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Responses of Alveolar Macrophages to Metals

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Responses of Alveolar Macrophages to Metals

I. Inhalation of Lead and Nickel

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Rats were subjected to the inhalation of soluble and insoluble aerosols of lead or nickel ($PbCl_2$, Pb_2O_3, $NiCl_2$, NiO) at concentrations near or below the levels proposed as acceptable for occupational exposures.

After at least two weeks of exposure to these contaminants, the number of alveolar macrophages available as measured by a standard washing procedure was significantly increased with exposure to NiO, significantly decreased with Pb_2O_3, and not significantly changed with $PbCl_2$ and $NiCl_2$. However, the distribution of sizes of cells in a population washed out was altered with the different exposures. A marked increase in mucus in the rats exposed to $NiCl_2$ was noted. The histopathological examination (light microscopy) of the lungs revealed changes after inhalation of both nickel compounds.

The defensive capacity of the alveolar macrophage against pulmonary infection has led to an intensive investigation of the factors that control the availability and function of these cells. Since the lungs of most persons, especially in the urban population, are subjected to a variety of gaseous contaminants (O_3, NO_2, SO_2) and particulates (dust, soot, metallic particulates, etc), the alterations in the number and function of alveolar macrophages induced by these agents are evidently relevant to the study of pulmonary disease. Indeed, the continuous disruption of these cells through long-term low-level exposure to certain agents may cause not only an impaired defense against infectious agents, but also may result in damage to the lung such as development of fibrosis[1] and accumulation of toxic particulates in the lung.

We reported a decrease in the number of alveolar macrophages as "free cells" lavaged from rats that have inhaled low concentrations of Pb_2O_3 (10μg/cu meter or 150μg/cu meter), compared with control rats breathing filtered air.[2] In an effort to determine whether a dose-response relationship could be demonstrated, rats were subjected to the inhalation at two concentrations of Pb_2O_3 for various periods of time, and the number of free cells in the lung determined. Following lavage of the lung of rats that had inhaled 150μg/cu meter Pb_2O_3, the number of cells recovered diminished significantly in one day and remained low for the duration of the exposure (three months). The numbers of phagocytic cells diminished more slowly in rats breathing 10μg/cu me-

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Table 1.—Concentration and Size of Metallic Particulates Used in Inhalation Experiments

Metallic Particulate	Concentration of Metal	Distribution	Mass Median Diameter, μ, (Unit Density)	Geometric Standard Deviation (σg)*
Pb_2O_3	150μg/cu meter	99% <1.0μ	0.15	3.33
$PbCl_2$	100μg/cu meter	95% <1.0μ	0.17	3.29
$NiCl_2$	109μg/cu meter	96% <1.0μ	0.32	1.51
NiO	120μg/cu meter	93% <1.0μ	0.25	2.50

* σg signifies geometric standard deviation (with the assumption of a log normal distribution Gg is equal to the 84.16% value of d_p† divided by the 50.00% value of d_p).
† d_p signifies diameter of particle corresponding to the given collection efficiency from cumulative number distribution curve.

ter, beginning to fall after three days and reaching their lowest number after the eight days of exposure.[3]

Another important consideration was whether or not the effects of inhaling the Pb_2O_3 were reversible. In the same experiments, rats were allowed to breathe filtered air for various periods of time after three weeks of prior exposure to Pb_2O_3, and the numbers of pulmonary free cells were then counted. The number of cells washed out of the lungs of rats that had inhaled either 10 μg/cu meter or 150μg/cu meter returned to control levels after three days of breathing filtered air.[3]

The question of specificity of the response to Pb_2O_3 continues to be an important issue and experiments to answer this question are presented. Another compound of lead, $PbCl_2$ (soluble), and two nickel compounds, NiO (insoluble) and $NiCl_2$ (soluble), were selected for comparison with Pb_2O_3.

The numbers of free cells that are found in the washings from the lungs of rats after the inhalation of Pb_2O_3, $PbCl_2$, NiO, or $NiCl_2$ were determined, and histological examination of the lungs and the cellular population lavaged from the respiratory tract were made.

Methods

The techniques for the exposure of animals to the inhalation of Pb_2O_3, $PbCl_2$, NiO, and $NiCl_2$ have been described.[2-4] The Pb_2O_3 was generated with and without the use of a system for neutralization of charges on the particles. The method for neutralization of charged particles was to pass the aerosol through by a tube surrounded by a ^{90}Sr source.[5]

The duration of the respiratory exposure reported in these experiments was 12 hours per day and six days per week for various periods of time up to several months, except in the case of Pb_2O_3 that was continuous. The concentrations of the various contaminants were determined with use of multistage impacter to collect the particles, and the samples were analyzed for the specific metal by atomic absorption spectroscopy. Details of the methodology are presented elsewhere.[4] The concentrations of the contaminants and their particulate dimensions are presented in Table 1.

The rats in the experiments were Greenacres controlled-flora rats. They are a Wistar-derived animal with little tendency to develop chronic respiratory disease in our laboratory. Young male rats weighing approximately 300 gm were used.

The lavage technique for recovering alveolar macrophages was that described by Brain and Frank,[6,7] as modified by Bingham et al.[2] After the number of cells in each washout fluid was determined, all nine washings from each rat were combined and centrifuged, and a slide was prepared from the pellet of cells. The slides were stained with Giemsa and eosin, and 150 cells were measured and assigned to a group of 10μ, 10μ to 20μ, or 20μ.

Whole lungs (not washed out) were taken for histopathological examination. The lungs were fixed by inflation with 10% formaldehyde solution after being collapsed under vacuum. Sections (5μ) were stained with hematoxylin and eosin.

In a small number of rats exposed by $NiCl_2$ and NiO, after removal of cells by centrifugation of the washings for 10 minutes at 500 g, the supernatant was centrifuged for 30 minutes at 16,000 g. This procedure separated out all cloudy or viscous material. The resultant pellet was analyzed for sialic acid with use of the procedure of Warren[8] in order to obtain a quantitative index of mucus.[9]

Results

The number of cells (in millions per gram of wet weight tissue) washed from the lungs of rats of various experimental groups was

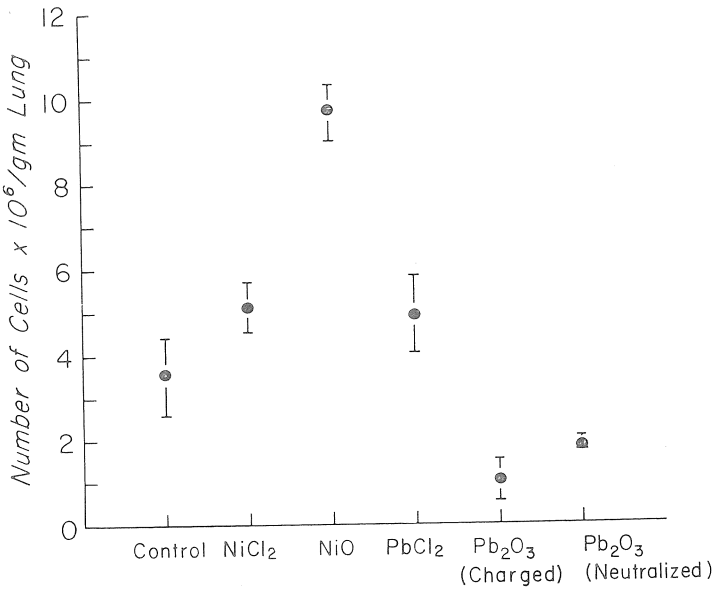
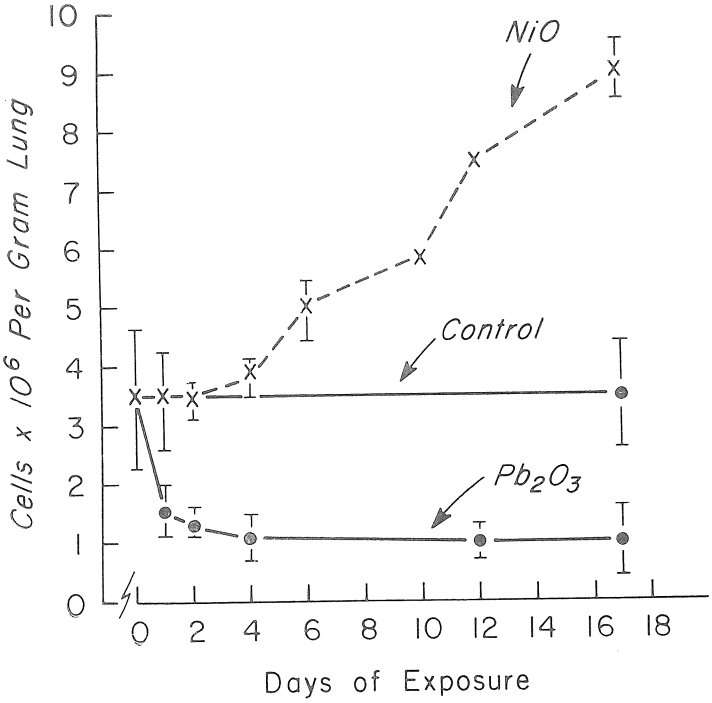


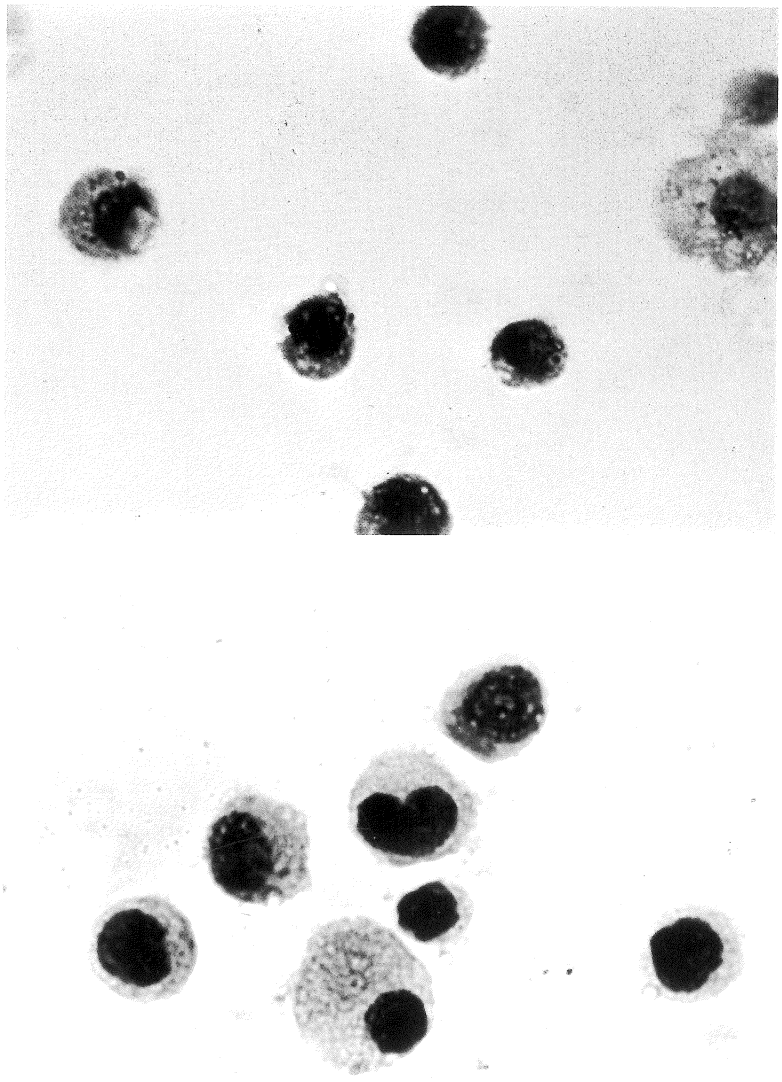
Fig 1.—Mean number and standard error of alveolar macrophages washed from rats breathing metallic particulates for two weeks.

Fig 2.—Mean number and standard error of alveolar cells washed from lungs of rats after inhalation of oxides of lead and nickel.



as follows: controls that breathed filtered air, 3.5×10^6/gm lung; $NiCl_2$ group, 5.1×10^6/gm lung; NiO group, 9.8×10^6/gm lung; animals exposed to Pb_2O_3, 1.0×10^6/gm lung and Pb_2O_3 (electrostatic charge neutralized) 1.8×10^6/gm lung; and $PbCl_2$ group, 4.9×10^6/gm lung. The average number of cells obtained by the use of each experimental regimen is given in Fig 1 and 2. Each point is the mean and standard error of at least ten rats, except the control that is the mean of 53 rats killed over the interval of the various experimental regimens. The number of cells washed from the lungs of rats that had inhaled Pb_2O_3 was significantly decreased, as previously reported, while the inhalation of NiO produced a marked increase in the number of cells. Continued inhalation of Pb_2O_3 did not result in significantly greater reduction in the number of cells; however, continued inhalation of NiO yielded larger numbers of cells. After four to six weeks of exposure, more than 1.1×10^7 cells were recovered per gram of lung. Although the quantity of cells washed from the lungs of rats that had breathed $NiCl_2$ or $PbCl_2$ was not significantly different from the quantity removed from the lungs of control rats, there were pathological changes in the lungs from rats subjected to the inhalation of $NiCl_2$.

Both nickel compounds, NiO and $NiCl_2$, produced changes visible with the light microscope. The inhalation of NiO for two weeks



Alveolar macrophages from rats after inhalation of filtered air (top) or NiO (bottom) (Giemsa and eosin, X 2,000).

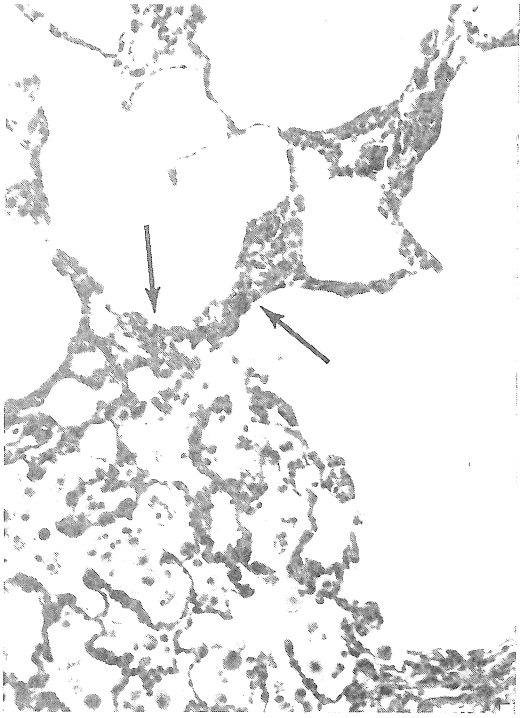


Fig 3.—Lungs from rats that had inhaled NiO. Note large numbers of alveolar macrophages and infiltrates of lymphocytes (arrows) (hematoxylin-eosin, ×350).

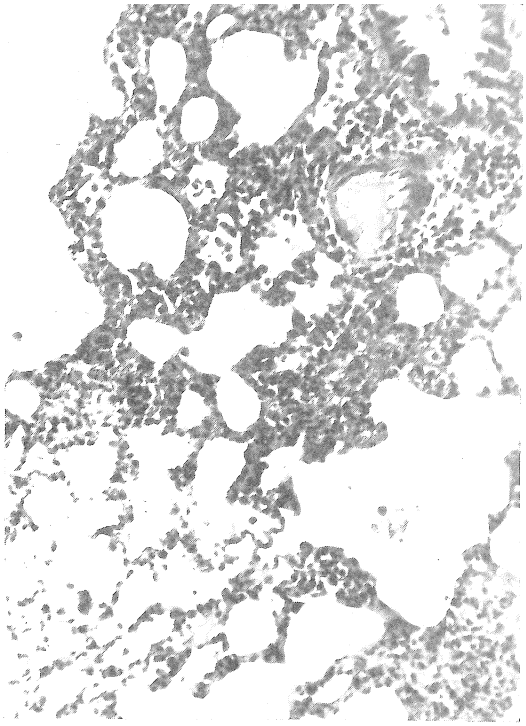


Fig 4.—Lungs from rats that had inhaled NiO. Note hypersecretion of mucus in bronchi and lymphocytic infiltration (hematoxylin-eosin, ×350).

caused significant accumulations of macrophages in the alveolar spaces (Fig 3). There appears to be some hypersecretion in the bronchial epithelium compared with controls (Fig 4). Focal infiltration by lymphocytes occurred in the alveolar walls and perivascular spaces (Fig 3 and 4). After longer exposure to NiO, the cellular infiltration subsided significantly; however, a thickening of the alveolar walls was distributed in a patchy manner throughout the lung (Fig 5). Occasionally the respiratory bronchus showed prominent walls. The number of intraalveolar macrophages had diminished. The cells washed from the lungs of rats inhaling NiO were quite variable in size and a representative population may be seen in the color Figure.

The most prominent effect of inhaled $NiCl_2$ was on the bronchial epithelium, as shown in Fig 6. Here, the epithelium was hyperplastic with evidence of marked mucus secretion. Peribronchial lymphocytic infiltration may be seen also. Alveolar macrophages were present but did not appear to be as abundant in these sections of lung as in the sections of lung obtained from rats inhaling NiO. Examination of the cells from the lavaged lung revealed a difference in distribu-

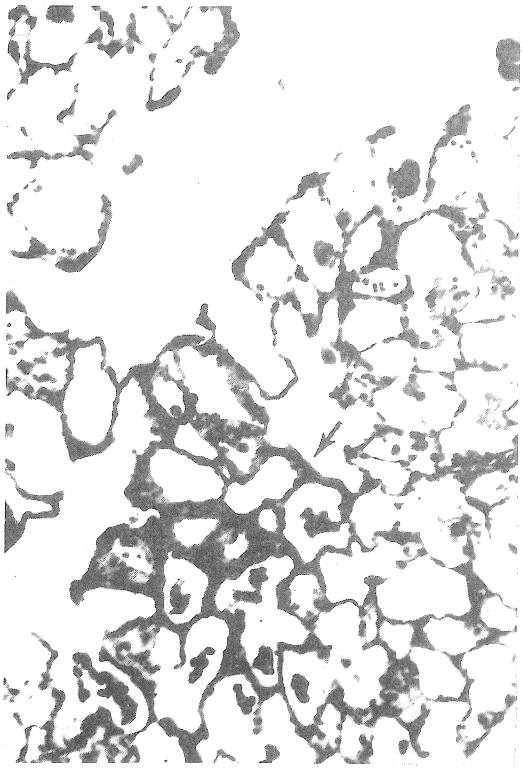


Fig 5.—Lungs from rats that had inhaled NiO. Note focal thickening of alveolar walls (arrow) (hematoxylin-eosin, ×350).

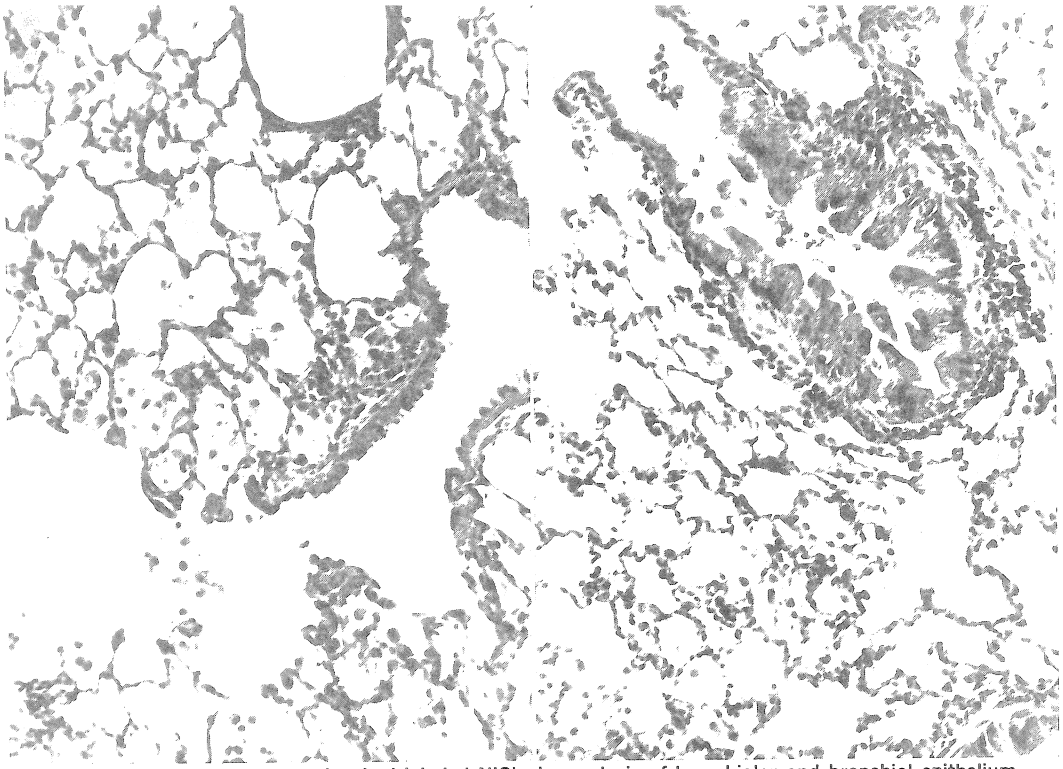


Fig 6.—Lungs from rats that had inhaled $NiCl_2$, hyperplasia of bronchiolar and bronchial epithelium with peribronchial lymphocytic infiltrates. Note small number of alveolar macrophages (hematoxylin-eosin, ×350).

Fig 7.—Lungs from rats after inhalation of Pb_3O_4 or $PbCl_2$. No significant pathologic alterations (hematoxylin-eosin, ×350).

Table 2.—A Comparison of the Washings From Lungs of Rats Subjected to Inhalation of Aerosols of Certain Metals

Type of Exposure	Number of Free Cells	Sizes of Cells: <10μ Number of Cells	Sizes of Cells: 10μ to 20μ Number of Cells	Sizes of Cells: >20μ Number of Cells	Appearance of Fluid Portion
Control	3.5×10^6/gm lung	64	67	19	Slightly cloudy and foamy in first and second lavages; mean weight of pellet, 52 mg; sialic acid, 0.03μg/mg of pellet
NiO	Markedly increased	25	120	5	Cloudy material, evident over five or six lavages; mean weight of pellet, 77 mg; sialic acid, 0.05μg/mg of pellet
$NiCl_2$	Not significantly changed	93	56	1	Very cloudy and viscous, all nine lavages are affected; mean weight of pellet, 131 mg; sialic acid, 0.1μg/mg of pellet
Pb_2O_3	Decreased	22	128	0	Did not differ from control
$PbCl_2$	Not significantly changed	21	125	4	Did not differ from control

tion of cell sizes in the populations produced by the different experimental regimens. These data are given in Table 2 along with a description of the fluid portion of washings.

The washings from the rats that had breathed the nickel compounds were viscous and cloudy. Inhalation of $NiCl_2$ for one week resulted in an especially cloudy washing that was still evident after the ninth lavage. The total weight of pellet available from each lavaged fluid and its content of sialic acid reflect the viscous and cloudy appearance of the fluid. The number of samples was too small for statistical analysis.

The lungs from rats which had inhaled $PbCl_2$, Pb_2O_3, or filtered air were indistinguishable (Fig 7). Both showed little or no inflammatory response.

Comment

The data on the number of populations of free cells washed from the lungs of rats subjected to the inhalation of different metallic particulates provide clear evidence that we cannot assume that the inhalation of particulates induces a nonspecific increase in the number of alveolar macrophages. In these experiments the number and distribution of sizes of the free cells and the quality of the fluid portion of the washings were dependent on the specific metallic particulate that was inhaled. The reaction of the lung to these particulates may depend on a number of factors such as the quantity, the specific metal, solubility, and physical properties. We cannot speculate as yet on the significance of the variations in the sizes of cell observed after the different exposures. The variability in the functional characteristics of these cells is currently under investigation.

Comparison of the responses of the lungs of rats to the inhalation of $PbCl_2$, NiO, $NiCl_2$, and Pb_2O_3 suggests that the reduction in number of alveolar macrophages in rats after inhalation of Pb_2O_3 is a specific response. The possible mechanism of this response is discussed elsewhere (E Bingham, unpublished data).

The results of the inhalation of metallic particulates described in this report suggest the possibility that the several metals found in coal dust may contribute to the alveolar macrophage response to inhaled coal dust. This is currently under investigation.

The type of experimental regimen may have influenced our results. In the past, most investigators introduced dusts intratracheally (insufflation or intubation of a suspension) and then determined the response of the lung,[7,10] and under these experimental conditions the number of macrophages washed from the lung increased. However, our experiments have been conducted using

inhalation of relatively low concentrations, always below the threshold limit value (TLV), of the specific metallic particulate. This procedure enables us to monitor the fluctuations in the size of the pool of alveolar macrophages by sampling the experimental animals after different lengths of exposure. It is possible that a relatively large dose of any particulate introduced all at one time into the lung will lead to an increased number of free cells in the alveoli. On the other hand, introduction of the material in a manner more like an actual exposure (inhalation) induces a response to the specific contaminant that may more nearly resemble the real life situation.

It has been noted by Lemon[11] that the respiratory system responds to different types of foreign particles in different ways, and Brain[7] has suggested that a specific experimental exposure may produce its own profile of cell types in the lung washings.

The origin of alveolar macrophages is probably of considerable importance in understanding the variations in the characteristics of the pool of free cells available after different experimental procedures. For example, if the alveolar macrophages arise almost wholly from the bone marrow, as suggested by the recent observation by Godleski and Brain,[12] the question arises whether the metals affect the bone marrow cells or act on the cells in the lung.

In view of the experimental results obtained with NiO and $NiCl_2$, the level of the current TLV for nickel (1,000μg/cu meter)[13] should be scrutinized. The data obtained in these experiments at levels of approximately one tenth of the current TLV suggest that 1,000μg/cu meter may be too high. Certainly additional experiments are required to make a scientifically sound judgment concerning the validity of the current TLV.

We are now engaged in experiments to provide information on the phagocytic capacity and biochemical characteristics of the populations of free cells washed from rats subjected to the inhalation of nickel compounds.

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