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Alveolar Macrophages and Lung Lesions after Combined Exposure to Nickel, Cobalt, and Trivalent Chromium

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In earlier inhalation exposures of rabbits, nickel increased the production of surfactant by type II cells, with secondary effects on morphology and function of alveolar macrophages. Cobalt induced mainly a nodular growth pattern of the type II cells. Trivalent chromium seemed to impair the capacity of macrophages to catabolize surfactant but did not affect the type II cells. We exposed rabbits by inhalation to combinations of nickel (0.6 mg/m³ as $NiCl_2$) and trivalent chromium [1.2 mg/m³ as $Cr(NO_3)_3$] (Ni–Cr), cobalt (0.5 mg/m³ as $CoCl_2$) and nickel (0.5 mg/m³) (Co–Ni), or cobalt (0.5 mg/m³) and chromium (1.2 mg/m³) (Co–Cr) for 4 months, 5 days/week, 6 hr/day. Alveolar macrophages, alveolar type II cells, and lung content of phospholipids were determined. All combined exposures induced more pronounced lung lesions than exposures for each of the metals. Phospholipid concentrations were significantly higher. There were significantly higher percentages of macrophages filled with surfactantlike inclusions and a smooth surface. Accumulations of macrophages in alveoli were more widespread. Chromium potentiated the effects of nickel and cobalt on the type II cells, which led to secondary effects on the macrophages. Nickel potentiated the specific effects of cobalt, i.e., type II cell nodule formation. The result indicates that noxious effects could also be induced in man by combined exposure to nickel, cobalt, and trivalent chromium in concentrations similar to those occurring in some occupational settings.

Introduction

In the working environment as well as in the general environment around industries, people may be exposed to combinatons of metals such as nickel, cobalt, and chromium. For more than a decade we have studied effects of inhaled metals on the alveolar part of the rabbit lung, with metal concentrations in the order of 1 mg/m³ and exposure times of 1–8 months, usually 4 months. The results after exposure to a single metal have been reviewed by Camner and Johansson (*1*).

Our studies revealed that soluble nickel increased the number and size of the alveolar epithelial type II cells and the lung content of surfactant lipids, with secondary effects on the alveolar macrophages. Soluble cobalt alters the growth pattern of the type II cells which occur in nodules. Around these nodules there is often accumulation of alveolar macrophages, which have a similar appearance to those after nickel exposure. Trivalent chromium in soluble form only induces clear effects on the alveolar macrophages. The macrophages had abnormal enlarged lysosomes and an increased content of surfactantlike inclusions, in spite of the fact that there was no significant increase in surfactant phospholipids. These changes suggest an increased ability to catabolize surfactant.

This paper presents data of three combination exposures of nickel, trivalent chromium, and cobalt: *a*) The effects after combined exposure to nickel and chromium were compared to those after nickel alone and an earlier exposure to chromium only (*2,3*). *b*) The effects after a combination of cobalt and nickel were compared with the effects after exposure to cobalt only and with nickel only in an earlier exposure (*4*). *c*) Effects after a combination of cobalt and trivalent chromium were compared with the effects after cobalt alone and with another experiment with chromium only (Johansson et al., unpublished data).

Materials and Methods

Groups of eight rabbits each were exposed to the metals. In experiment 1 (Ni–Cr) the rabbits were exposed to nickel (0.6 mg/m³ as $NiCl_2$) or to a combination of nickel and trivalent chromium [0.6 mg/m³ as $NiCl_2$ and 1.2 mg/m³ as $Cr(NO_3)_3$]. In experiment 2 rabbits were exposed to cobalt (0.5 mg/m³ as $CoCl_2$) or a combination of cobalt and nickel (0.5 mg/m³ as $CoCl_2$ and 0.5 mg/m³ as $NiCl_2$), and in experiment 3 rabbits were exposed to cobalt (0.6 mg/m³ as $CoCl_2$) or to a combination of cobalt and trivalent chromium [0.6 mg/m³ as $CoCl_2$ and 1.2 mg/m³ as $Cr(NO_3)_3$]. In each of the three combination experiments, a control group was exposed to filtered air. The exposure time was 4 months, 5 days/

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Table 1. Number and distribution of cells recovered by lavage from the right lung of rabbits exposed to combinations of metals (means ± SD).

Metal exposure	Number of cells, $\times 10^6$	Macrophages, %	Neutrophils, %	Eosinophils, %
Ni–Cr	126 ± 32	95.3 ± 2.3	3.6 ± 1.8*	0.6 ± 1.1
Ni	40 ± 32	86.3 ± 10.8	12.2 ± 10.7*	1.0 ± 0.8
Controls	17 ± 6	98.5 ± 1.2	0.2 ± 0.2	0.1 ± 0.2
Co–Ni	70 ± 22	97.4 ± 1.5	1.6 ± 1.2*†	0.3 ± 0.4
Co	24 ± 20	98.4 ± 1.4	0.4 ± 0.4	0.1 ± 0.2
Controls	14 ± 5	98.3 ± 1.4	0.3 ± 0.4	0
Co–Cr	45 ± 32	90.8 ± 4.2	5.4 ± 2.9‡	0.9 ± 0.7‡
Co	35 ± 24	93.3 ± 5.1	2.9 ± 3.4	0.6 ± 0.4
Controls	18 ± 6	97.6 ± 3.2	1.2 ± 1.7	0.1 ± 0.2

*$p < 0.01$ compared to controls.
†$p < 0.05$ compared to single metal exposure.
‡$p < 0.05$ compared to controls.

Table 2. Ultrastructural data on macrophages obtained by lavage (means ± SD).

Metal exposure	Surfactantlike inclusion profiles/cell profile			Smooth surface, %
	0–3, %	4–10, %	> 10, %	
Ni–Cr	41 ± 12 *†	25 ± 4	35 ± 15 *†	67 ± 15 *†
Ni	70 ± 8	18 ± 3	12 ± 8 *	23 ± 16 **
Controls	85 ± 9	15 ± 9	0.3 ± 0.5	3 ± 6
Co–Ni	57 ± 14 *§	17 ± 5	26 ± 16 *‡	40 ± 15 *†
Co	76 ± 14	17 ± 8	7 ± 7//	8 ± 9
Control	84 ± 10	15 ± 8	2 ± 2	2 ± 1
Co–Cr	50 ± 15 *§	20 ± 5	31 ± 13 *§	35 ± 7 **
Co	67 ± 10 **	12 ± 4	21 ± 8 *	23 ± 23
Controls	85 ± 8	13 ± 7	2 ± 2	11 ± 12

*$p < 0.001$ compared to controls.
†$p < 0.001$ compared to single metal exposure.
‡$p < 0.01$ compared to single metal exposure.
§$p < 0.05$ compared to single metal exposure.
//$p < 0.05$ compared to controls.
**$p < 0.01$ compared to controls.

week, for 6 hr/day. Metal aerosols were produced with an ultrasonic nebulizer, and the mass medium aerodynamic diameter was about 1 μm. Details are given by Johansson et al. (*2–4*).

The upper left lung lobe was used for light microscopy. From the left lower lobe, three pieces, 1–2 μm^3 each, were taken for electron microscopic examination and measurement of the volume density of the type II cells. The remainder of the lobe was used for phospholipid analysis. The right lung was lavaged and the morphology of the cells studied with light and electron microscopy. The function of the cells was tested by measuring their oxidative metabolic activity at rest and upon stimulation with *E. coli* bacteria and by measuring their phagocytic activity (*2*).

Results

In all three combination experiments significantly more cells were washed out from the lungs of the combined exposed rabbits than from the lungs of control animals or animals exposed only to one metal. The combination of nickel and chromium caused the highest increase (Table 1). The vast majority of the cells were macrophages, but all groups exposed to combinations of metals and the Ni group showed a significant increase in the percentage of neutrophils (Table 1).

The number of surfactantlike inclusions were increased in macrophages from all exposed groups compared to controls (Table 2). The percentage of macrophage profiles with more than 10 such inclusion profiles (Fig. 1) was significantly increased in all groups exposed to a combination of metals compared to corresponding single-exposed groups. About 60–70% of the macrophages from rabbits exposed to chromium also contained lysosomal complexes with membrane fragments and precipitated chromium, i.e., as previously seen in macrophages

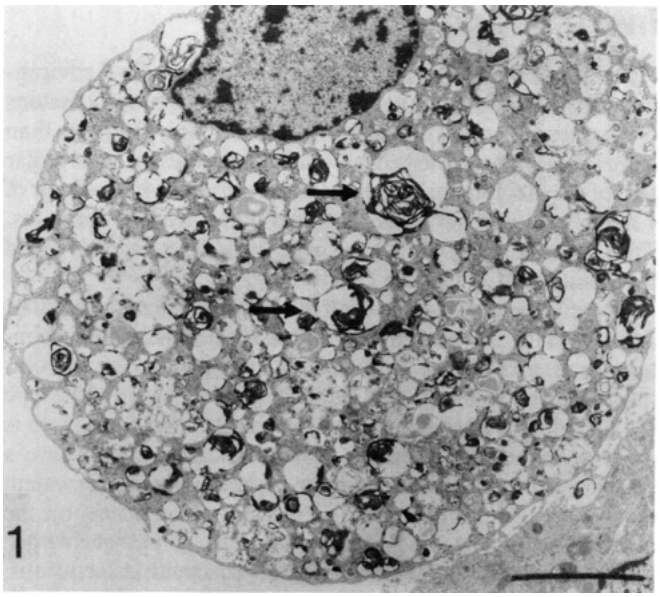
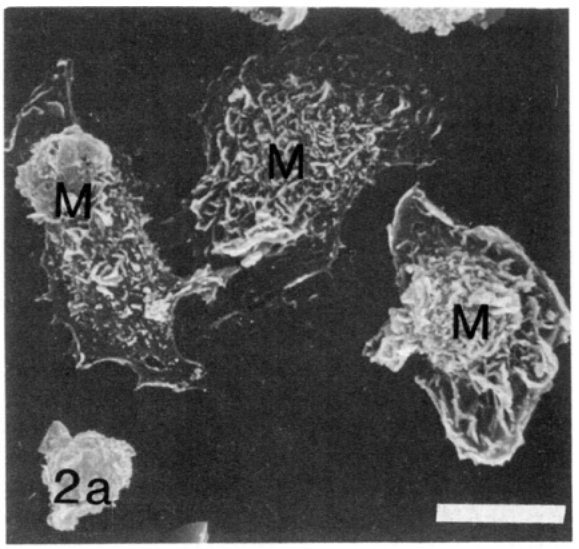


FIGURE 1. Alveolar macrophages filled with laminated, surfactantlike inclusions (arrows) from a rabbit exposed to Co^{2+} and Ni^{2+}. Bar = 5 μm.



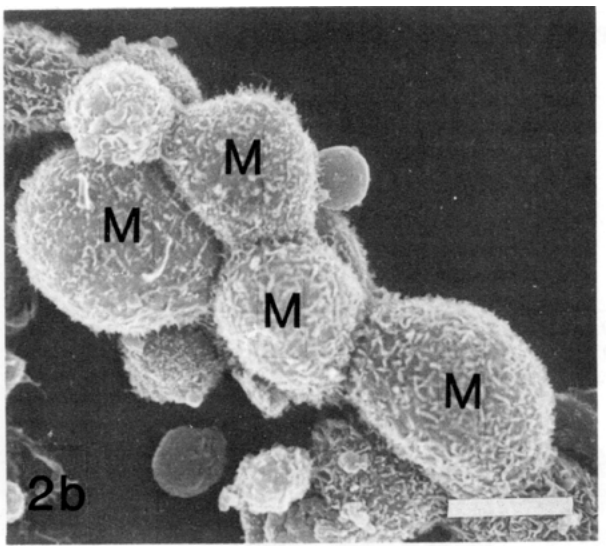


FIGURE 2. Alveolar macrophages (M) from (*a*) a control rabbit and (*b*) a rabbit exposed to Co^{2+} and Cr^{3+}. Bar = 5 μm.

from rabbits exposed to trivalent chromium alone (*5,6*). The high number of surfactantlike inclusions was often associated with a smooth surface (Fig. 1), and the percentage of such cells was significantly higher in the groups exposed to a combination of metals compared to controls or corresponding single-exposed groups (Table 2).

Scanning electron microscopy from the Co–Ni and Co–Cr experiments revealed that many macrophages from the exposed animals were poorly spread on the supporting glass surface, stuck together, and had short microvilli covering the upper surface (Fig. 2*a*), whereas cells from control animals spread out on the glass surface, appeared as single cells, and had an undulating surface with broad lamellipodia (Fig. 2*b*). The percentage of "ball-shaped" cells was highest in the double-exposed groups.

The ability of the macrophages to reduce nitroblue tetrazolium (NBT) to formazan was significantly increased in the Ni, Ni–Cr, Co, and Co–Cr groups, both at rest and after stimulation with bacteria, and increased significantly more in the Co–Cr group than in the Co group. Also, the phagocytosis of yeast particles was increased in these groups, but there was no significant change between the double- and single-exposed groups.

Accumulations of enlarged, vacuolated macrophages were found in alveolar spaces from all double-exposed rabbits, and in some of the single-exposed ones (Table 3 and Fig. 3). These aggregates appeared as naked granulomas or were associated with interstitial infiltrations of neutrophils, eosinophils, and lymphocytes. The reaction was significantly more pronounced in double-exposed than in the single-exposed groups and was highest in the Ni–Cr group, in which all eight rabbits had severe lesions.

Table 4 shows the volume density of alveolar type II cells. All double-exposed groups showed significantly higher values than corresponding single-exposed groups. The combination of Ni and Cr showed the highest increase. Alveolar spaces in large areas were filled with surfactant and macrophages in all double-exposed groups. In the Ni–Cr group, type I cell damage was seen, and the alveolar epithelium was thickened due to type I cell replacement by type II cells, which often were enlarged and stuffed with lamellar bodies (Fig. 4). Co^{2+} caused a decrease in percentage of single type II cells and an increase in percentage of cells found in nodules (Fig. 5). Both Ni^{2+} and Cr^{3+} appeared to enhance the nodular growth of type II cells caused by Co^{2+}.

Table 3. Macrophage reaction in lung tissue.

Metal exposure	Granulomatous macrophage reaction (positive/total)
Ni–Cr	8/8
Ni	6/8
Controls	0/8
Co–Ni	8/8
Co	2/8
Controls	0/8
Co–Cr	7/8
Co	1/8
Controls	1/8

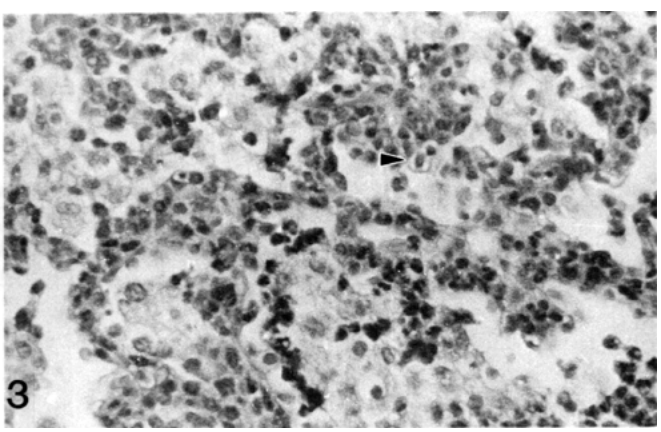


FIGURE 3. Lung tissue from a rabbit exposed to Co^{2+} and Ni^{2+}. Alveolar spaces are filled with enlarged macrophages, and a type II cell nodule is marked by arrowhead. × 300.

Table 4. Volume density of type II cells (means ± SD).

Metal exposure	Volume density
Ni–Cr	0.18 ± 0.05*†
Ni	0.10 ± 0.03*
Controls	0.04 ± 0.01
Co–Ni	0.08 ± 0.02*
Co	0.06 ± 0.05
Controls	0.05 ± 0.01
Co–Cr	0.08 ± 0.03†‡
Co	0.04 ± 0.02
Controls	0.04 ± 0.01

*$p < 0.001$ compared to controls.
†$p < 0.01$ compared to single metal exposure.
‡$p < 0.01$ compared to controls.

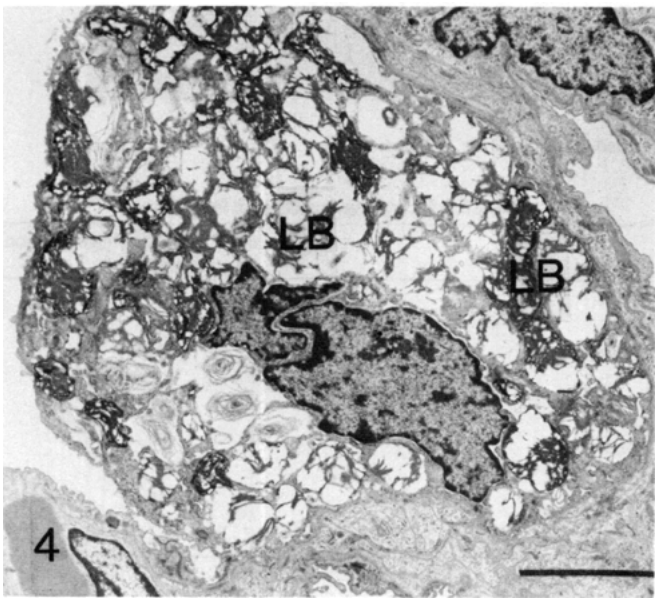


FIGURE 4. Alveolar type II cell from a rabbit exposed to Ni^{2+} and Cr^{3+}. The cytoplasm is filled with lamellar bodies (LB). Bar = 5 μm.

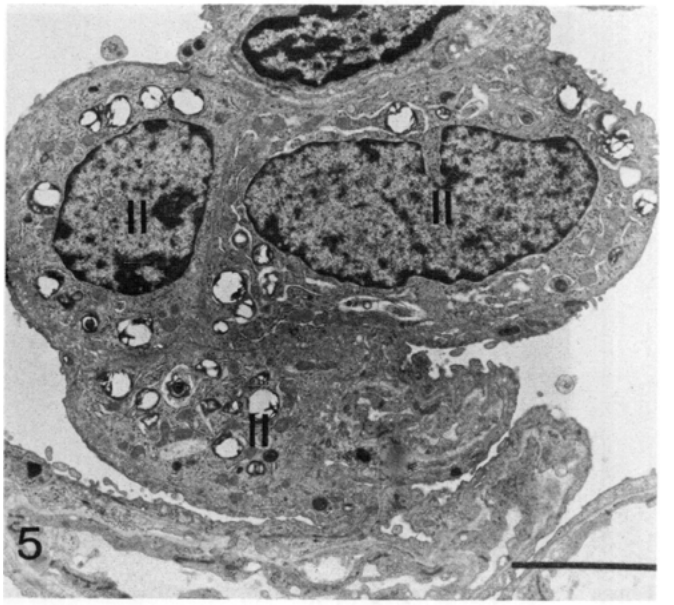


FIGURE 5. Type II cell (II) nodule from a rabbit exposed to Co^{2+} and Ni^{2+}. Bar = 5 μm.

The lung content of phospholipids showed a significant increase in all double-exposed rabbit groups compared to corresponding single-exposed ones. Table 5 shows the concentrations of the total phospholipids and the surface-active 1,2-dipalmitoylphosphatidylcholine and the quotient between concentrations in exposed groups and controls.

Discussion

This paper presents data from three inhalation experiments where effects on combined metal exposures were studied: Ni^{2+} and Cr^{3+}, Ni^{2+} and Co^{2+}, and Co^{2+} and Cr^{3+}. For practical reasons the effects of the combined inhalations in each experiment could be compared with the effects of only one of the two

Table 5. Data on phospholipids in lung tissue (left lower lobe; means ± SD).

Metal exposure	Total phospholipids, μmole/g wet lung	Quotient of total phospholipids between exposed and control values	Phosphatidylcholines, μmole/g wet lung	1,2-Dipalmitoylphosphatidylcholine, mole % of phosphatidylcholines
Ni–Cr	73 ± 28*†	2.8	55 ± 24*†	45 ± 4*†
Ni	32 ± 8‡	1.2	19 ± 6†	36 ± 5‡
Controls	26 ± 2		16 ± 2	31 ± 2
Co–Ni	33 ± 13*†	2.3	19 ± 9*§	43 ± 3§//
Co	17 ± 3	1.2	9 ± 3	39 ± 3*
Controls	15 ± 1		7 ± 1	34 ± 2
Co–Cr	58 ± 4*#	2.3	33 ± 9//#	47 ± 1§//
Co	30 ± 6	1.2	14 ± 4‡	45 ± 2//
Controls	25 ± 4		10 ± 2	39 ± 2

*$p < 0.01$ compared to controls.
†$p < 0.01$ compared to single metal exposure.
‡$p < 0.05$ compared to controls.
§$p < 0.05$ compared to single metal exposure.
//$p < 0.001$ compared to controls.
#$p < 0.001$ compared to single metal exposure.

metals in the combination: Ni in the Ni–Cr combination, Co in the Co–Ni combination, and Co in the Co–Cr combination. However, we have recently exposed rabbits to Cr^{3+} only using about the same concentration and exposure time (*6*).

Exposure to Cr^{3+} alone and in a concentration twice as high as in the present study (2 mg/m³) produced no significant effect on the volume density of the type II epithelial cells or on phospholipid concentration (*6*). In combination with Ni^{2+} and Co^{2+}, the volume density of the type II cells was significantly higher than after exposure to Co^{2+} or Ni^{2+} alone. The combination with Co^{2+} and Cr^{3+} seemed to increase the type II cells both in nodular and singular forms. Cr^{3+} thus seems to potentiate the effect of both Ni^{2+} and Co^{2+} by inducing an increase on type II cells. Ni^{2+} in combination with Co^{2+} seems to lead to prominent nodule formation in type II cells more than exposure to Co^{2+} alone; however, the combination of Ni^{2+} and Co^{2+} did not increase the volume density more than Ni alone.

In all three experiments, the combined exposures induced markedly larger increases in content of phospholipids and especially of 1,2-dipalmitoylphosphatidylcholine, which is a main component in the surfactant, than the additive increases after the single exposures to the metals. In the combinations Cr–Ni and Cr–Co, the increases in phospholipids are probably caused by an increased production of surfactant by type II cells and a decreased catabolism of the surfactant by alveolar macrophages caused by Cr^{3+}. In the combination Ni–Co, the increase in volume density of type II cells was not higher than after Ni alone, but still the phospholipids were markedly increased. This may indicate another mechanism behind the increase occurring in the combination Ni–Co than for the other combinations, or the volume density might have been underestimated due to the uneven distribution of type II cells.

One common feature after all three combination exposures was the large amount of surfactant in the lungs. The increase in surfactant was reasonably coupled to the marked granulomatous macrophage reaction in the lung tissue after combined exposures. Most of the morphological and functional changes in the macrophages was probably caused by this increase in surfactant (*1*).

The effect pattern with high surfactant content in alveolar spaces and enlarged macrophages filled with surfactant and a smooth surface is similar to alveolar lipoproteinosis seen in rats after exposure to quartz dust (*7,8*) and seen in the human disease pulmonary alveolar proteinosis (*9*).

In summary, our studies indicate that combinations of nickel, cobalt, and chromium can cause larger effects on type II cells and surfactant than addition of effects of each metal alone and that the increase in surfactant probably affects the macrophages. The mechanism behind the combined effects of Ni^{2+} and Co^{2+} may be different from those of Ni^{2+} and Cr^{3+} and Co^{2+} and Cr^{3+}. This implies that the combined effects of all three metals might be even stronger. The concentrations in our experiments were not far from occupational threshold limit values, which are 0.1 mg/m³ for Ni^{2+}, 0.05 mg/m³ for Co^{2+}, and 0.5 mg/m³ for Cr^{3+} in Sweden. It is thus important to investigate effects of combined exposures to metals in the occupational environment.

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