and plasma. The mean nickel concentration for the nasal mucosa is 273.9 µg per 100 g wet weight (table II). The highest value for mucosal nickel was 3460 µg per 100 g wet weight. In 271 subjects (85.8 percent), the values for mucosal nickel concentration were above normal limit. The correlation between the nickel concentrations for plasma and nasal mucosa is not statistically significant ($r = 0.02, P > 0.10$) (table III).

The retired nickel workers. In figure 5 is demonstrated the individual measurement for nickel concentrations in
The mean nickel concentration for plasma and urine are 2.9 µg per liter and 11.3 µg per liter, respectively (table II). The correlation between plasma and urine nickel concentrations is statistically significant \((r = 0.79, P < 0.001)\) (table III). The highest nickel concentrations in plasma was 7.0 µg per liter and in urine 42 µg per liter. Five pensioners (33 percent) had raised nickel values for either plasma or urine.

In figure 6 is shown the correlation between the nickel concentration of nasal mucosa and plasma \((r = 0.70, P < 0.001)\) (table III). The highest value for nickel in the nasal mucosa was 720.0 µg per 100 g wet weight. In six subjects (40.0 percent), the mucosal nickel concentration was above normal.

**Relation of Nickel Concentrations to Nickel Amounts, Compounds and Duration of Nickel Exposure**

The mean nickel concentrations for nasal mucosa, plasma and urine in nickel workers, retired nickel workers and in controls, and the mean values for these parameters related to three categories of work are summarized in table II. The mean values for the various nickel concentrations in the control group are all significantly lower than the corresponding values for both active and retired nickel workers \((P < 0.01\) and \(P < 0.05\), respectively). The difference in the mean plasma nickel between active and retired nickel workers is also statistically significant \((P < 0.01)\), but not the difference in the mean for urine and tissue nickel.

The differences in the mean nickel concentration for plasma and urine between the three working categories are statistically significant \((P < 0.01)\). Highest mean values were found in subjects working with electrolysis, subsequently followed by workers from the roasting/

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**TABLE III**

Correlations Between Nickel Concentrations in Plasma, Urine and Tissue from Nasal Mucosa for Nickel Workers, Retired Nickel Workers and Controls

<table>
<thead>
<tr>
<th>Category of Subjects/Work</th>
<th>Number of Subjects</th>
<th>Correlation Coefficient ((r)) Between Tissue &amp; Plasma (r_1)</th>
<th>Correlation Coefficient ((r)) Between Tissue &amp; Urine (r_2)</th>
<th>Correlation Coefficient ((r)) Between Plasma &amp; Urine (r_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roasting/smelting</td>
<td>97</td>
<td>0.10</td>
<td>0.26*</td>
<td>0.77+</td>
</tr>
<tr>
<td>Electrolysis</td>
<td>144</td>
<td>0.14</td>
<td>0.10</td>
<td>0.68†</td>
</tr>
<tr>
<td>Non-process work</td>
<td>77</td>
<td>0.12</td>
<td>0.12</td>
<td>0.36†</td>
</tr>
<tr>
<td>All nickel workers</td>
<td>318</td>
<td>0.02</td>
<td>0.00</td>
<td>0.69†</td>
</tr>
<tr>
<td>Retired nickel workers</td>
<td>15</td>
<td>0.70†</td>
<td>0.77†</td>
<td>0.79†</td>
</tr>
<tr>
<td>Controls</td>
<td>57</td>
<td>0.01</td>
<td>0.03</td>
<td>-0.09</td>
</tr>
</tbody>
</table>

\(*0.01 < P < 0.05\) †† < 0.01
smelting department and non-process workers. In the nasal mucosa, however, the highest mean nickel concentration was found in subjects from the roasting/smelting department, followed by non-process and electrolytic workers. The difference between roasting/smelting and each of the other working categories is statistically significant ($P < 0.01$), but not between electrolytic and non-process work.

The correlations between the nickel concentrations in nasal mucosa, plasma and urine for the various groups and working categories are summarized in table III.

In table IV are shown the correlations between working categories and nickel concentrations in nasal mucosa, plasma and urine, respectively. Close and statistically significant correlation is present between roasting/smelting work and raised nickel content of the nasal mucosa ($r = 0.35, P < 0.0001$), and between electrolytic work and raised plasma or urine nickel concentration ($r = 0.42$ and $0.39$, respectively, $P < 0.0001$). There are also highly significant correlations between length of nickel exposure and nickel concentrations in nasal mucosa, plasma or urine (table IV).
RETENTION AND RELEASE OF NICKEL IN THE NASAL MUCOSA

Figure 7 illustrates the negative correlation between the logarithmical values of the mucosal nickel concentration and the number of years from retirement in the 15 former nickel workers. The correlation coefficient (r = -0.434) is statistically significant by a one-sided test (P < 0.035) (supposing that nickel concentration is not raised by time following retirement). From the equation for the regression line:

\[ \ln Y = 4.9 - 0.2X \]

the half-life of nickel release from the nasal mucosa is estimated to about 3.5 years.

Discussion

Most of our knowledge on nickel metabolism and toxicology are reviewed by Sunderman et al\textsuperscript{23} in a comprehensive monograph with extensive references and in a follow-up report.\textsuperscript{22} Nickel is now accepted as an essential trace metal for growth, intestinal iron absorption and for activity of some enzymes in rats.\textsuperscript{11,19,20,21} It has also been discovered that urease in jack beans is a nickel metalloenzyme,\textsuperscript{4} and a nickel containing protein has been isolated from both human and rabbit serum.\textsuperscript{8,15} In relation to human disease raised nickel in serum has been found after myocardial infarction and "acute stroke," whereas hyponickelemia has been reported in patients with hepatic cirrhosis and uremia.\textsuperscript{14}

Certain nickel compounds induce malignant tumors on experimental animals. The carcinogenic effect of these compounds appears to be inversely correlated to their solubilities in water.\textsuperscript{23} Insoluble dusts of nickel subsulphide and oxides have been pointed out as possible carcinogens also in man.\textsuperscript{23}

The nickel workers participating in this study represent 86 percent of the primary selected number of subjects that were wanted to participate. The nonparticipation was fairly even in the different
TABLE IV
Correlations Between Working Category or Duration of Nickel Exposure and Nickel Concentration in Nasal Mucosa, Plasma and Urine

<table>
<thead>
<tr>
<th>Category of Subjects/Work</th>
<th>Nasal Mucosa</th>
<th>Plasma</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roasting/smelting</td>
<td>0.35†</td>
<td>-0.06</td>
<td>-0.08</td>
</tr>
<tr>
<td>Electrolysis</td>
<td>-0.11*</td>
<td>0.42†</td>
<td>0.39†</td>
</tr>
<tr>
<td>Non-process work</td>
<td>-0.03</td>
<td>-0.14†</td>
<td>-0.16†</td>
</tr>
<tr>
<td>Duration of nickel exposure</td>
<td>0.21†</td>
<td>0.28†</td>
<td>0.19†</td>
</tr>
</tbody>
</table>

*0.01 < P < 0.05 †P < 0.01

working categories, and there is no reason to believe that any severe bias has been introduced by not achieving 100 percent participation. The equal age distribution and duration of nickel exposure for the three working categories at the plant make them suitable for comparison regarding the influence of the working conditions on the distribution of nickel in tissue and body fluids. The matched age distribution of the control group makes it appropriate for estimation of normal values.

Electrothermal atomic absorption spectrometry is a sensitive analytical method which allows determination of trace amounts of nickel in small samples. The procedures and the accuracy for the applied analytical methods have been described and discussed previously by the authors. The obtained values for nickel concentrations in nasal mucosa, plasma and urine in our controls are compatible with previous results. The wide range in mucosal nickel concentration probably reflects both biological variation and accumulated variability in collecting and analyzing the samples, whereas contamination of the biopsies by the surgical instrument seems negligible. To reduce the influence of urine dilution on the nickel concentration, the urine values were based on analysis of samples collected before breakfast. Ongoing studies in our laboratory suggest, however, that urine nickel concentration in nickel exposed individuals is scarcely influenced by diluted urine following liquid intake.

McNeely el al provided the first evidence that nickel in serum and urine could serve as indices of environmental nickel exposure, and nickel determinations in blood and urine are to date the most widely used and accepted methods for monitoring nickel exposure. The close correlation between plasma and urine nickel found in both active and retired nickel workers in the present investigation indicates that these parameters may be regarded as equal in monitoring the individual nickel burden in exposed subjects. The lack of correlation between plasma and urine nickel in our control group does not invalidate this assumption as it may be explained by technical inaccuracy near the detection limit for the analysis.

The significantly raised nickel concentration in nasal mucosa, plasma and urine in active and retired nickel workers shows that all these parameters are usable for distinguishing between nickel-exposed and non-exposed individuals. An objection to the use of plasma and urine nickel in quantitating the degree of nickel exposure is the lack of correlation between the
amounts of nickel in the occupational atmosphere and in the body fluids, previously stated by several investigators \(^2,16\) and also found in the present study.

Raised nickel in the nasal mucosa was found in representatives from all working categories. Highest mean and individual mucosal nickel concentrations were measured in workers at the roasting and smelting department, where also the highest amounts of nickel in the atmosphere were registered. The amount of accumulated nickel in the nasal mucosa, therefore, seems to be a more reliable parameter for expressing the total nickel exposure to the upper respiratory tract than are the concentration of nickel in plasma or urine.

The qualitative composition and physical characteristics of the nickel compounds in the atmosphere obviously influence the uptake of nickel in the organism and thereby its concentrations in nasal mucosa, blood and urine. The higher nickel concentrations in body fluids from electrolytic workers than from subjects at the roasting and smelting department give reason to suppose that nickel compounds in aerosols are more readily absorbed and thereby tend to give a higher general accumulation of nickel in the organism, than do the nickel composites inhaled as furnace dust particles. On the other hand, the concentration of nickel in the nasal mucosa seems to reflect the local nickel exposure to the upper respiratory tract from such particles. As there is a constant turn-over of surface cells in the nasal epithelium, it seems probable that the main part of the accumulated nickel is stored in the underlying mucosal stroma, although electron probe x-ray microanalysis of the nasal mucosa in nickel workers has failed in localizing such deposits.\(^27\)

Physiological studies have demonstrated that airborne particles have a tendency to deposit on the anterior curvature of the middle nasal turbinate,\(^7\) where our biopsies were taken. In this area, the ciliated cells are frequently replaced by non-ciliated cells,\(^29\) unable to transport and remove the particles from the surface. How this surface deposited nickel reaches the stroma and results in gradual accumulation of nickel is still to be explained. Our study on retired nickel workers shows that nickel, once accumulated in the mucosa, is retained during a considerable period of time, and that it is released at a fairly constant rate, according to a half-life of 3.5 years.

As already mentioned in the introduction, there is a significantly increased incidence of nasal carcinoma among employees at nickel refineries, particularly in workers involved in roasting and smelting procedures. Torjussen and Solberg\(^28,29\) have previously shown that these workers also have the highest incidence of epithelial dysplasia in the nasal mucosa. The results of the present study, showing that the same workers have the highest average content of nickel in the nasal mucosa, fits well with the assumption that one or more nickel-related substances induce nasal carcinoma by a local carcinogenic effect. Nasal carcinomas in nickel workers are often diagnosed several years after end of active work.\(^28\) If nickel-related compounds are main carcinogens also in such cases, our findings of raised mucosal nickel in retired nickel workers implies that the local carcinogenic effect on the nasal mucosa may persist for many years following termination of the nickel exposure.

References
