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ADVERSE EFFECTS OF METALS ON THE ALVEOLAR PART OF THE LUNG

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Abstract

Rabbits were exposed to low levels of metal dust or metal ions by inhalation for 1-8 months, 5 days/ week and 6h/ day. Following exposure lung tissue was examined by light and electron microscopy, the lung content of phospholipid was analyzed and the morphology and function of alveolar macrophages were investigated.

Metallic nickel dust as well as soluble nickel chloride produced accumulation of macrophages and laminated structures in alveoli and increased volume density of alveolar type II cells. The amount of phospholipids was elevated, mainly due to an increase in disaturated phosphatidylcholine. After one month exposure to metallic nickel dust or soluble nickel chloride, the alveolar macrophages contained surfactant inclusions and were functionally activated. After 3 and 6 months exposure to metallic nickel the macrophages were 'overfed' and inactive. A similar reaction is seen in the human disease pulmonary alveolar proteinosis. Exposure to cadmium chloride gave a similar reaction pattern as nickel did but in addition interstitial alveolitis. One month exposure to cobalt chloride affected the growth pattern of type II cells which formed nodules projecting into the alveolar lumen. Four months exposure to cobalt chloride resulted in further developed type II cell nodules, areas of hyperreactive type II cells, and interstitial inflammation. Copper chloride produced no effects apart from a slight increase in volume density of type II cells. Hexa- and trivalent chromium mainly affected the alveolar macrophages which showed enlarged lysosomes. Thus, different metal ions, in similar concentrations produced different pathological effects in the lung.

KEY WORDS: alveolar type II cells, surfactant, macrophages, lung ultrastructure, nickel, cadmium, copper, cobalt, manganese, chromium.

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Introduction

Airborne particles deposited in the alveoli initially come in contact with alveolar macrophages and alveolar epithelial cells such as type I cells (membranous pneumocytes) and type II cells (granular pneumocytes). Alveolar macrophages represent the main defense mechanism against foreign agents in the alveoli. Type I cells are very attenuated and thus vulnerable cells. They form, together with the endothelial cells of blood capillaries and the basal membrane, the blood-air barrier. The type II cells have two main functions (Mason and Williams 1977). They produce the surfactant layer which consists mainly of disaturated phosphatidylcholines, especially 1,2 dipalmitoylphosphatidylcholine. Type II cells also serve as precursors to type I cells in case of injury to the alveolar epithelium.

The present paper compares effects of dusts of metallic nickel, cadmium, copper, cobalt, manganese, and chromium in soluble form, on alveolar type II cells, lung content of phospholipids, and alveolar macrophages.

Materials and Methods

Male rabbits (size 2-3 kg) were exposed to metal dusts or metal ions, usually for one month but sometimes for up to 8 months (5 days/ week and 6h/ day). The concentrations were usually in the range 0.1-1 mg/m^3, i.e., the same order of magnitude as the actual occupational limit values for several of the metals. Following exposure alveolar tissue, lung phospholipids, and the alveolar macrophages were studied. The left upper lung lobe was fixed in 10% formalin for light microscopical examination. From the left lower lobe samples were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer for electron microscopical studies including determination of the volume density of type II cells. The samples were postfixed in 1% OsO_4 in 0.1M cacodylate buffer, dehydrated

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in graded alcohol and embedded in Epon 812 or Polarbed 812. Thin sections were examined in a JEOL 100S electron microscope. On the rest of that lobe, the phospholipid content was analyzed by reversed phase chromatography (Curstedt 1974). The right lung was lavaged and the alveolar macrophages were studied morphologically and functionally. For further details see Johansson et al. 1983b and Wiernik et al. 1983.

Results and Discussion

Metals in metallic form

Following exposure to 0.2 and 1.0 mg/m^3 of respirable metallic nickel dust the lung weight increased significantly (Camner et al. 1978). After 3 and 6 months exposure to 1 mg/m^3, alveolar spaces contained many enlarged macrophages with foamy or granular cytoplasm (Johansson et al. 1981). After 6 months exposure also interstitial lymphocytic infiltrations occurred. Electron microscopy showed laminated structures similar to the lamellar bodies of alveolar type II cells in the alveolar spaces. These structures were especially abundant after 6 months exposure. The volume density showed a 2- to 3-fold increase following exposure to nickel dust for 1, 3, and 6 months (Johansson et al. 1981; Johansson and Camner 1980). No damage to type I cells was observed. Exposure to nickel markedly increased the amount of phospholipid per gram of lung tissue (Casarett-Bruce et al. 1981; Curstedt et al. 1974). This increase was mainly due to elevated levels of 1,2-dipalmitoylphosphatidylcholine.

The lung lavage fluid had an opaque, milky appearance and the number of alveolar macrophages was significantly increased (Camner et al. 1978, Casarett-Bruce et al. 1981). After one month exposure to 1 mg/m^3 of nickel dust the macrophages exhibited numerous slender protrusions on the surface. The cell size varied remarkably and the cytoplasm contained laminated inclusions, probably consisting of ingested surfactant (Camner et al. 1978). The oxidative metabolic activity of the macrophages, as measured by their ability to reduce nitroblue tetrazolium (NBT) to fromazan, was enhanced 'at rest' and further increased with the cells incubated with *E. coli* bacteria. The phagocytic capacity of the macrophages was increased. After 3 and 6 months exposure most macrophages had a smooth surface and the cytoplasm contained closely packed laminated inclusions and lipid droplets (Johansson et al. 1980a). The oxidative metabolic activity was increased 'at rest' but could not be further stimulated with *E. coli* bacteria. These results indicate an impaired function of the macrophages. Incubation of normal alveolar macrophages *in vitro* with native surfactant from nickel exposed rabbits produced similar effects on the macrophages as one month exposure to metallic nickel dust did (Wiernik et al. 1981). Therefore, the effects on the macrophages following nickel dust exposure seemed to be produced by the high amounts of surfactant produced by the hyperplastic type II cells. The effect pattern in the lung showed many similarities with alveolar lipoproteinosis induced in rats by exposure to quartz (Corrin and King 1970, Heppleston et al. 1970) and the pathological picture in the human disease pulmonary alveolar proteinosis (Rosen et al. 1958).

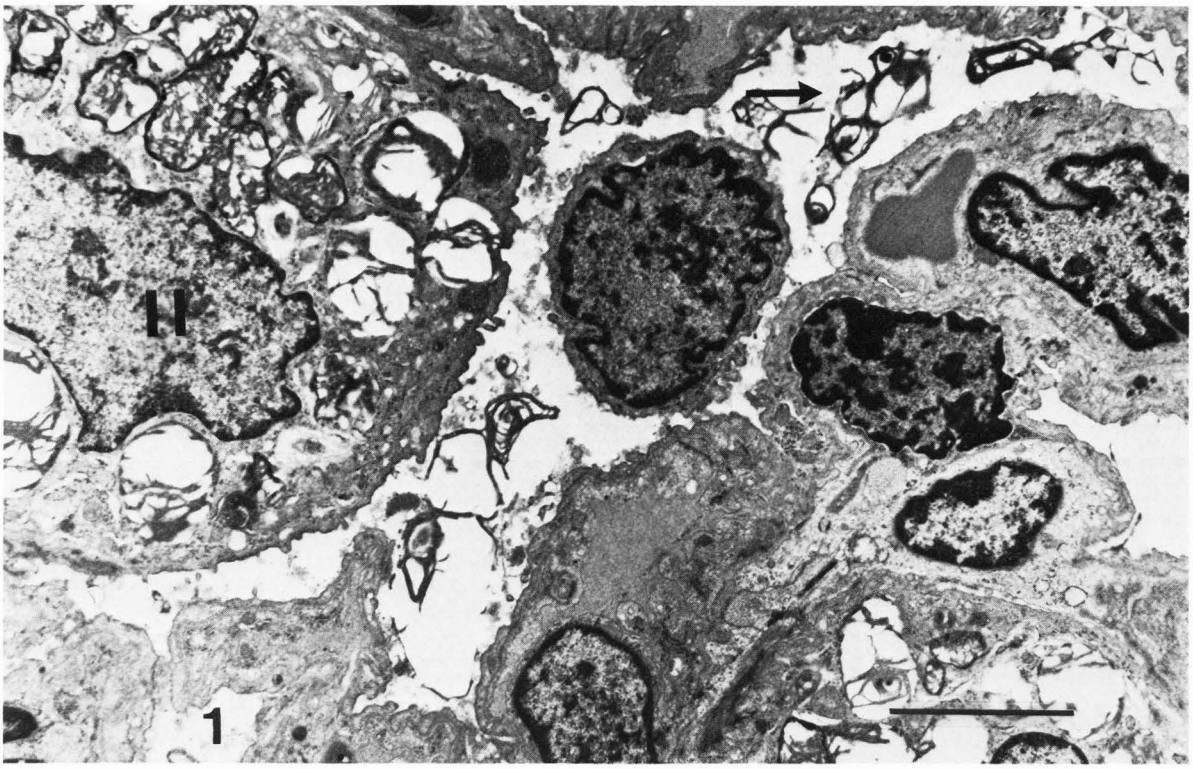
Exposure of rabbits to dust of metallic iron, cobalt and chromium in concentrations similar to that used in the nickel experiments did not produce the same pattern of effects in the lungs as nickel did (Johansson et al. 1980c). The metal particles were studied with respect to solubility in fluids and more nickel ions were found to be dissolved from the nickel particles than the other ions from their respective particles. This led us to expose rabbits to soluble nickel in order to study whether the nickel ions produced the effects.

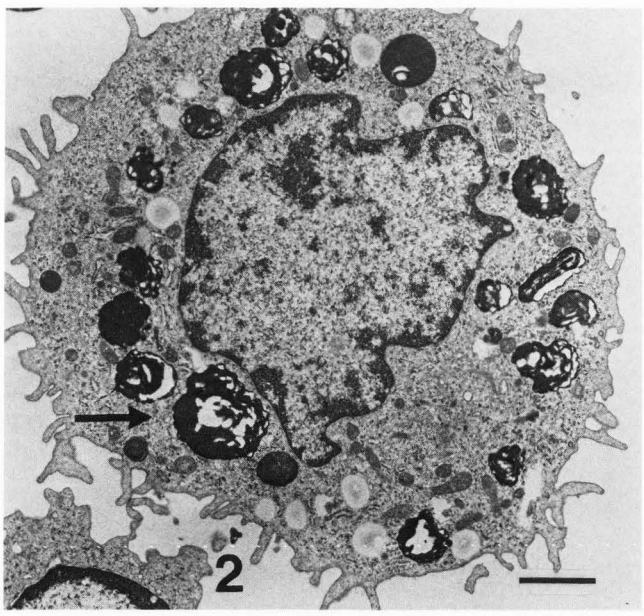
Metals in soluble form

The most characteristic findings are summarized in Table 1.

Nickel. Exposure to nickel chloride, 0.3 mg/m^3 as nickel, for one month produced an effect pattern in the lungs similar to that of metallic nickel dust. In the light microscope, focal accumulations, up to 2 mm, of large macrophages were seen in alveolar spaces (Johansson et al. 1983b). Electron microscopy revealed large type II cells engorged with lamellar bodies and abundant laminated material, surfactant, in alveolar spaces (Fig 1). The volume density of the type II cells was increased by a factor of two. This increase was due to an increase in size. The phospholipid content was increased by 40%, mainly due to an increase of 1,2-dipalmitoylphosphatidylcholine (Johansson et al. 1983b). The number of alveolar macrophages in the lavage fluid was significantly increased as was the variation in cell diameters (Wiernik et al. 1983). The surface of most macrophages was rich in microvilli and the cytoplasm contained many laminated inclusions (Fig 2). These cells had fewer lysosomes than control macrophages (Fig 3). Some of the macrophages had a smooth surface and contained densely packed laminated structures (Fig 4). The oxidative metabolic activity tended to be increased 'at rest' and was significantly higher upon stimulation with *E. coli* bacteria. No change in phagocytic activity was, however, observed. The lysozyme levels were significantly reduced in macrophages and in lavage fluid following Ni^{2+} exposure (Lundborg and Camner 1982, 1984). When macrophages from lavage fluid were separated with respect to size, the cell fraction with the highest contribution of 'normal' macrophages, i.e., macrophages of normal size and few laminated inclusions showed the lowest lysozyme level (Johansson et al., 1986d). Thus, apart from the effects the augmented levels of surfactant caused on the macrophages, nickel ions apparently exert a direct influence on these cells.

Cadmium. Cadmium chloride, 0.4 mg/m^3 as cadmium, produced a reaction pattern in the lung similar to that of nickel chloride, i.e., accumulation of foamy macrophages in alveolar spaces, increased volume density of alveolar





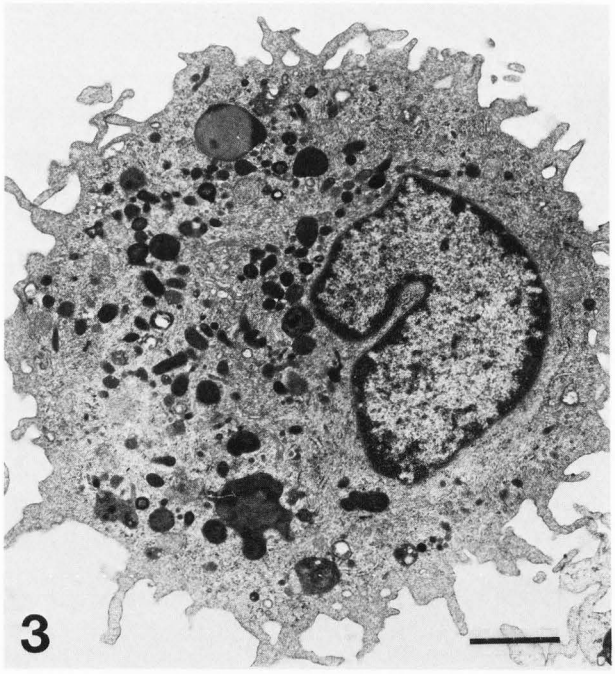


Fig 1: Alveolar tissue from a rabbit exposed to $NiCl_2$ for one month showing laminated material in alveolar spaces (arrow) and an enlarged type II cell (II). Bar = 5µm.

Fig 2: Alveolar macrophage from a rabbit exposed to $NiCl_2$ for one month. The cytoplasm contains many laminated inclusions (arrow). Bar = 2µm.

Fig 3: Alveolar macrophage containing abundant lysosomes from a control rabbit. Bar = 2µm.

type II cells, increase in phospholipids and an increased metabolic activity in the macrophages (Johansson et al. 1983a, Johansson et al. 1984). In addition, Cd^{2+} gave interstitial infiltration of neutrophils and lymphocytes.

Table 1

Comparison of the most characteristic findings in lung tissue from rabbits exposed to metals in soluble form for one month

Metal	Conc mg/m^3	Increased lung wt	Increased phospholipid concentration	Interstitial inflammation	Macrophage stimulation	Increased volume density of type II cells	Nodular proliferation of type II cells
$NiCl_2$	0.3	+	+	0	+	++	0
$CdCl_2$	0.4	+	+	+	+	++	0
$CoCl_2$	0.5	0	0	0	+	0	+
$CuCl_2$	0.6	0	0	0	0	+	0
$MnCl_2$	1.1 3.4	0	0	0	0	0	0
Cr(VI) Na_2CrO_4	0.9	0	0	0	0	0	0
Cr(III) $Cr(NO_3)_3$	0.6	0	0	0	+	+	0

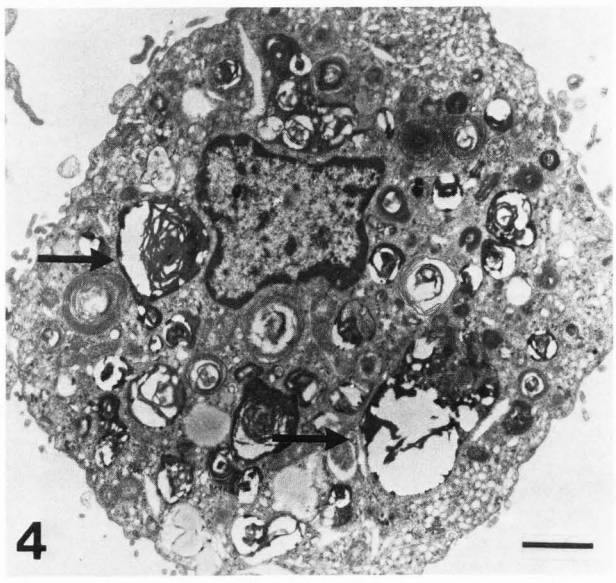
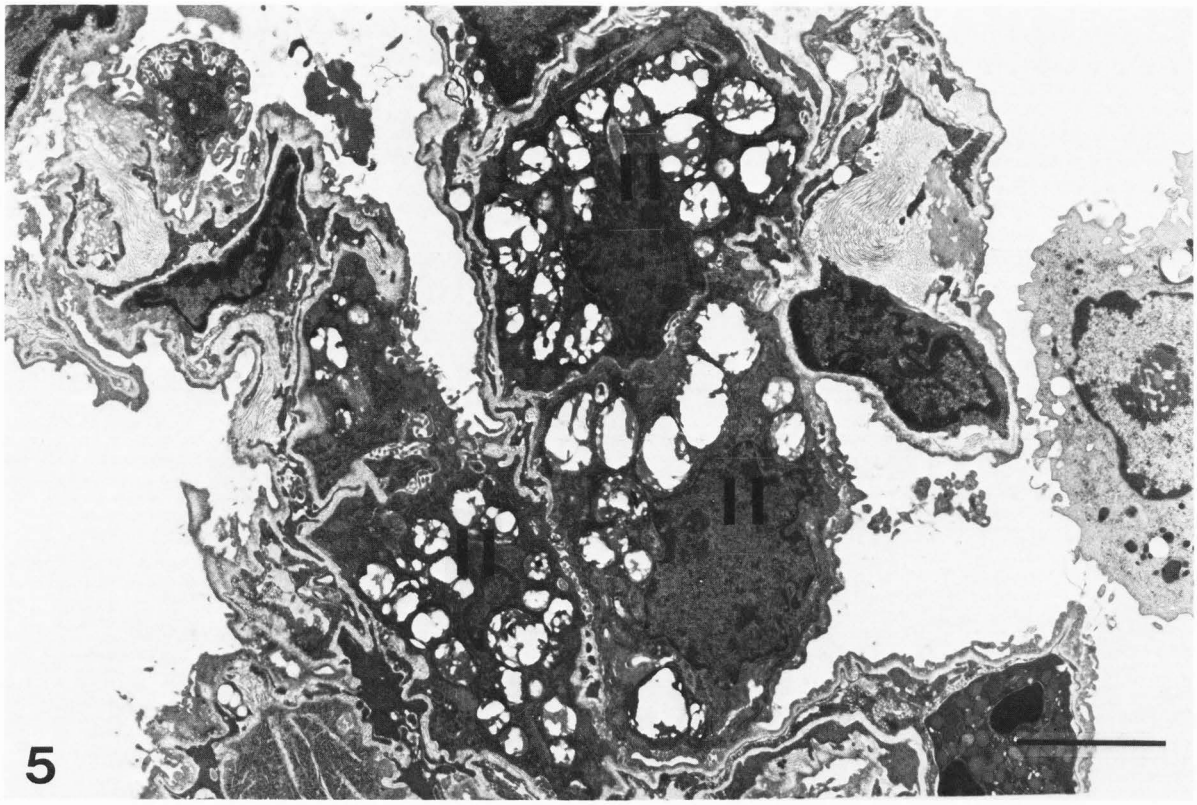


Fig 4: Alveolar macrophage with a smooth surface and the cytoplasm filled with laminated inclusions (arrow) and lipid droplets from a rabbit exposed to $NiCl_2$. Bar = 2μm.

Many alveolar macrophages exhibited cytoplasm filled blebs on the surface (Johansson et al. 1983b). The lysozyme levels in lavage fluid and macrophages were increased (Lundborg and Camner 1984). No damage to alveolar type I cells was observed at this Cd^{2+} concentration.

Cobalt. Exposure to cobalt chloride, 0.5 mg/m^3 as cobalt, for one month produced an altered growth pattern of the alveolar type II cells. They formed clusters projecting into the alveolar lumen (Fig 5) (Johansson et al. 1984). No increase was found in the volume density of the type II cells or in the concentration of phospholipids. The alveolar macrophages showed an enhanced metabolic activity but there were no morphological changes (Johansson et al. 1983a).

In a follow-up study rabbits were exposed to cobalt chloride, 0.5 and 2.0 mg/m^3 as cobalt for 4 months (Johansson, to be published). This investigation showed a more pronounced nodular hyperplasia of type II cells and also accumulation of enlarged macrophages in reactive areas. Laminated material filled the alveoli in some parts of the lung. This reaction was especially prominent in 'white spots' on the lung surface and in hyperreactive areas more centrally in the lung. A similar reaction has been described by Kitamura et al. (1978) in a lung autopsy from a woman with cemented tungsten carbide pneumoniosis. Some type II cells had a swollen apical part and lacked microvilli. There was no significant increase in the volume density, because areas between type II cell clusters contained fewer type II cells than in controls. Oedema in type I cells was observed in focal areas but was not a prominent finding.



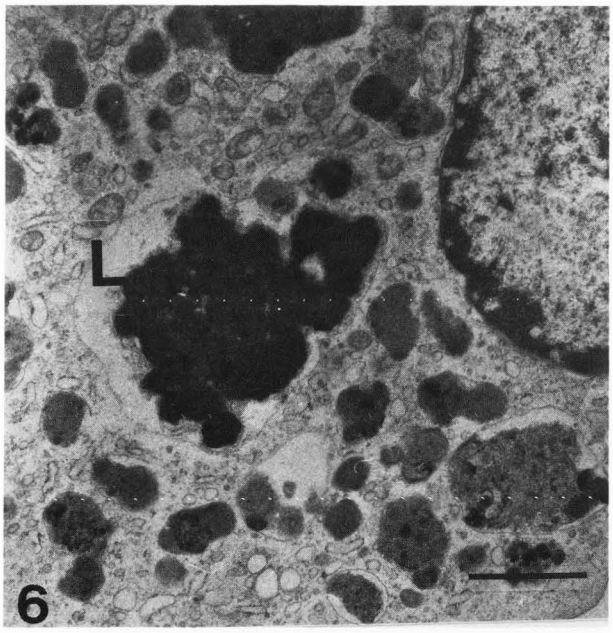


Fig 5: Cluster of alveolar type II cells (II) in the lung parenchyma of a rabbit exposed to $CoCl_2$ for one month. Bar = 5µm.

Fig 6: Alveolar macrophage containing enlarged lysosomes (L) from a rabbit exposed to Na_2CrO_4 for one month. Bar = 2µm.

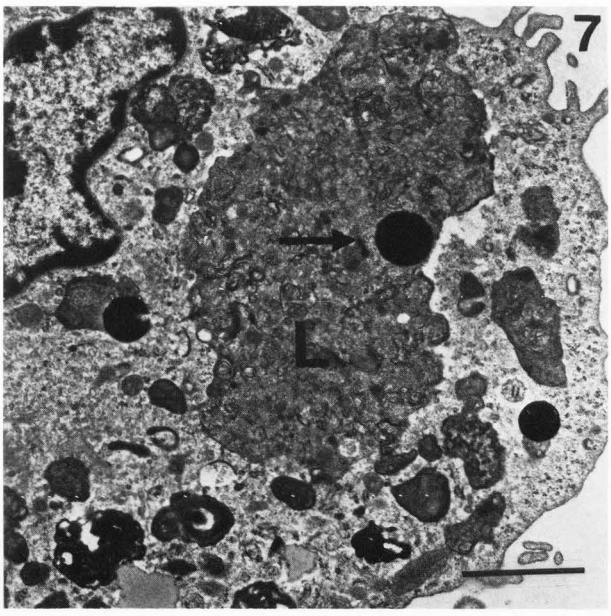


Fig 7: Alveolar macrophage from a rabbit exposed to $Cr(NO_3)_3$ for one month. An enlarged lysosome (L) with chromium-containing bodies (arrow) is present in the cell. Bar = 2µm.

The morphology of the macrophages showed great variations. Some cells were large, smooth and filled with laminated inclusions. Others appeared similar to macrophages from control animals (Johansson et al. 1986a). The oxidative metabolic activity was increased but the phagocytic activity was unchanged. With knowledge of the growth pattern of type II cells it is likely that the large smooth macrophages emanated from hyperreactive areas in the lung.

Copper. Exposure to copper, 0.6 mg/m^3 as copper chloride, for one month produced no effects in rabbit lungs apart from a slight increase in volume density of type II cells (Johansson et al. 1984).

Manganese. Manganese chloride, 1.1 and 3.9 mg/m^3, administered for one month gave no effects on lung tissue, lavaged macrophages or phospholipid content in the lung (Camner et al. 1985).

Chromium. Exposure to hexavalent (Na_2CrO_4) as well as to trivalent ($Cr(NO_3)_3$) chromium in concentrations of 0.9 and 0.6 mg/m^3, respectively, for one month tended to give nodular accumulations of macrophages but produced otherwise no effect on the lung tissue (Johansson et al., 1986b). Prominent effects were, however, found in the alveolar macrophages (Johansson et al., 1986c). Lysosomes in macrophages from Cr(VI) exposed rabbits were large and contained short membranous fragments (Fig 6). Also lysosomes of macrophages from Cr(III) exposed rabbits were enlarged but contained long membrane structures. These cells had rounded electron-dense inclusions often, but not always, located in lysosomes (Fig 7). X-ray microanalysis demonstrated chromium to be present in these inclusions. Laminated structures like those seen following nickel exposure, were frequent but the amount of phospholipid in the lungs was unchanged. Only Cr(III) produced any functional changes in the macrophages. The metabolic activity was increased but the phagocytic capacity was impaired (Johansson et al., 1986d). Chromium thus seems to primarily affect the alveolar macrophages. The accumulation of laminated inclusions, membranous lysosomes and the concomitant functional impairment following exposure to Cr(III) might be due to a reduced capacity to catabolize surfactant.

Conclusions

Many of the metals studied here, i.e., nickel, cadmium, cobalt, and chromium produced a number of effects on the lung. Several of these effects, such as nodular accumulation of macrophages, interstitial inflammation, hyperplasia of type II cells, accumulation of surfactant in alveolar spaces, impaired function of alveolar macrophages, and decreased lysozyme levels can be regarded as pathological. The effect patterns were characteristic and differed among metals. The effects were often complex and involved interaction among cell systems.

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Discussion with Reviewers

G.M. Roomans: Are the effects of the different metals on the alveolar part of the lung so characteristic that one might identify the metal from its ultrastructural effects?
Authors: We have so far studied only a limited number of metals, although exposure to these metals are common in industrial work. The metals have been administered in pure form, i.e., we have not so far looked at combinations of different metals or metal ions. It might, however, be possible, if we have some information about exposure history, to suggest which metal could have caused certain effects.

B.A. Afzelius: When you expose alveolar macrophages in vitro to surfactants from nickel exposed animals you get certain effects which you ascribe to the surfactants. Can you be sure that those effects are due to the surfactant rather than to nickel? In other words how free from nickel is the surfactant?
Authors: The nickel concentration in the surfactant is not detectable by X-ray microanalysis which means that it must be rather low (<300 ppm). In experiments similar to the in vitro exposures of normal rabbit alveolar macrophages to crock surfactant from nickel exposed animals, human monocytes reacted in the same way when they were exposed to a purified phospholipid fraction from normal pig surfactant (Wiernik et al., in preparation). Thus, the functional activation of the cells seems to be caused by the surfactant phospholipids. We have, however, found one effect which most likely is caused directly by nickel exposure: a decrease in the macrophage content of lysozyme (Lundborg et al., in preparation).

B. Forslind: Has the E. coli other effects in your experimental system than to stimulate oxidative metabolic activity? Would other bacteria serve the same purpose, e.g., pneumococci?
Authors: E. coli bacteria have been found to be very suitable to use in the NBT-test because of their high ability to stimulate the metabolism of macrophages as well as blood monocytes and granulocytes. Pneumococci are less effective.

B. Forslind: Could the difference in chromium (VI) and chromium (III) effects on lysosomes in macrophages be attributed to concentration differences caused by different cell membrane permeability of the two chromium species?
Authors: That might be one explanation. There has also been demonstrated a difference in clearance time; hexavalent chromium clears more rapidly than trivalent chromium from the lungs (Beatjer et al, 1959). In our study, $Cr(NO_3)_3$ showed an ability to precipitate in neutral pH and dissolve in acid pH. The chromium-rich bodies in the lysosomes might have been phagocytized by the cells and dissolve in the acid milieu in the lysosomes. The local chromium concentration could thus be comparatively high and remain high for a long time.

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