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# Effect of Nickel Chloride on Primary Antibody Production in the Spleen

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**Mice immunized intraperitoneally with sheep erythrocytes were treated with nickel chloride, a common particulate air pollutant. Primary antibody production in the spleen was examined using a hemolytic plaque technique. A negative linear dose-response relationship ($p < 0.05$) was observed between the logarithm of (plaques/$10^6$ cells) and the nickel concentration administered. Mice injected with 3.09 $\mu$g $Ni^{2+}$/g body weight displayed lymphocyte function similar to that of control mice. However, injection of 9.26–12.34 $\mu$g $Ni^{2+}$/g caused significant immunosuppression ($p < 0.05$).**

## Introduction

As a particulate air pollutant, nickel (Ni) has been found to have deleterious health effects in both man and animals. Nickel compounds can cause dermatitis, pneumonitis, pulmonary alveolar lesions, nephritis, cardiovascular and respiratory disease, and possibly pulmonary cancer (*1*).

This laboratory has previously demonstrated that *in vitro* exposures to $NiCl_2$ reduced the phagocytic (*2*) and ATP (*3*) levels of alveolar macrophages and reduced viability and increased lysis of alveolar macrophages (*4*) and human fetal lung fibroblasts (*5*). Also, intratracheal injections of NiO enhanced hamster mortality after a bacterial aerosol challenge (*6*).

As a part of an integrated research program on the effects of nickel exposure, it was decided to examine the effect of $Ni^{2+}$ on the humoral immune system, since this system is essential for health in that it protects the host from infectious disease. It was decided to begin by studying primary humoral responses which are first observable in lymph nodes and the spleen. Since the spleen is the largest lymphatic organ and cells are easily obtainable, the effect of $NiCl_2$ on antibody production in this organ was tested.

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## Materials and Methods

### Animals

Six-week-old, female pathogen free, Swiss albino mice, strain CD-1 (Charles River Farms, Inc., Wilmington, Massachusetts) weighing 20–25 g were used for all experiments.

### $NiCl_2$ Exposures

The appropriate amounts of $NiCl_2$ (International Nickel Co., Inc., Suffern, N.Y.) were weighed and dissolved in Dulbecco's saline. Most of the metal was in solution as $Ni^{2+}$, but there was a fine precipitate that remained. Aliquots of this suspension were intramuscularly (IM) injected. All exposures were in an approximate volume of 0.25 ml.

For each daily experiment, six mice were randomly assigned to each of five treatment groups. Four of the groups received IM injections of 12.34, 9.26, 6.17, or 3.09 $\mu$g $Ni^{2+}$/g body weight, while the fifth group was a control and received no $Ni^{2+}$

## Immunization

Sheep red blood cells (SRBC) (Gibco, Grand Island, N.Y.) were washed three times and resuspended in physiological saline to a concentration of 4.5–5.5 × $10^6$ SRBC/ml. Immediately after $NiCl_2$ treatment, all mice, including controls, were immunized with 0.5 ml of the SRBC suspension injected intraperitoneally.

Prior work had shown that nonimmunized mice exhibited no antibody production in this system, and therefore such a group was not included in this study.

## Source of lymphocytes

On the fifth day after immunization, mice were sacrificed by cervical dislocation, and their spleens were removed and weighed. The spleens were minced by pressing them through a size 60 stainless steel wire mesh. Cells and debris were washed through the screen with RPMI 1640 medium supplemented with 5% heat-inactivated newborn calf serum (all media were obtained from Gibco, Grand Island, N.Y.). The cell suspension was then poured through a syringe barrel that contained cotton gauze which had been previously moistened with the supplemented medium. Approximately 3 ml of the media was passed through this filtering apparatus, resulting in a filtrate that contained a cell suspension in which less than 5% of the lymphocytes were clumped. This suspension was washed twice at 250*g* for 7 min at room temperature. The cells were resuspended in the supplemented medium to an approximate concentration of 0.5 × $10^6$ lymphocytes. Precise cell counts were determined later using a hemocytomer.

Since a hemocytometer was used, it was possible to only count cells that had the appearance of lymphocytes. Visual inspection showed that only a few macrophages and other nucleated cell types were present.

The cells were then held at 4°C for approximately 30 min until they were used in the hemolytic plaque procedure. Lymphocyte viability as determined by trypan blue dye exclusion was > 98% (*7*).

## Hemolytic Plaque Technique

A modification of the direct Jerne plaque technique was utilized to test the IgM antibody-producing capabilities of the recovered lymphocytes (*8*). A 1-ml portion of a 1.5% Seaplaque agarose (Microbiological Associates, Bethesda, Maryland) solution in Hanks balanced salt solution (HBSS) and 0.5 ml of a 5% suspension of SRBC in HBSS was added to a test tube which was maintained in a 45°C water bath. After the addition of 0.5 ml of the lymphocyte suspension, the entire contents of the tube were poured into a plastic petri dish (60 x 15 mm) containing 1 ml of a solidified base layer of 1.5% Seakem agarose (Marine Colloids, Inc., Rockland, Maine) in HBSS. Lymphocytes from each individual mouse were plated in duplicate. The dishes were swirled to insure a uniform dispersal of lymphocytes and SRBC. These plates were held at 37°C for 1.5 hr under an atmosphere containing 4% $CO_2$. Following this incubation, 1 ml of a 10% solution of guinea pig complement in HBSS was added to each plate. After a further incubation for 45 min, the plates were placed in the refrigerator and examined the next day. The number of plaques was not altered by refrigeration. During the initial incubation, functioning lymphocytes produced antibody specific for SRBC, which diffused outwards and became fixed to SRBC. When complement attached to the SRBC–hemolysin complexes, the SRBC lysed, froming a clear plaque (*8*). The number of plaques/plate was determined under 12 × magnification on a light microscope. This number was then converted to the number of plaques/$10^6$ cells.

## Statistical Analysis

The data were analyzed by using analysis of variance. Since preliminary studies revealed significant daily variation in the mean number of control plaques, daily variability was eliminated as a source of experimental error by testing each treatment group on each day. Whenever the hypothesis of no treatment effect was rejected, Duncan's multiple range test was used to determine the pattern of significant treatment differences. In some cases, analyses were conducted with Dunnett's test, which compares each treatment group against controls. Since the variability about the mean variance increased as the mean response level increased for both the number of plaques/$10^6$ cells and the spleen

weight to body weight ratio, the analysis was performed on the common logarithms of the measurement for these two parameters.

## Results

The results from the analysis of variance are given in Table 1. It can be seen that nickel had a significant effect upon all parameters studied. Lack of a significant nickel concentration × day interaction indicates that the pattern of treatment differences was consistent from day to day. The desirability of including days as essentially a blocking factor in the analysis can be seen from the fact that this source of variation was significant for log (plaques/$10^6$ cells).

Table 2 contains the results of Duncan's multiple range test on the treatment means for the various parameters studied. The 12.34 $\mu$g $Ni^{2+}$/g treatment caused significantly lowered body weight, spleen weight, and the logarithm of the spleen weight to body weight ratio in comparison to controls and all other concentrations of $Ni^{2+}$. With respect to the mean logarithm of plaques/$10^6$ cells, the control group had a significantly higher mean response than did the 9.26 and 12.34 $\mu$g $Ni^{2+}$/g treatment groups. This indicates that these $Ni^{2+}$ concentrations caused significant splenic immunosuppression. Dosages of 3.09, 6.17, and 9.26 $\mu$g $Ni^{2+}$/g were not significantly different from each other, nor were 6.17, 9.26, and 12.34 $\mu$g $Ni^{2+}$/g. Concentrations of 6.17 $\mu$g $Ni^{2+}$/g depressed log (plaques/$10^6$ cells). When the data were analyzed with Duncan's multiple range test, this depression was not statistically significant. However, when Dunnett's test, which compares each treatment against control, was used, the immunosuppression was significant ($p < 0.05$).

Further analysis using linear regression is depicted in Figure 1. There was a statistically significant ($p < 0.05$) negative linear dose-response relationship between the concentration of metal and log (plaques/$10^6$ cells).

In an attempt to determine the concentration of $Ni^{2+}$ in the spleen, another experiment was performed. Twenty-five mice received 6.17 $\mu$g $Ni^{2+}$/g. Immediately after injection and 1, 2, 3, and 4 days later, five mice were sacrificed and their spleens removed. The spleens were ashed, solubilized, and assayed for Ni with an atomic absorption spectrophotometer. No nickel was detected at any of these times.

## Discussion

This preliminary investigation has shown that $Ni^{2+}$ adversely affects primary antibody production in the spleen. In mice, antibody production against

**Table 1. Anova summary of significance probabilities of various biological parameters.**

| Source of variation | Body weight | Spleen weight | Log $\frac{\text{Spleen wt}}{\text{Body wt}}$ | Plaques/ $10^6$ cells | Log (plaques/ $10^6$ cells) |
|---|---|---|---|---|---|
| Days | 0.32 | 0.65 | 0.67 | 0.0006 | 0.0044 |
| Ni concn | 0.0001 | 0.0014 | 0.034 | 0.023 | 0.024 |
| Days × Ni concn | 0.35 | 0.94 | 0.86 | 0.73 | 0.68 |

**Table 2. Means of measurements of various biological parameters.[a]**

| $Ni^{2+}$, $\mu$g/g | Body wt, g | Spleen wt, g | Log $\frac{\text{Spleen wt}}{\text{Body wt}}$ | Log (plaques/ $10^6$ cells) | Sample size |
|---|---|---|---|---|---|
| 0 | 24.8 | 0.163 | -2.187 | 2.629 (512)[b] | 11 |
| 3.09 | 26.0 | 0.164 | -2.202 | 2.573 (423) | 12 |
| 6.17 | 24.6 | 0.157 | -2.196 | 2.473 (343) | 12 |
| 9.26 | 24.3 | 0.147 | -2.229 | 2.366 (289) | 11 |
| 12.34 | 20.8 | 0.113 | -2.287 | 2.285 (245) | 12 |

[a]Any two treatment means connected by a vertical line DO NOT differ significantly from each other at the 0.05 probability level (using Duncan's multiple range test).
[b]Detransformed mean.

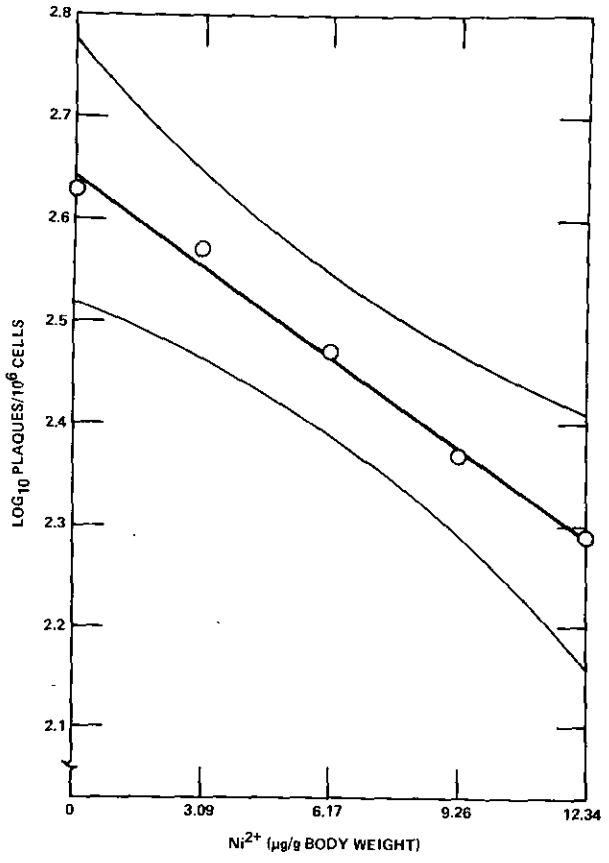


FIGURE 1. There was a significant ($p < 0.05$) negative linear dose-response relationship between the concentration of Nickel injected and the resulting level of plaque production. The regression equation ($Y = 2.64 - 0.028\ X$) along with its 95% confidence limits is presented.

sheep erythrocytes is dependent on B and T cell interaction (*9*). Therefore, if $Ni^{2+}$ caused defects in either of these cells, immunosuppression could result. It is further possible that $Ni^{2+}$ caused a shift in the relative populations of B and T lymphocytes in the spleen. Since the Jerne plaque technique measures the number of antibody-producing cells per million total cells, if a $Ni^{2+}$-exposed mouse had different proportions of B to T lymphocytes, this could cause alterations in the immune response.

It is further possible that $Ni^{2+}$ caused changes in the proportions of macrophages and their precursors to lymphocytes. With a light microscope, small macrophages are undistinguishable from large lymphocytes. If $Ni^{2+}$ caused an influx of such macrophages, they would be counted as lymphocytelike cells; and since they do not produce antibodies, the plaques/$10^6$ cells would be skewed.

Macrophages also participate in immunogenesis. However, it is not known whether their role relates to their plasma membrane or phagocytic activity (*10*). Previous studies have shown that concentrations of $Ni^{2+}$ as low as 35 µg/ml depress phagocytosis by alveolar macrophages *in vitro*. This phagocytic defect could be due to membrane or cytoplasmic damage, but in any case, it still is not possible to relate such defects to the immunosuppression seen in these current experiments.

For these reasons, additional research in this laboratory will focus on further Jerne plaque tests as well as the effect of $Ni^{2+}$ on B and T cell transformation after phytohemagglutinin and lipopolysaccharide stimulus and on the proportion of B and T lymphocytes within the spleen. In view of the toxic effects of Ni on primary humoral immunity, it would be of great interest to test the effect of Ni on cell-mediated immunity, especially since Ni has been implicated as a possible carcinogen or cocarcinogen (*1*).

In addition to the metal studied in this paper, other environmental pollutants such as lead (*12, 13*), nitrogen dioxide (*14, 15*), sulfur dioxide (*16*), silica (*17*) and cigarette smoke (*18*) can depress the immune response. Thus, it would be advisable to see if other pollutants also cause immunosuppression.

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This report has been reviewed by the Office of Research and Development, EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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