Scand J Work Environ Health 1977;3(1):1-41

<https://doi.org/10.5271/sjweh.2798>

Issue date: 1977

Acute pulmonary toxicity of manganese dioxide.

by [Bergström R](#)

Key terms: [acute pulmonary toxicity](#); [manganese dioxide](#); [pulmonary toxicity](#)

This article in PubMed: www.ncbi.nlm.nih.gov/pubmed/905805

This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/).

Introduction

Manganese is widely distributed in nature and is calculated to constitute about 0.10 % of the earth's crust. It does not naturally occur in the pure metal form. The oxides are the most important ore minerals of manganese, of which pyrolusite, a mineral form of manganese dioxide (MnO_2), is one of the more common. As a trace element, manganese is also found in soil, seawater, and freshwater, and present knowledge indicates that all plants and animals contain manganese in minute quantities (50).

In industry, manganese is used in the production of steel as a reagent to reduce oxygen and sulfur and as an ingredient in special alloy steel. It is also used as MnO_2 in the manufacturing of dry cell batteries and in the chemical industry as an oxidizing agent for the production of potassium permanganate (KMnO_4) and other manganese chemicals (68).

Industrial exposures occur primarily by inhalation. Exposure to airborne dust containing manganese may cause a chronic disorder of the central nervous system

with symptoms similar to Parkinsonism. The first cases of manganese poisoning were reported in 1837 by Couper (19) in Glasgow, who observed the symptoms in five manganese ore crushers. Since then, manganese poisoning has been reported by several authors (17, 52, 53, 55, 56, 69).

Exposure to airborne manganese dust may also affect the lungs. The pulmonary effects of manganese are not as well documented as the neurological effects, and there are discrepancies concerning the interpretation of the pulmonary toxicity of manganese.

The aim of the present work was to investigate the pulmonary toxicity of manganese with special reference to the mechanisms behind the acute cellular reactions in the lung. As a background, a review of the literature on epidemiologic and experimental studies is presented. The methods, measurements, and results of experimental studies on the effects of acute MnO_2 exposure in guinea pigs is then described, followed by a general discussion of the methods and results.

General background

PULMONARY EFFECTS AFTER EXPOSURE TO MANGANESE DIOXIDE

Epidemiologic studies

The first cases of croupous pneumonia in a manganese mine, where 5 out of 10 workers died within 27 months, were reported in 1913 by Brezina [cited by Elstad (22)].

Baader (2) considered manganese dust to be an etiological factor in the development of croupous pneumonia. In a description of a patient with a lethal case of croupous pneumonia, he associated the development of the disease with the patient's exposure to manganese dust at work in a factory producing dry cell batteries. His conclusions were based upon the findings of Brezina (13), Bakradse (4), Schopper (64), Bubarev (14), Freise (27), his own studies in Morocco (1), Gallego (28), Vigliani (73) and Thiis-Evensen (71), all of whom described similar symptoms. He concluded that pneumonia should be regarded as an occupational disease among manganese workers.

Bakradse (4) reported a 31 % mortality rate among workers suffering from pneumonia in a Russian manganese mine during the years 1906 to 1912. Several observations by other authors were subsequently made on an increased rate of pneumonia and mortality from this disease among workers in manganese mines. These studies were summarized by Wasermann and Mihail (74).

In respect to exposed workers, Thiis-Evensen (71) reported that, among workers in a ferromanganese plant, the incidence of croupous pneumonia was 0.012/1,000 (6/50) during one year. Three of the cases resulted in death. Bauer (5) found that, among 104 workers in manganese mines and dry cell factories observed from

1927 to 1937, there were 14 cases of croupous pneumonia, of which 11 resulted in death.

Heine (33) found a high incidence of pneumonia among workers in an alloy producing factory in Aachen during the period 1932 to 1941. A statistical evaluation of the industry's personnel records showed that the morbidity and mortality rates of pneumonia were 0.138/1,000 and 0.073/1,000 during the period studied. These data were based on the mean number of persons employed per year, which ranged between 118 and 617 persons. As a control group Heine used male members of an insurance society in Leipzig. The mortality rate of this group during the same time period was 0.0054/1,000. The morbidity rates, as well as the number of persons in the control group, were not given.

In a further analysis of his material, the author compared the mortality rates during two periods, from 1936 to 1938 and from 1939 to 1941. During these times the number of employees ranged between 441 to 617 and 567 to 570, respectively. The mortality rates for pneumonia were 0.128/1,000 for the earlier and 0.017/1,000 for the latter period. According to the author, the low mortality rate during the 1939-to-1941 period was due to the employees' greater care concerning personal hygiene.

By investigating the incidence of pneumonia at certain places within the factory, the author found no correlation between high incidence of pneumonia and a high concentration of manganese in the air in different areas. He also reported that the majority of pneumonia cases occurred during the winter and stated that such climatological factors as temperature, humidity and draft contributed to the development of pneumonia.

Heine concluded finally that factors other than manganese, such as draft, weather conditions, unemployment and malnutrition, were predisposing factors for the development of pneumonia.

Using medical records for male employees in a potassium permanganate factory, Lloyd Davies (42) made a statistical analysis of the years 1938 to 1945. During this time the group varied in number from 40 to 124 persons. The control group for this study consisted of male members of an insurance society who were employed in chemical and pharmaceutical manufacturing and retail business during the same time period. This group varied in size from 4,645 to 6,751 persons during the period in question. The incidence of pneumonia in the control group during the years 1938 to 1944 varied from 0.47/1,000 to 1.20/1,000, with an average of 0.73/1,000. In the exposed group he found an incidence of lobar pneumonia or bronchopneumonia which varied from 15/1,000 to 0.63/1,000 with an average of 26/1,000. In all cases the diagnosis of pneumonia was made by the patient's medical attendant and confirmed by communication with the doctor, and all cases were diagnosed as lobar pneumonia or bronchopneumonia. In two cases it was reported that the patient's temperature and general condition responded more slowly than usual to sulfonamides.

Environmental studies in the plant showed that 41 to 66 % of the dust was MnO_2 . A particle size analysis showed that approximately 80 % of the particles were less than $0.2 \mu m$ and nearly all particles were less than $1 \mu m$ in size.

Davies concluded that it was difficult to establish whether the diagnosed cases of pneumonia were entirely due to the chemical action of manganese or whether the action of manganese increased the possibilities for a secondary infection by the flora of the nose and mouth. On the basis of clinical experience and subsequent animal experiments, he stated that manganese had a specific action on the whole respiratory tract and that "manganese pneumonitis" would be a better term than "manganese pneumonia."

Wassermann and Mihail (74) conducted an epidemiologic study of bronchopneumonia during the period 1957 to 1959. Their subjects were groups of manganese

miners, carbon miners and forest workers, all working in comparable geographical areas. Air samples taken at different places in the manganese mine showed that the dust consisted of 12 to 30 % manganese and 16 to 35 % silica (SiO_2). The concentration ranges of the dust were 28 to 840 mg/m^3 . The range of particles less than $5 \mu m$ was 34 to 81 % and 51 to 71 % for manganese and SiO_2 , respectively. The diagnoses were based on medical certificates and general clinical examinations of the manganese miners. They found that the incidence of bronchopneumonia was 0.26/1,000 to 0.38/1,000 for manganese miners, 0.008/1,000 to 0.036/1,000 for carbon miners, and 0.078/1,000 to 0.24/1,000 for forest workers. The author's conclusion was that the frequency of pneumonia and bronchopneumonia was increased in manganese workers as compared to the two other groups.

Studies by Van Beukering (72) on workers in a manganese mine and an iron mine in the same region in South Africa from January 1963 to August 1965 showed that the risk of contracting pneumonia, diagnosed by X-ray, was significantly higher in the manganese mine than in the iron mine. Of 1,156 iron miners and 3,674 manganese miners, the proportion with pneumonia was 5.10 and 8.08 %, respectively. The number of deaths among the manganese miners was 10, in comparison to 1 death among the iron mine workers.

Rodier (55) in a study of manganese poisoning in Moroccan miners, cites Cauvin (18) who found a high death rate caused by pneumonia during the winter of 1939—1940 in Morocco. A similar incidence of a high death rate from pneumonia was not seen until 1947. This finding led Rodier to suppose that manganese could not be the only etiological factor involved. Furthermore, the author reported that approximately 3.7 % of the workers in the mines developed pneumonia or other respiratory diseases (excluding bronchitis) each year. The average morbidity was 11 %. However, no data on controls were reported. He concluded that manganese could "undoubtedly be an aggravating element" in the development of pneumonia.

The clinical signs of manganese pneumonia were reported by Rodier (55) and

later confirmed by Dervillée et al. (20). They are characterized as:

Acute alveolar inflammation ... extremely violent character of this condition which becomes very serious almost at once, Dyspnea is marked, respiration shallow and gasping. Cough and expectoration are rare A fatal issue may ensue from heart failure between the fifth and the tenth days. It may also occur suddenly in patients regarded as cured. The usual drugs, in particular sulphonamides and antibiotics, mostly are without effect [55].

Because of difficulties in obtaining X-ray films and necropsies the author concluded that it was difficult to determine whether manganese pneumonia was a pulmonary infection aggravated by manganese or a subacute edema resulting from a toxic action of manganese.

Pulmonary effects related to manganese exposure have also been found in the general environment. A plant producing alloys of manganese by electrical melting is situated in Sauda, Norway. During periods of calm and fog, the valley in which the town of Sauda is located used to be covered with a cloud of smoke from the plant. Elstad (22) used data compiled from official registers and found that the incidence in croupous pneumonia morbidity and mortality in the general population of Sauda during the years 1924 to 1937 was, respectively, four and eight times higher than that of the whole country. Also, the mortality rate of croupous pneumonia was high; 32.3 % of all deaths in the district during the period studied were caused by this disease. For the whole country the mortality rate from croupous pneumonia was 3.65 %.

The particular disease noted in Sauda was described as being sudden in onset, with high fever and shivers prior to a cooling of the body. The patient was recorded as having stitch and as being cyanotic with coughing and red-brown expectorate. From the first day of sickness the patient was often in bad physical condition and had a weak, unstable pulse. The mortality rate was high; death occurred within two to four days. Analysis of lung tissue from 11 individuals in Sauda who died of croupous pneumonia showed a range of 35 to 163 μg of manganese per 100 g of wet tissue (54).

He concluded that these abnormal pneu-

monia conditions were caused by the exposure to manganese dust found in the smoke emitted from the plant. The plant was built in 1924. Before that time cases of pneumonia occurred only rarely. Elstad showed also that the morbidity, as well as the death rate, from pneumonia was parallel to the production of alloys of manganese, which varied between 323,000 to 69,000 tons per year during the period 1930 to 1937.

Pancheri (51) reported on a similar situation in the town of Aosta, situated in a valley in Italy where manganese dust was emitted from a metallurgical plant producing alloy steel. Doctors reported an increased incidence of pneumonia among people living in the town. No data were given on the incidence and type of pneumonia, and the association between the plant's emissions and the incidence of pneumonia was not thoroughly investigated.

Stocks (67) made an attempt to correlate pneumonia with the presence of trace elements in 23 British cities by comparing the standardized mortality ratios for pneumonia in selected areas with the amount of trace metals in the air collected on filters from those areas. The correlation for manganese was 0.310 for males and 0.190 for females. The highest correlations were found for beryllium, with 0.760 in males and 0.783 in females, and for vanadium, with a correlation of 0.805 for males and 0.711 for females.

Experimental studies

Ehrishmann (21) exposed rabbits to an aerosol of MnO_2 dust. One group of animals was exposed to MnO_2 with a concentration of 10 to 20 mg/m^3 of air and a particle size of less than 5 μm . The exposure time was 4 h/day for three months. In a second set of experiments, four cats were exposed to approximately 10 $\text{mg MnO}_2/\text{m}^3$ of air for 21 h/day during a period of about 15 months. Thereafter, three of the cats were exposed to about 150 $\text{mg MnO}_2/\text{m}^3$ of air, 4 h/day for an additional 15 months.

From results of chemical analyses taken randomly from both experiments the author concluded that there was no accumulation of MnO_2 in the lungs. The mean

concentration of MnO_2 in the lungs of exposed animals was about $10 \mu\text{g/g}$ of wet weight. The concentration of MnO_2 in the liver varied between 3 and $6 \mu\text{g/g}$ of wet weight; in muscles it was $3 \mu\text{g/g}$ and in the kidneys $13 \mu\text{g/g}$, compared to approximately $0.1 \mu\text{g/g}$ of wet organ for the control animals. The author could not find any signs of pneumonia or pneumoconiotic changes in the animals studied. However, no details were given of the criteria for the histological evaluation of the lungs.

Jötten et al. (36) exposed 32 rabbits to MnO_2 dust 1 h/day for 39 days in the first of a set of experiments. No data were given on the aerosol concentration. The particle size was about $1 \mu\text{m}$. During the first 19 days of exposure, nine animals died. Histological examination showed that one of the animals had bronchopneumonic foci and the others a leukocyte infiltrate in the peripheral alveolar region. The remaining 23 animals were exposed to MnO_2 1 h/day during the following 20 days, but 16 were intranasally infected with pneumococci type I and II. Eight of these animals were also exposed to draft for 15 min prior to MnO_2 exposure. Four additional rabbits, not exposed to MnO_2 , were intranasally infected with pneumococci, and they served as a control group.

At the end of the MnO_2 exposure period, the mortality rates among the groups were as follows: 57 % (4/7) of those exposed to MnO_2 only, 75 % (6/8) of those exposed to pneumococci + MnO_2 , 88 % (7/8) of those exposed to pneumococci + airstream + MnO_2 and 0 % (0/4) of those exposed to pneumococci only.

A histological examination of the animals exposed to MnO_2 and infected with pneumococci — with or without exposure to draft — showed that about 50 % had bronchial lesions with a massive confluent infiltrate of leukocytes and lymphocytes within 15 days after pneumococcal infection. These changes were not seen in the group exposed to pneumococci only. No signs of a croupous fibroid pneumonia could be seen in either group.

The aim of a second set of experiments was to investigate the possible similarities in the etiology of manganese pneumonia, particularly the form of pneumonia called

Thomasslachen pneumonia earlier described by Gundel and Fischer (31).

One group of 23 rabbits was exposed to a mixture of 90 % limestone and 10 % MnO_2 — the composition of Thomasslachen smoke. Another group of 23 rabbits was exposed to limestone only. The particle size of the limestone was 2 to $3 \mu\text{m}$. No data on the concentration were given for either exposure. The exposure time, duration and exposure to pneumococci and stream of air were similar to that in the first set of experiments.

Six animals died within 18 days of exposure to limestone + MnO_2 , in comparison to three animals exposed to limestone only for the same period. Subsequent exposure to airstream and/or pneumococci resulted in a higher mortality rate during the following 20 days than among animals exposed only to limestone or limestone + MnO_2 .

Histological examinations of the rabbits exposed to limestone + MnO_2 revealed mostly focal pneumonic changes, with confluent infiltrate of leukocytes and lymphocytes in both the bronchial and alveolar region. The findings were more pronounced in animals exposed to airstream and/or pneumococci. No pathological lung changes were seen in animals exposed to limestone only. When the animals were exposed to airstream and/or pneumococci, only one showed pathological evidence of pneumonia.

From the data of these experiments, and citing earlier findings in clinical and epidemiologic studies by Buttner and Lenz (15) and Gundel and Fischer (31), the authors concluded that it was probably the MnO_2 in Thomasslachen dust that was the etiological factor in Thomasslachen pneumonia.

In summarizing these experiments, Jötten and his co-workers concluded that exposure to MnO_2 or 10 % MnO_2 + 90 % limestone led to a mortality rate twice as high as that resulting from an exposure to limestone only. The effect was more pronounced when additional exposure to airstream and/or pneumococci was performed. The histological examinations led to the conclusion that exposure to MnO_2 gives rise to more pronounced pathological lung changes than exposure to MnO_2 + limestone. It was also stated that manganese has a specific local action

on the tissues which is probably due to the mechanical and chemical properties of the MnO_2 particles.

In order to elucidate the pathogenetic importance of the presence of pneumococci for the development of manganese pneumonia, Jötten performed a third set of experiments. Mice were exposed to an aerosol of MnO_2 for a period of 29 days. No data were given for the exposure time per day or concentration of MnO_2 .

The exposed animals were divided into four groups. One group was exposed to MnO_2 only. A second was immunized with killed pneumococci on the sixth day after the beginning of MnO_2 exposure. A third group was exposed to viable pneumococci type I and II at 19 days after the start of MnO_2 exposure. The fourth group was immunized on the sixth day after the beginning of exposure and then exposed to viable pneumococci on the 19th day after the beginning of MnO_2 exposure.

The mortality rates within 20 days after MnO_2 exposure were 23 % (12/53) for those exposed to MnO_2 only, 36 % (9/25) for those immunized with killed pneumococci, 79 % (30/38) for those exposed to viable pneumococci and 64 % (16/25) for those immunized and exposed to viable pneumococci.

Histological observations of lung tissue showed no pneumonic changes in the MnO_2 animals. In 8 out of the 31 examined animals exposed to viable pneumococci, one had severe, genuine croupous pneumonia. All showed massive bronchopneumonic changes. Of the animals exposed to killed pneumococci, 8 out of 9 showed severe massive confluent bronchopneumonic changes. All of the animals exposed to killed and viable bacteria showed severe confluent bronchopneumonia.

The authors' conclusion was that, in the mice exposed to MnO_2 , immunized with killed pneumococci and thereafter exposed to viable pneumococci, the observed bronchopneumonic changes were more severe than in the animals exposed to MnO_2 + viable pneumococci. In those exposed to MnO_2 only, no bronchopneumonic changes were seen.

In the first of a set of experiments, Heine (33) exposed 50 guinea pigs to ferromanganese dust in an alloy producing industry. The animals were placed in

cages within the plant for 8 h/day for a period of 178 days. The concentration of dust was 2.35 g/m^3 of air. Data were not given on particle size or the method of measuring the concentration. No animal died during the exposure period. Histological examinations revealed no pathological changes in the lungs. Some incorporation of dust in the lungs, as well as a threefold increase of manganese content in the lungs of the exposed animals, was observed.

A similar experiment was performed during the winter to investigate the eventual role of climatological factors. Seventy-five guinea pigs were exposed to ferromanganese dust for 8 h/day for 198 days. The concentration used during the first month was 170 mg/m^3 of air; during the rest of the period the concentration was 60 mg/m^3 of air. No pathological changes were seen in the lungs. No data were given on the amount of manganese in the lungs of the exposed animals, though the author noted a somewhat increased amount in the exposed animals.

In a third set of experiments the aim was to investigate whether exposure to ferromanganese dust could cause a predisposition to pneumonia. Groups of 35, 45 and 62 mice were exposed to ferromanganese dust, containing between 88 and 90 % manganese, for 15 min/day for 31-, 102- and 54-day periods, respectively. No data on the size or the concentration of the particles were given, and the method of exposure was not described. During the exposure to manganese, the mortality rates were 17 % (6/35) within 28 days, 27 % (12/45) within 98 days and 17 % (5/30) within 45 days.

In the 54-day experiment, unexposed animals and animals which had been exposed to ferromanganese dust were nasally infected with viable pneumococci type II at the end of the exposure period. In both groups, some of the animals were immunized with killed pneumococci 20 days before being infected. The method of immunization was not described.

Most of the unexposed and exposed animals died within 20 to 100 h after infection and no significant differences between the groups were seen concerning the observed lung changes. Among the animals immunized with killed pneumococci the mortality rates were found to

be 30 to 40 % lower than among nonimmunized animals.

To further study the effect of immunization with killed pneumococci, an experiment was performed on 100 guinea pigs. The animals were exposed to ferromanganese dust of a concentration of 2.35 g/m³ of air for 8 h/day for 226 days. A total of 50 animals were immunized with killed pneumococci on the 17th day before termination of exposure. The type of pneumococci used was not reported. Neither the exposure method nor the method for immunization was described.

After the exposure, 20 unexposed, 20 unexposed + immunized, 50 exposed, and 50 exposed + immunized animals were nasally infected with viable pneumococci type I. Within 12 to 17 days after the infection the mortality rates in the different groups were 30 % (6/20) of those unexposed, 25 % (5/20) of those unexposed + immunized, 36 % (18/50) of those exposed and 28 % (14/50) of those exposed + immunized.

From the results of these experiments, the author concluded that the inhalation of ferromanganese dust alone did not cause pneumonia and that no decreased resistance towards infection was observed. He suggested that other factors, such as draft, humidity and temperature changes, must be considered in the development of pneumonia.

Lloyd Davies (42) exposed groups of 60 to 70 mice in three different experiments (I, II, III) to dust collected from the ledges and walls of a plant which produced potassium permanganate. The animals used in experiment I were exposed to dust containing 70 % MnO₂, and those in experiments II and III to dust with a 74 % MnO₂ content. In a control experiment (IV), 60 mice were exposed to calcium carbonate dust (CaCO₃), used as a nontoxic dust similar to the dust in the plant with respect to specific gravity and particle size.

The animals were exposed in a small chamber with controlled climate. Dust counts were performed with a thermal precipitator and correlated to the size and concentration of the particles in the chamber air.

For experiment I the exposure times were 2 × 120 min/day for 14 days followed by 2 × 70 min/day for eight days.

The particle count per milliliter of air ranged between 923 and 2,786. The exposure time for experiments II, III, and IV was 2 × 70 min/day during 15 to 21 days, the dust count varying between 900 and 5,000 counts/ml.

After the dust exposures, control and exposed animals were intraperitoneally injected with a single dose of 0.5 ml pneumococcus type II (experiments I, II, IV), pneumococcus type IV (experiment II) or Streptococcus Haemolyticus (experiments II, IV) in concentrations of 20 to 240,000, 8,400 and 200 to 245 organisms/ml, respectively. In experiment III, control animals and dust exposed animals were exposed to an aerosol of serum broth with pneumococci type IV for 15 min only, or for 15 min/day for a period of three days. One group was exposed to serum broth only and a second group was unexposed.

During the dust exposure period the highest mortality rate — 28 % (17/60) — was seen in experiment I (pneumococci). In experiments II, III, and IV (MnO₂ and bacteria) the rates were 15 % (9/60), 25 % (16/63) and 20 % (12/60), respectively. Histological examination showed dust-laden cells in bronchi, alveoli and interstitial spaces with slight to intense infiltration of leukocytes and large mononuclear cells around bronchi and arteries.

No significant differences in mortality rates were seen between the control animals and the animals exposed to dust after intraperitoneal injection with pneumococcus type II and IV or Streptococcus Haemolyticus or after exposure to an aerosol of serum broth with or without pneumococci type IV. The author reported, however, that dust exposed animals in experiments I and II were in weak condition and showed such symptoms as weight loss, blindness and hind leg paralysis.

The author concluded that inhalation of manganese dust did not give rise to an increased susceptibility to pneumococci or streptococci. He also concluded that manganese is toxic to the respiratory epithelium and exerts a specific chemical action resulting in an intense mononuclear proliferation and infiltration of the lung tissues leading to cellular death and necrosis. From analyses of body, skin, brain and lung, together with determinations of sol-

ubility in serum, it was concluded that all manganese particles — phagocytized or not — pass into solution in tissue fluids.

In a further study, Lloyd Davies and Harding (43) intratracheally injected 10 mg of MnO_2 in 1 ml of saline into the lungs of rats. Eighty per cent of the particles were less than 1 μm . After the injection the animals were sacrificed at intervals varying from 15 min to 18 months and the lungs were histologically examined.

The lungs of control rats injected with saline only showed no histological abnormalities. In the exposed animals the epithelial cells of the bronchi discharged their mucus, and the epithelium became ragged and could be loosened from the basal membrane after 15 min of exposure. After approximately 24 h, an intense histiocytic mononuclear reaction appeared in the interstitial tissue and the alveoli contained many histiocytic cells. Shortly thereafter, large hydropic cells appeared, often in large numbers. An early granulomatous reaction occurred after 14 days. Six weeks later giant phagocytic cells were observed.

The authors concluded that manganese acts as an irritant to the bronchial and alveolar epithelium and causes a characteristic mononuclear cell reaction. They also suggested that the presence of hydropic cells was related to the degree of tissue irritation produced by chemical agents.

Their overall conclusion was that "manganese dust in suitable particle size introduced into the respiratory system will, without the presence of other factors, cause pneumonitis."

Maigretter et al. (45) exposed SPF mice to an aerosol of 109 mg MnO_2/m^3 of air. The exposures lasted 1, 2 and 3 h per day for up to four days. The method of measuring air concentration was not described. The dust was generated by a Wright dust feed apparatus. About 97 % of the particles were less than 5 μm , and 50 to 60 % were less than 0.7 μm .

The amount of manganese found in the lungs 10 min after a 3-h exposure was 95.6 $\mu\text{g/g}$ of dry lung — almost twice the amount analyzed after 1- and 2-h exposure. At 5 h after, and again at 14 days after, a 3-h exposure, the amount of manganese in the lungs was 67.4 and 4.3

$\mu\text{g/g}$ of dry lung, respectively; 2.1 $\mu\text{g/g}$ was found in the lungs of unexposed mice.

A microscopic examination of lung tissue from the MnO_2 exposed animals revealed an acute to subacute inflammatory infiltrate with slight alveolar septal edema and congestion. These changes were the most marked after the fourth exposure to MnO_2 aerosol.

Among mice exposed to MnO_2 3 h/day during 1, 2, 3 and 4 days and then challenged with *Klebsiella pneumoniae* type A at 1 or 5 h after the final MnO_2 exposure, the overall mortality rate was significantly higher. The survival time was also considerably decreased in animals challenged with *K. pneumoniae* 5 h after the final MnO_2 exposure. No deaths were observed among 40 control mice exposed to MnO_2 + 0.2 % bovine serum albumin aerosol. In the group exposed to *K. pneumoniae* 1 h after MnO_2 , a positive linear relationship was present between mortality rate and the frequency of exposure to MnO_2 aerosol. This relationship was not present among animals exposed to *K. pneumoniae* 5 h after MnO_2 .

In a second set of experiments mice were challenged with airborne mouse-adapted influenza A/PR8/34 virus at 24 or 48 h prior to a 3-h MnO_2 exposure lasting up to three days. The animals challenged with the virus 48 h prior to 1- and 2-day MnO_2 exposures showed significantly increased mortality rates and lung consolidation and a decreased survival time, when compared with animals challenged 24 h before MnO_2 exposure.

The authors suggested that the susceptibility to the infectious agent was dependent upon the dose of inhaled MnO_2 and that the cytotoxic effects of MnO_2 on macrophages could be, in part, responsible for the increased mortality rates due to bacterial pneumonia in MnO_2 exposed mice. They concluded from results of the virus experiment that the time of infectious challenge in relation to the MnO_2 exposure was critical.

Zaidi et al. (77) conducted experiments on three groups of guinea pigs, each consisting of 30 animals. One group was inoculated with *Candida albicans*, (500 $\mu\text{g}/1.5$ ml saline), a second with MnO_2 (50 mg/1.5 ml saline) and a third with MnO_2 + *C. albicans* (50 mg + 500 $\mu\text{g}/1.5$ ml saline). The dust used contained 68.30 %

MnO₂, 10.50 % SiO₂, 5.00 % Fe₂O₃, 4.60 % Al₂O₃ and 0.06 % NiO; the particle size was less than 5 μ m. Two animals from each group were killed and autopsied immediately after inoculation. At least two animals from each group were sacrificed and autopsied at different intervals between 2 to 180 days after inoculation.

The mortality rates between 15 to 40 days after inoculation were higher for the MnO₂ + bacteria exposed animals (30 %) than for animals exposed to bacteria only (13 %). No animal died in the MnO₂ group.

Viable postinoculation counts of organisms from the cardiac lobe showed the presence of organisms up to 90 days in the group exposed to *C. albicans*, no counts in the MnO₂ exposed group, and up to 120 days in the MnO₂ + bacteria exposed group.

In the group exposed to *C. albicans* histological examinations showed an acute inflammatory reaction in the lumen of bronchi and bronchioles at early stages; by 90 days the reaction had subsided. In the group exposed to MnO₂ a marked proliferation of alveolar macrophages, together with increased phagocytosis of dust, was seen at seven days. From 30 to 180 days the lymph nodes contained small amounts of dust, and a slight to marked fibroblastic proliferation was seen. At 180 days, unphagocytized particles and dust-laden macrophages were still seen in the alveolar lumen. A benign type of pneumoconiosis appeared at this time with a grade II fibrosis classified according to Belt and King (6). No pneumonic changes were seen. In the group exposed to MnO₂ + *C. albicans* a widespread infiltration of polymorphonuclear leukocytes, together with macrophage reaction with phagocytosis of dust and organism, was seen at seven days. Bronchopneumonia developed within 15 days; by 30 days the leukocytic reaction subsided. Dust was found primarily in the alveolar lumen, and lymph nodes revealed the presence of dust. At 150 days the reaction became chronic and fibroblastic response was seen. At 180 days the fibrosis consisted of thick reticulin and collagen fibers and became grade III.

The authors concluded that the transport of MnO₂ dust to regional lymph nodes was not effective and that *C. albicans* and

manganese dust interact and develop lesions earlier and produce more fibroses than would the dust or *C. albicans* alone.

Shanker et al. (66), using an experimental design similar to Zaidi et al. (77), inoculated 30 guinea pigs intratracheally with 50 mg MnO₂ in 1.5 ml of saline. The animals were killed and autopsied between 24 h and 180 days after inoculation, and the tracheobronchial lymph nodes were histologically examined.

At seven days, scattered manganese particles were seen extracellularly. At 30 days, particles were seen extracellularly; they were associated with macrophages but were not phagocytized. At 60 days a few macrophages appeared to be laden with particles. At 90 days some large collections of dust-laden macrophages were seen which showed varying degrees of degenerative changes. By 120 days a marked degree of lymphopoenia was seen, as well as a marked density of lymphocytic cell population. At 180 days a significant increase in the density of particles was noticeable throughout the section. Many densely packed macrophages were seen — some of which had degenerated — and particles appeared extracellularly in various places. Furthermore, infiltration of eosinophils and an increase in the reticulin fibers were observed. None of the control animals showed any pathological changes.

It was concluded that MnO₂ particles, primarily in the extracellular state, are apparently quickly brought to the lymph nodes via lymphatic clearance mechanisms. Subsequently, there is proliferation of macrophages and some phagocytosis. The cytotoxic effects of manganese were a degeneration of dust-laden macrophages and a slight proliferation of fibroblasts, together with some reticulosis.

Shanker's final conclusion was that, due to the reducing action of lymph during its filtration through the manganese dioxide loaded nodes, manganese may dissolve slowly into ionic form and be carried into the general circulation.

CONCLUSIONS AND COMMENTS

The epidemiologic studies conducted by Baader (2), Elstad (22), Lloyd Davies (42) and Van Beukering (72) indicate a rela-

tionship between manganese exposure and the development of a pneumonialike disease. In Wassermann and Mihail's study (74) it was concluded that the incidence of manganese pneumonia was high among manganese workers, although the statistical analysis on which the conclusion was based was not described. Other studies by Brezina [cited by Elstad (22)], Bakradse (4), Thiis-Evensen (71) and Vigliani (73) also suggest that manganese might be the etiological factor of a pneumonialike disease.

Contrary to these conclusions, Heine (33) stated that factors other than manganese are important for the development of pneumonia among workers exposed to manganese. His conclusions, based on statistical evaluations and environmental studies in an alloy producing industry, were that the increased incidence of pneumonia was due to predisposing factors, such as draft, humidity, temperature changes, unemployment, and malnutrition, and that manganese had no specific influence.

Certain other authors, who conducted studies on workers in manganese mines and who were cited by Wassermann and Mihail (74), also failed to find cases of pneumonia.

The aforementioned studies show that there are discrepancies in different authors' conclusions concerning manganese as an etiological factor in the development of pneumonia among manganese exposed workers.

Also, the definition of the term "pneumonia" varies in the cited studies. Elstad (22), Baader (2), and Rodier (55) used the term croupous pneumonia. Lloyd Davies (42) used pneumonitis, Wassermann and Mihail (74) used pneumonia, and Van Beukering (72) reported on contracting pneumonia.

The clinical signs often described were a sudden onset of symptoms, intense dyspnea, high temperature (40—41 °C), radiological signs of acute lobar pneumonia, and little expectoration (often hemorrhagic). The pneumonia is also accompanied by a high mortality rate — four times higher than that for lobar pneumonia (20). In relation to treatment, Lloyd Davies (42), Rodier (55), and Dervillé et al. (20) found that in some cases the usual anti-

biotics used for pneumonia treatment were not effective.

Results from experimental studies attempting to produce pneumonia in animals exposed to manganese are also contradictory. Jötten et al. (36) showed that exposure to MnO_2 dust, or to manganese as a 10 % part of Thomasslachen smoke, resulted in an increased mortality rate and typical pneumonialike changes in lung tissue of rabbits. In combined exposures to draft and pneumococci the effect was increased. The authors concluded that MnO_2 seemed to exert a local cytotoxic effect, which resulted in severe alterations in the lung epithelium. The cytotoxic effect also seemed to be correlated to particle size, i.e., smaller particles gave rise to increased alterations in the lung tissue. In another set of experiments by the same authors, no pneumonic changes were seen in mice exposed to MnO_2 for 29 days. However, the concentration and exposure time per day were not given in their experiment. Ehrishmann (21) and Heine (33) failed to produce pneumonia or pneumonialike changes in the lungs of rabbits exposed to MnO_2 by inhalation. The discrepancy in the results of the studies might be explained by several factors, such as differences in particle size, dust concentrations and the types of exposure conditions used. However, the lack or inaccuracy of data concerning these factors makes a comparison difficult.

Histological examinations of lung tissue of rabbits exposed to MnO_2 via inhalation showed infiltration of leukocytes in the peripheral alveolar region within 19 days of exposure (36). In another experiment on mice by the same author, no pneumonic changes were seen within 20 days after MnO_2 exposure. Lloyd Davies (42) reported an intense mononuclear proliferation and slight to intense leukocyte infiltration of the lung tissue of mice exposed for about 20 days to dust from a potassium permanganate industry. Maigretter et al. (45) found an acute to subacute inflammatory infiltrate with slight edematous changes in the lungs of mice exposed to MnO_2 dust.

In contrast to these findings, Heine (33) did not find any pathological changes in the lungs of guinea pigs exposed to ferromanganese dust 8 h per day for up to

200 days. Furthermore, in experiments on rabbits and cats, exposed for up to 15 months to an aerosol of MnO_2 , Ehrishmann (21) could not find any signs of pneumonia or pneumoconiotic changes in the lung tissue. The method used for the histologic examination of the lung tissue was, however, not described.

The studies by Jötten et al. (36), Lloyd Davies (42), and Maigretter et al. (45) suggest that manganese exerts an irritant effect upon the respiratory epithelium and that the level of response is dependent upon the duration of exposure. Contradictory findings were reported by Heine (33) and Ehrishmann (21). Lloyd Davies (42) concluded that manganese is toxic to the respiratory epithelium; this conclusion was supported in a later study by Lloyd Davies and Harding (43). Maigretter et al. (45) suggested that MnO_2 had a cytotoxic effect on macrophages — a conclusion which was also presented by Shanker et al. (66).

Certain studies have attempted to evaluate the importance of a simultaneous exposure to MnO_2 and other agents, notably bacteria. Jötten et al. (36) found that the mortality rates among mice and rabbits were more pronounced when they were exposed to MnO_2 + killed and viable pneumococci. These findings could not be confirmed, however, by Heine (33) in his own experiments on guinea pigs and mice. Lloyd Davies (42) also failed to produce an increased susceptibility to pneumococci or streptococci. Maigretter et al. (45) stated that the susceptibility to pneumonia might be enhanced when certain inhaled doses of manganese were attained. They also concluded that the time of infection in relation to the time of manganese exposure was crucial. Zaidi et al. (77) found a greater pulmonary reaction when MnO_2 and *C. albicans* were intratracheally injected together than when either agent was given alone.

In summary, the information on the pulmonary toxicity of MnO_2 is still incomplete and sometimes contradictory, particularly in respect to the changes which take place in the lung immediately after exposure and thus the relation to the development of acute pneumonia. The importance of bacteria in the development of pulmonary effects is also unclear, as well as the clearance time of the manganese dust once it is deposited in the lung.

SCOPE OF THE INVESTIGATION

The aim of the present investigation was to study further the mechanisms involved in the action of manganese on the pulmonary cells and tissue after acute exposure. With reference to the results from experimental studies by previously cited authors, the following factors were considered important in the study:

- a. the pulmonary clearance of manganese as a determinant of the contact time between the agent and the lung tissue, as well as the accumulation of manganese in the lungs;
- b. the number of free lung cells present in the airways, i.e., those indicating the phagocytic capacity (macrophages) and presence of inflammation (polymorphonuclear leukocytes);
- c. the phagocytic activity of the alveolar macrophages at different times after exposure to manganese, indicated by the number of alveolar macrophages containing phagocytized manganese particles and the clearance of inhaled viable bacteria; and
- d. the effects of exposure to bacteria on the clearance of manganese, the inflammatory response, and the phagocytic activity of macrophages.

Material and methods

EXPERIMENTAL TECHNIQUES

Animals

Full-grown male and female guinea pigs of high quality were used in the experiments. For assurance of the absence of pulmonary infection in the test animals, a count was made of the macrophages and leukocytes in the lung lavage fluid of five animals selected at random from the group. During the period of an experiment, the animals used were kept and handled separately so that possible cross-infections among animals housed in the animal unit would be avoided; six to seven animals were kept in the same cage ($55 \times 33 \times 18$ cm). Pelleted standard guinea pig food and tap water containing 25 g of ascorbic acid per liter was given *ad libitum*.

Manganese dioxide aerosol

Analytical grade of MnO_2 was used in the experiments. According to the manufacturer's analysis, it contained 99.5 % MnO_2 , 0.01 % Cl, 0.05 % NO_3 , 0.05 % SO_4 , 0.05 % insoluble in acid and 0.2 % alkalies and earths.

Generation. The dust aerosol was generated with a Rag Pe generator which has been earlier described by Rylander et al. (63) and Hellström (34). The apparatus consists of a fan which circulates air through a circular plastic tube. A perspex tower is mounted on the top of the apparatus. Smaller particles are carried up to the top of the tower and through a connecting tube into the exposure chamber. The generator is equipped with a dosage mechanism which automatically gives a dust dose at certain, preset times.

Particle size and dust concentration. The size of the particles and their distribution in the aerosol were measured with a ROYCO optical particle counter model 225. In this apparatus, a light beam is focused on a small chamber through which particles pass and the intensity of the scattered light from the particles is measured. As the geometrical diameter of the particle is proportional to the intensity of the scattered light, the particle size can be determined.

The concentration of the dust was monitored by sucking air through preweighed Millipore membrane filters ($0.8 \mu\text{m}$ MAWP) and weighing the filters after the sampling period. Table 1 gives the measured concentration of MnO_2 and the proportion of particles below $3 \mu\text{m}$ for all clearance experiments.

Exposure conditions. For MnO_2 exposure the animals were put in four cages, each holding six to seven animals. The cages were placed in a stainless steel chamber with a volume of 0.67 m^3 . MnO_2 dust, generated by the Rag Pe generator and mixed with sterile filtered air, was introduced into the chamber from the top and sucked through the chamber at a flow of $8 \text{ m}^3/\text{h}$. A negative pressure of 5 mm of water was maintained within the chamber during the exposure. The duration of the exposure was 24 h.

Table 1. Means (M) and standard deviations of the concentration and proportion of particles equal to or smaller than $3 \mu\text{m}$ in the clearance experiments.

Number of experiments	Concentration $\text{mg MnO}_2/\text{m}^3 \text{ air}$		Size $\% \leq 3.0 \mu\text{m}$	
	M	SD	M	SD
9	22	± 9.2	87	± 13.4

Bacterial aerosol

In the experiments a strain of *Enterobacter cloacae* was used. The bacteria were cultured on Drigalski agar plates at 37° C overnight and suspended in sterile distilled water or phosphate buffer. The suspension was aerosolized with a Collision spray (57).

The concentration of the bacterial aerosol was determined by the measurement of the percentage of transmittance of the bacterial suspension in a colorimeter (Unicam). The concentration used was 10⁹ bacteria/ml of suspension, corresponding to a transmittance of 40 %. No determinations were made of the number of viable bacteria in the exposure chamber air.

An aluminium chamber with a volume of 37 l was used for the bacterial exposures. The flow through the chamber was kept at 4 m³/h. During the exposure, a negative pressure of 40 mm of water was maintained. The exposure lasted 40 min.

In certain experiments the animals were given the bacterial aerosol at different time intervals with relation to the MnO₂ exposure.

In other experiments the number of viable *E. cloacae* was determined in the lungs of animals at different time intervals after exposure.

Exposure regimes

The exposure regimes used in the different experiments are illustrated in fig. 1. In the following presentation of the results, the different exposure groups will be referred to as:

E₁ + M = *Enterobacter* 1 day prior to MnO₂
 E₀ + M = *Enterobacter* immediately before MnO₂
 M + E₀ = *Enterobacter* immediately after MnO₂
 E = *Enterobacter*
 MnO₂ = MnO₂

Preparations and measurements

Determination of manganese dioxide in the lung. Groups of two to four animals were sacrificed at the termination of MnO₂ exposure and at varying times thereafter.

Fig. 1. Time scheme in days after exposure to *Enterobacter* (E) and manganese dioxide (MnO₂) in clearance experiments.

The lung and trachea were removed, placed in porcelain crucibles, weighed, and then dried for a period of 15 h at 110 to 115° C.

The dry weight was determined, and ashing was done in a muffle oven for 2 × 15 h at 445° C. The ash weight was then calculated, and 15 ml of 1N HNO₃ was added to the crucibles, which were then left overnight. The sample was stirred thoroughly and transferred to test tubes. The concentration of manganese in the sample was determined at a wave length of 279.5 μm by flame atomic absorption spectrophotometry (Perkin Elmer 403, equipped with deuterium background correction). The amount of manganese was expressed as micrograms per gram of dry lung. The method used was in principle similar to the one earlier described in detail by Kjellström et al. (37).

Number of free lung cells. The number of free lung cells in the airways was determined with a lavage technique described in detail by Rylander (58). Ten milliliters of saline was gently flushed into the lungs and withdrawn. The same fluid was then

Table 2. Time relations between exposure to manganese dioxide (MnO₂) and *Enterobacter* for the different combined exposed groups.

Days after <i>Enterobacter</i> exposure	Days after MnO ₂ exposure				
	0	1	2	3	4
0	M + E ₀				
1	E ₀ + M	M + E ₀			
2	E ₁ + M	E ₀ + M	M + E ₀		
3		E ₁ + M	E ₀ + M	M + E ₀	
4			E ₁ + M	E ₀ + M	M + E ₀

flushed in again and withdrawn. The process was repeated ten times, and then 0.5 ml of the fluid was transferred into a test tube and stained with Türck's solution. The preparation was studied under the microscope, and the number of macrophages and polymorphonuclear leukocytes counted in a Bürker chamber. The number of cells reported in the results should be multiplied by 5×10^4 for the total number of cells in the lung.

Determination of macrophages with phagocytized manganese dioxide particles. After 0.5 ml of the lavage fluid was withdrawn for the cell count, the remaining sample was centrifuged at 2,000 rpm for 5 min and a smear was made from the precipitate. The percentage of macrophages containing phagocytized particles was determined by the count of 50 cells per smear under a microscope (x 400). Only cells with five or more particles were considered as cells containing phagocytized MnO₂ particles. This procedure was followed so that methodological errors would be reduced, inasmuch as other kinds of particles present in macrophages could be mistaken for MnO₂ particles.

Measurement of clearance of viable bacteria. The animals were killed at 0, 1, 3, 5 and 24 h after a 10-min exposure to *E. cloacae*. The lungs and trachea were removed under aseptic conditions and homogenized in 10 ml of a phosphate buffer solution. Dilution series of 1:10, 1:100 and 1:1,000 were made of the preparations. Duplicate samples of 0.5 ml per dilution were mixed with Drigalski agar. After incubation at 37° C overnight, the number of colonies were counted and the mean value from the duplicate sample per dilution was calculated.

Histological evaluation of lung section. Histological sections of lung tissue were prepared according to standard histological techniques and stained according to the Giemsa method. The stained preparations were scanned in a microscope at a magnification of x 100. About 40 vision fields were required for the scanning of the preparate, and the number of vision fields containing areas of leukocyte infiltration was counted. The degree of infiltration was evaluated as follows:

Number of vision fields containing leukocyte infiltrate	Degree of infiltration	Symbol
0	none	—
1—2	occasional	+
3—4	rather spread	++
5—6	spread	+++
> 7	widespread	++++

TREATMENT OF DATA

Mean values and standard deviations were calculated from individual data. In the experiments on clearance the mean from all the animals examined at the same time after exposure was considered as representing the retention value for that experiment. Means were then calculated from several experiments. Differences between different experiments were tested for significance with the two-sided Student's *t*-test.

The data for the different observations are usually given with relation to the MnO₂ exposure. In some cases the time-corresponding value after *Enterobacter* exposure is referred to as the TRE value.

Table 2 clarifies the time relations for the combined exposed groups with reference to the time of exposure to either agent.

Results

LUNG CLEARANCE OF MANGANESE DIOXIDE PARTICLES

The pulmonary clearance of MnO_2 at various time intervals after a 24 h exposure is demonstrated in table 3 and illustrated in fig. 2.

The table demonstrates that, of an initially deposited mean amount of 438 (221) $\mu\text{g MnO}_2/\text{g}$ of dry lung, about 50 % remained after 1 day. Approximately 27 %, 16 % and 3 % remained after 2, 3 and 7 days, respectively.

The table and the figure also report data from animals exposed to *Enterobacter* 1 day prior to MnO_2 . The initially deposited mean amount in these animals was 340 (154) $\mu\text{g MnO}_2/\text{g}$ of dry lung. This value was not significantly different from that of the group exposed to MnO_2 only.

In comparison to animals exposed only to MnO_2 , a significantly higher retention was found in the animals exposed to *Enterobacter* at day 1 and day 3 after exposure. The value for the retention at 2 days after MnO_2 exposure was also higher, but the difference was not significant. At 7 days the retention was about

the same. The retention data thus indicate that a decreased clearance was present up to 3 days after exposure in animals exposed to *Enterobacter* prior to MnO_2 .

The results from the pilot study to investigate whether the time of bacterial

Fig. 2. Clearance of manganese dioxide (MnO_2) from lungs of guinea pigs at various times after exposure, expressed as the percentage of the initial deposition.

Table 3. Manganese dioxide (MnO_2) in lungs at various times after exposure. Data expressed as percentage of initial deposition. ($E_1 + M$ = animals exposed to *Enterobacter* 1 day prior to MnO_2 ; ID = initial deposition in micrograms of MnO_2/gram of dry lung weight; N = number of experiments; figures in parentheses = standard deviation)

Days after MnO_2 exposure	MnO_2 ID = 438 (221)		$E_1 + M$ ID = 340 (154)	
	N	%	N	%
0	9	100.0	4	100.0
1	5	49.7 (5.4)	4	73.3 (6.6)***
2	4	27.2 (8.6)	2	40.7 (2.7)
3	7	16.3 (4.8)	4	26.2 (4.9)**
7	6	3.2 (0.6)	4	4.2 (0.8)

** $p \leq 0.01$; *** $p \leq 0.001$.

Table 4. Manganese dioxide (MnO_2) in lungs at various times after exposure. Data expressed as percentage of initial deposition. ($E_0 + M$ = *Enterobacter* immediately before MnO_2 ; $M + E_0$ = *Enterobacter* immediately after MnO_2 ; ID = initial deposition in micrograms of MnO_2 /gram of dry lung weight; N = number of experiments; figures in parentheses = standard deviation)

Days after MnO_2 exposure	MnO_2 ID = 438 (221)		$E_0 + M$ ID = 126 (40)		$M + E_0$ ID = 313 (28)	
	N	%	N	%	N	%
0	9	100.0	1	100.0	2	100.0
1	5	49.7 (5.4)	1	49.8	2	37.6 (12.6)
2	4	27.2 (8.6)	1	31.5	2	23.1 (9.8)
3	7	16.3 (4.8)	1	25.0	2	10.1 (2.1)
7	6	3.2 (0.5)	1	5.2	2	2.5 (0.2)

exposure might be important to the clearance of MnO_2 particles are shown in table 4.

It can be seen in the table that the high retention found at day 1 in the $E_1 + M$ group (table 3) was not present in the $E_0 + M$ or the $M + E_0$ group. The retention in the $E_0 + M$ group was about the same as in the group exposed to MnO_2 only. In the $M + E_0$ group the value was lower, but the difference was not statistically significant. The same was true for the slight differences seen in the two groups at 2, 3 and 7 days after exposure.

NUMBER OF MACROPHAGES

Table 5 reports the number of macrophages found in the airways of animals exposed to MnO_2 , E, and $E_1 + M$.

Table 5. Mean (M) number of macrophages in animals at various times after exposure. The mean value for the controls was 115 (62) based on 59 animals. (MnO_2 = animals exposed to manganese dioxide; E = animals exposed to *Enterobacter*; N = number of animals; figures in parentheses = standard deviation)

Days after exposure	MnO_2		E	
	N	M	N	M
0	23	46 (31)***		
1	19	127 (70)	10	141 (123)
2	18	114 (62)	10	133 (54)
3	26	172 (94)***	10	208 (133)***
7	13	180 (63)***		

*** $p \leq 0.001$.

For the MnO_2 group, the results show that, in comparison to control animals, the number of macrophages was significantly reduced immediately after the exposure. At 1 and 2 days no difference was found. At days 3 and 7 the number of macrophages was significantly increased.

In animals exposed to *Enterobacter* only the number of macrophages was almost the same as in control animals at 1 and 2 days after exposure. At 3 days a significant increase was found.

The results from experiments in which *Enterobacter* were given at different time intervals in relation to MnO_2 exposure are illustrated in fig. 3 and reported in the appendix (table A-1). In the figure the mean number of macrophages in the exposed groups is plotted against the time after MnO_2 exposure. Each point represents the mean from between 4 to 12 animals. The number of macrophages for unexposed animals is indicated with "C".

In comparison to the control animals a significantly increased number of macrophages was seen in the $E_1 + M$ group at 0, 1, 2 and 7 days after exposure ($p \leq 0.01$, $p \leq 0.05$, $p \leq 0.001$ and $p \leq 0.05$, respectively). In the $E_0 + M$ group an increased number was seen at 1 and 2 days ($p \leq 0.05$ and $p \leq 0.01$, respectively). In the $M + E_0$ group an increase was seen at 1, 2, 3 and 7 days ($p \leq 0.001$).

In comparison to the animals exposed only to MnO_2 , the figure shows that in the $E_1 + M$ group the number of macrophages was higher at 0 and 2 days after exposure ($p \leq 0.001$). For the $E_0 + M$

group the number of macrophages was increased at 0, 1 and 2 days after MnO_2 exposure ($p \leq 0.001$). Animals exposed to $\text{M} + \text{E}_0$ showed an increased number of macrophages at 1, 2, 3 ($p \leq 0.001$) and 7 days ($p \leq 0.05$) after MnO_2 exposure.

The figure also shows that the peak increase at 1 and 2 days was higher for the $\text{E}_0 + \text{M}$ and $\text{M} + \text{E}_0$ groups than for $\text{E}_1 + \text{M}$ exposed animals. This difference was statistically significant ($p \leq 0.05$ and $p \leq 0.01$, respectively). No significant difference was seen between the $\text{E}_0 + \text{M}$ and $\text{M} + \text{E}_0$ groups. At 3 days the values were also higher than the values for the $\text{E}_1 + \text{M}$ group ($p \leq 0.01$). At this time a significant difference was also present between the groups ($p \leq 0.05$).

At the times when the number of macrophages in the groups given combined exposures was significantly higher than in the animals exposed to MnO_2 only, the difference in the time related number of macrophages in the animals exposed to *Enterobacter* only (TRE values) was evaluated. Data from table A—1 in the appendix show that all values for the animals given combined exposures were significantly higher ($p \leq 0.05$) than corresponding TRE values except at 0 days in the $\text{E}_0 + \text{M}$ group, although the same tendency was still found at that time.

Fig. 3. Number of macrophages (5×10^4) in lungs of guinea pigs at different times after exposure to manganese dioxide (MnO_2). (Day 0 represents the time immediately after MnO_2 exposure; C represents control values for each group).

Table 6. Mean (M) number of leukocytes in animals at various times after exposure. The mean value for controls was 74 (50) based on 59 animals. (MnO_2 = animals exposed to manganese dioxide; E = animals exposed to *Enterobacter*; N = number of animals; figures in parentheses = standard deviation)

Days after exposure	MnO_2		E	
	N	M	N	M
0	23	63 (38)		
1	19	124 (79)**	10	507 (283)***
2	18	98 (56)	10	371 (226)***
3	26	151 (94)***	10	330 (190)***
7	13	93 (37)		

** $p \leq 0.01$; *** $p \leq 0.001$.

In summary, the data on the number of macrophages show that, in comparison to control animals, the MnO_2 exposed animals showed a decrease immediately after exposure and then an increase several days after exposure. Exposure to *Enterobacter* resulted in an increased number 3 days after exposure. A combined exposure to MnO_2 and *Enterobacter*, in comparison to when either agent was given alone, produced a significantly increased macrophage response, with a peak 2 days after MnO_2 exposure. When *Enterobacter* was given in immediate connection to MnO_2 , the response was increased in comparison to the response in the animals exposed to *Enterobacter* 1 day prior to MnO_2 .

NUMBER OF LEUKOCYTES

The number of leukocytes in the MnO_2 and *Enterobacter* exposed animals is shown in table 6.

The table shows that, in comparison to the control animals, the number of leukocytes was significantly increased in the MnO_2 group at 1 and 3 days after MnO_2 exposure. At 0, 2 and 7 days the number was not significantly different from the controls.

In animals exposed to *Enterobacter*, the number of leukocytes was significantly increased at 1, 2 and 3 days after exposure in comparison to both the control and MnO_2 exposed animals.

Fig. 4. Number of leukocytes (5×10^4) in lungs of guinea pigs at different times after exposure to manganese dioxide (MnO_2). (Day 0 represents the time immediately after MnO_2 exposure; C represents control values for each group)

Illustrated in fig. 4 and reported in the appendix (table A—2) is the number of leukocytes found at different times after the exposures of the MnO_2 , $\text{E}_1 + \text{M}$, $\text{E}_0 + \text{M}$, and $\text{M} + \text{E}_0$ groups. The mean number of cells in from 4 to 12 animals are plotted against the time after MnO_2 exposure. The number of leukocytes in unexposed animals is indicated by "C".

The figure shows that, in general, the number of leukocytes was higher than in the controls at 0, 1, 2 and 3 days after exposure ($p \leq 0.05$). At 7 days the values were not significantly different in the $\text{E}_1 + \text{M}$ and $\text{E}_0 + \text{M}$ groups.

For the $\text{E}_1 + \text{M}$ exposed animals the figure shows that the number of leukocytes was increased at 0, 1 and 2 days after MnO_2 exposure, in comparison to the MnO_2 exposed animals ($p \leq 0.001$, $p \leq 0.05$ and $p \leq 0.01$, respectively). In animals exposed to $\text{E}_0 + \text{M}$ an increase was seen at 0, 1 and 2 days ($p \leq 0.001$). For the $\text{M} + \text{E}_0$ group an increased number of leukocytes was seen at 1, 2, 3 and 7 days ($p \leq 0.001$).

The figure also shows that the increased leukocyte number at 1, 2 and 3 days was higher for the $\text{E}_0 + \text{M}$ and $\text{M} + \text{E}_0$ exposed animals than for the $\text{E}_1 + \text{M}$ animals. This difference was statistically significant ($p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.01$, respectively). No significant difference was present between $\text{E}_0 + \text{M}$ and $\text{M} + \text{E}_0$ groups at 1 day after exposure. At 2, 3 and 7 days the number was higher in the $\text{M} + \text{E}_0$ group ($p \leq 0.05$, $p \leq 0.05$ and $p \leq 0.001$, respectively).

At the times when the number of leukocytes in the groups given combined exposures was significantly higher than in the animals exposed to MnO_2 only, the difference to the TRE values was evaluated. Data from table A—2 in the appendix show that no difference was seen for the $\text{E}_1 + \text{M}$ group. In the $\text{E}_0 + \text{M}$ group a significantly higher number was seen at 1 and 2 days ($p \leq 0.001$ and $p \leq 0.01$, respectively). In the $\text{M} + \text{E}_0$ group a higher number was seen at 1, 2 and 3 days after exposure ($p \leq 0.01$, $p \leq 0.001$ and $p \leq 0.01$, respectively).

In summary, the data on the number of leukocytes show that, in comparison to controls, an increase was seen 1 and 3 days after MnO_2 exposure. Exposure to *Enterobacter* resulted in an increased number for 3 days following exposure. No difference was found between the $\text{E}_1 + \text{M}$ group and the group exposed only to *Enterobacter* at corresponding times. When *Enterobacter* was given in immediate connection to MnO_2 , a significant increase was seen in the 3 days following exposure. This increase was marked in the $\text{M} + \text{E}_0$ group. The data indicate that, similar to the macrophage response, the leukocyte response was also related to the time of *Enterobacter* exposure.

PHAGOCYTOSIS OF MANGANESE DIOXIDE

Table 7 shows the proportion and total number of macrophages with phagocytized particles (MWP) for the different exposure groups.

It can be seen that about 50 % of the macrophages in the MnO_2 group contained phagocytized particles immediately after the 24-h exposure. The proportion de-

creased thereafter, and at 7 days after exposure only 15 % contained phagocytized particles. The total number of macrophages with particles was higher at 1, 2 and 3 days after exposure as than at 0 and 7 days ($p \leq 0.001$, $p \leq 0.001$ and $p \leq 0.05$, respectively).

In the group exposed to $E_1 + M$, the proportion of MWP was significantly decreased in comparison to the MnO_2 group at 0, 1, 2 and 3 days after MnO_2 exposure. The total number of MWP was increased at 0 days, decreased at 1 day, and about the same at day 2. In the days that followed, a decrease was seen which was significant at day 3.

In the $E_0 + M$ group the proportion of MWP was about the same as in the MnO_2 group except on day 1, when a slight decrease was seen. The total number of MWP was increased at 0, 1 and 2 days after MnO_2 exposure and almost the same at days 3 and 7.

In the $M + E_0$ group, the proportion of cells with particles was significantly decreased at 1, 2, 3 and 7 days. The total number of MWP was decreased at day 0, somewhat lower at day 1, and increased at day 2. On days 3 and 7 the total number of MWP was about the same as in the MnO_2 exposed animals.

In summary, the results show that a decreased phagocytic capacity of the population of pulmonary macrophages can be

found in animals exposed to *Enterobacter* in combination with MnO_2 . The occurrence in time of this decrease is dependent upon when *Enterobacter* is given. The decreased capacity is more pronounced in animals exposed to $E_1 + M$ and $M + E_0$. For the $E_1 + M$ group, the phagocytic capacity was decreased immediately after MnO_2 exposure, and this reduced capacity persisted for 3 days. In the $M + E_0$ group, the same effect occurred 24 h after the *Enterobacter* exposure. In animals exposed to $E_0 + M$ hardly any effect was found, even if the total number of MWP was highly increased during the 2-day period after MnO_2 exposure.

CLEARANCE OF VIABLE BACTERIA

Table 8 reports the number and proportion of *E. cloacae* remaining in the lungs of control animals and MnO_2 exposed animals at different times after exposure. The clearance pattern is illustrated in fig. 5.

It is seen that a lower percentage of viable bacteria remained at 1 h in the MnO_2 group as compared to control animals. At 3, 5 and 24 h after bacterial exposure, a higher percentage of bacteria remained in the lungs of the MnO_2 exposed animals.

Table 7. Macrophages containing manganese dioxide (MnO_2) particles at various times after exposure. The data are presented as the percentage (%) and number (n) of macrophages with particles (MWP). ($E_1 + M$ = animals exposed to *Enterobacter* 1 day prior to MnO_2 ; $E_0 + M$ = animals exposed to *Enterobacter* immediately before MnO_2 ; $M + E_0$ = animals exposed to *Enterobacter* immediately after MnO_2 ; N = number of animals; M = mean number of macrophages; figures in parentheses = standard deviation)

Days after MnO_2 exposure	MnO_2				$E_1 + M$				$E_0 + M$				$M + E_0$			
	N		M		N		M		N		M		N		M	
			MWP				MWP				MWP				MWP	
			%	n			%	n			%	n			%	n
0	23	46	54 (14)	25 (6)	9	231	33*** (12)	76*** (28)	5	232	41 (9)	95*** (21)	6	39	47 (13)	18* (5)
1	19	127	47 (13)	60 (17)	9	172	25*** (12)	43* (21)	5	608	30* (9)	182*** (55)	8	371	12*** (6)	45 (22)
2	18	114	36 (13)	41 (15)	6	219	22* (7)	48 (15)	5	579	37 (15)	214*** (87)	12	481	13*** (7)	63* (34)
3	26	172	34 (14)	58 (24)	9	210	16*** (5)	34** (11)	4	265	24 (8)	64 (21)	12	400	14*** (4)	56 (16)
7	11	185	15 (9)	28 (17)	6	149	9 (6)	13 (9)	4	206	22 (11)	45 (23)	6	272	6* (3)	16 (8)

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Table 8. Clearance of viable *Enterobacter cloacae* in unexposed animals and in animals exposed to manganese dioxide (MnO_2) for 24 h. The data represent the mean number of colonies in a two tenfold diluted sample of lung homogenate at different times after exposure. Each datum represents a mean value from 9 animals except for the 24 h values where data are based on 4 animals. (%) = the percental retention of bacteria; figures in parentheses = standard deviation)

Hours after <i>Enterobacter</i> exposure	Controls		MnO_2 Exposed	
	Colonies	Retention (%)	Colonies	Retention (%)
0	177 (35.9)	100 (20.3)	170 (19.8)	100 (11.7)
1	146 (31.0)	82.1 (17.5)	88 (21.5)	51.5 (12.6)***
3	20 (10.5)	11.2 (5.9)	50 (23.9)	29.5 (14.0)**
5	5 (1.3)	2.7 (0.7)	23 (11.7)	13.3 (6.9)***
24	1 (0.5)	0.7 (0.3)	3 (0.8)	1.8 (0.5)**

** $p \leq 0.01$; *** $p \leq 0.001$.

Fig. 5. Viable *Enterobacter cloacae* in lung homogenates at various times after exposure.

In summary, the results show an increased clearance of viable bacteria during the first hour after exposure in the MnO_2 group, followed by a decreased clearance at 3, 5 and 24 h.

CHANGES IN LUNG TISSUE AFTER EXPOSURE TO MANGANESE DIOXIDE

Table 9 reports the results of the examination of lung tissue sections taken from animals exposed to MnO_2 at different

Table 9. Degree of infiltration of leukocytes in lung tissue of individual animals at different times after manganese dioxide (MnO_2) exposure in exposed and unexposed animals. (— no infiltration; + occasional infiltration; ++ rather spread infiltration; +++ spread infiltration; ++++ widespread infiltration)

Days after exposure	Unexposed	MnO_2 exposed
0	— — ++ ++ ++++	+ + + ++ ++++
1	— — + ++ +++	— — ++ +++ ++++
7	+ ++ +++ +++ ++++	+ + ++ +++ ++++

times after exposure and from unexposed animals at corresponding times.

The table shows that the interindividual variation was large within the two groups at all the time intervals studied. High degrees of infiltration as well as no infiltration were found in both unexposed and exposed animals. No differences were found between exposed animals at different times after exposure.

General discussion

METHODS

Methods of studying the lung clearance of chemical, bacteriological or radioactive materials have been described by several authors and in reviews presented by Hatch and Gross (32) and Holma (35). In one type of study, the agent to be cleared is an inert substance, and alterations in the clearance of this substance have been used as a method of studying the effect of exposure to noxious agents. Examples of this type of study are the experiments by Ferin and Leach (24), who studied the clearance of titanium dioxide (TiO_2) particles in rats after exposure to sulfur dioxide (SO_2) and nitrogen oxides (NO_x), and by Bair and Dilley (3), who used $^{59}\text{Fe}_2\text{O}_3$ and $^{51}\text{Cr}_2\text{O}_3$ particles to study clearance in rats and dogs exposed to cigarette smoke.

A second method is to investigate the clearance per se of different agents; the clearance kinetics of the agents used are compared with regard to such factors as particle size, solubility, and chemical structure. Klosterkötter and Bunemann (38), for example, studied in rats the clearance of SiO_2 particles with different chemical and physical properties, TiO_2 and alumina (Al_2O_3) particles. The differences in clearance rates were explained in terms of routes of elimination, initial dust loads, particle size, solubility, and sex-linked differential elimination rate.

In the present investigation a combination of these methods was used to study clearance. Clearance of the noxious agent, MnO_2 , was studied per se, as well as in connection with *Enterobacter*. In the experiments, one animal had to be sacrificed at each time interval for the analysis of the content of MnO_2 in the lungs. The reasons for choosing this method were several, i.e., it offered the possibility of studying both the retention

and the free lung cell response in the same animal, it is also a relatively simple method, and the equipment is not very costly. Some of the disadvantages of the method must be noted as well. The clearance could not be followed in the same animal and, consequently, a larger number of animals had to be used so that errors due to biological variation among the animals could be reduced. Also, the technique is rather time consuming, as multiple experiments had to be performed for reproducible results.

To generate the MnO_2 aerosol, a Rag Pe generator was used. The generator has earlier been used in studies by Rylander et al. (63) and Rylander and Bergström (61), and the technical characteristics have been described by Hellström (34). This method was employed because it made the production of a rather stable aerosol possible, in terms of both concentration and particle size, throughout the experiments. This stability is verified by the analysis of the dust aerosol. In comparison to other dust generators, such as the Wright dust feed mechanism described by Wright (76) and used by several authors, such as Maigretter et al. (45) and Bingham et al. (10), the Rag Pe generator had the advantage of being simple to operate and low in cost.

The atomic absorption spectrophotometry used to analyze MnO_2 in biological material is a reliable and well established technique for the determination of the content of metals in biological tissue. It was considered to be the best method for the present investigation for the detection of small amounts of manganese retained in lung tissue at different times after exposure.

Biological variations must be taken into consideration in animal experiments. If the same animal is used for the study of clearance during a certain period of time

(35, 48), the statistical variation is minimized. In order to study both clearance and the free lung cell response, as in the present study, it was unfortunately not possible to use the same animal. However, so that the number of animals needed for reproducible results would be reduced, care was taken to use only high quality animals who were free from upper respiratory infection. The importance of using such animals in inhalation toxicology experiments was also pointed out in the work of Rylander (59).

The number of free lung cells in the airways can be influenced by different inhaled agents, as has been demonstrated earlier (10, 11, 25, 30, 40, 60). Certain aspects of the role of free lung cells, as well as their quantitation and regulation, was described by Brain (12). The technique of lung lavage, the definition of free lung cells, and the interpretation of the results differ among the authors. In this study, the free lung cells were defined as the number of alveolar macrophages and leukocytes determined in the lavage fluid by a standardized light microscope counting technique. The leukocytes were mainly comprised of polymorphonuclear leukocytes, most of them neutrophils and eosinophils. Less than 10 % of the total cell population consisted of monocytes or lymphocytes, and the number was not affected by the exposures undertaken. A more-detailed differentiation between these cell types could not, however, be made with the technique used.

As an index of the phagocytic capacity of alveolar macrophages, smears of lung lavage cells were evaluated with a count of macrophages with five or more phagocytized particles. With this method, information can be received on the relative number of macrophages engaged in the phagocytosis of MnO_2 particles deposited in the lung. This procedure was chosen in order to minimize methodological errors, as it was seen in a pilot study that about 20 % of the macrophages in control animals contained up to four particles.

A drawback of this method is that only macrophages with five or more particles were counted. Another method to measure the rate of particle phagocytosis in vitro by rabbit alveolar macrophages was described by Myrvik et al. (49) and modi-

fied by Lundborg and Holma (44). In this method, the particles to be studied were added to a macrophage suspension, and the number of particles per macrophage was counted after incubation at 37° C for 1.5 h. The method has more recently been used by Camner et al. (16) to study the effect of a subacute aerosol exposure to nickel (Ni) in rabbits. In their study the authors used the number of Ag-coated 5- μm teflon particles phagocytized per macrophage as an index of the rate of phagocytosis in the exposed and control animals. Although the described method makes it possible to study the phagocytosis of single particles and is thus a more sensitive method than the one used in the present experiment, the present method was chosen because it offers a way of studying the phagocytosis of the inhaled agent per se in vivo.

The technique used to study the clearance of viable bacteria has been previously described in detail, and methodological studies have shown it to be accurate and the results to be reproducible (57).

Histological examination of lung sections was performed from control and exposed animals and the degree of leukocyte infiltration was used as an index of response. This criterion was chosen as other authors — Lloyd Davies (42), Levena and Robachevsky (41), Zaidi et al. (77) and Mägreter et al. (45) — reported on slight to intense leukocyte infiltration in MnO_2 exposed animals.

Finally, inhalation was the method selected to administer MnO_2 into the animals' lungs. This method is preferable to intratracheal administration in lung clearance studies because it offers a more realistic exposure situation. An intratracheal injection results in a sudden application of a dose which may overwhelm the lung clearance mechanism and open up other clearance pathways and may thus affect the clearance rate of the agent administered. Furthermore, the distribution of the injected agent in the lung will presumably be nonuniform and will result in differences in burden from one part of the lung to another.

RESULTS

Lung clearance

The results from the investigation have shown that the clearance of MnO_2 from the lungs of guinea pigs under the conditions of the experiments is rapid and is almost terminated 7 days after exposure. In earlier studies by Morrow et al. (48) a biological half-time of 38 days was reported when dogs were exposed to tagged $^{54}\text{MnO}_2$ particles in a concentration of 0.5 mg/m^3 and a particle size of $0.07 \mu\text{m}$ (CMD). In other experiments on humans (47) a half-time of 65 days was found during a measurement period of up to 120 days after exposure to 4 mg/m^3 with a particle size of $0.9 \mu\text{m}$ (MMD). These particles were considerably smaller than those used in the present study.

The previously cited study by Maigretter et al. (45) was performed using MnO_2 particles of a size distribution which was similar to the one used in the present study. The authors found that 30 % of an initially deposited amount of 95 mg/g of lung was cleared in mice 5 h after exposure and about 98 % after 14 days. Although a different animal species was used, the findings agree with the results of the present investigation.

The reason for the discrepancy between the present results and those of Morrow et al. may be due to differences in the particle size distribution of the aerosol used. It is known that larger particles, as used in the present experiments, favor a deposition in the upper part of the respiratory tree (70), i.e., in the ciliated region, where the particles are deposited on the mucous layer and subsequently rapidly cleared by the mechanical clearance mechanism.

It is also conceivable that the difference in the amount of material deposited in the pulmonary region has influenced the clearance. In the present experiments the mean initial lung burden was $430 \mu\text{g MnO}_2/\text{g}$ of dry lung in comparison to the $80 \mu\text{g}$ reported by Morrow et al. (48). La Belle and Brieger (39, 40) showed that a high initial lung burden might alter the clearance rate in comparison to a low initial lung burden. A possible explanation, suggested by Klosterkötter and Bune-

mann (38), Morrow (46), and Ferin (23), is that a higher dose level might open up clearance pathways via the lymphatics and result in faster clearance.

In some of the present experiments an attempt was made to analyze MnO_2 content in the tracheal lymph nodes of the guinea pigs at different times after exposure using the same method as used for the lung determinations. No MnO_2 could however be detected.

The clearance of MnO_2 from the lungs of exposed animals was affected by exposure to *Enterobacter*. For animals exposed to *Enterobacter* one day prior to MnO_2 the clearance was decreased up to three days after MnO_2 exposure. The time of bacterial exposure in relation to the time of particle exposure seems to be crucial for the effect on clearance. The tentative results from the pilot study, in which animals were exposed to bacteria immediately before MnO_2 , indicate no significant difference in clearance in comparison to MnO_2 exposed animals. However, when *Enterobacter* was given immediately after MnO_2 exposure, a tendency toward an increased clearance was noticed. The data from the pilot experiment are limited, and additional experiments must be performed before a definite conclusion can be drawn.

Previous experience concerning the effect of Gram-negative bacteria, or their endotoxins, on the reticuloendothelial system and subsequent effect on particle clearance have been reported by Benacerraf and Sebestyen (7). The authors found that the clearance of injected carbon particles from the blood was decreased in rabbits which had received a small dose of endotoxin intravenously prior to the carbon particles. The decrease was maximal at 12 h and still observed one day after endotoxin administration. On day two a change to an increased clearance took place.

In the present study the data on *Enterobacter* exposure at different time intervals indicate that, in animals exposed to *Enterobacter* one day prior to MnO_2 , the decrease in clearance was maximal three days after *Enterobacter* exposure, slightly decreased at four days and still decreased at five days. The times when a decrease in clearance was seen do not agree with the results from Benacerraf

and Sebestyen's study. The reason for the discrepancy between the studies is not clear. However, factors like route of administration, type of endotoxin used, and species variation are probably important. From a principal point however, the results support each other in that Gram-negative bacteria influence the clearance of particles in the body and that the effect is related to time of exposure.

Macrophages

In the present experiments a decrease in the number of macrophages was seen immediately after the 24-h exposure to MnO_2 . Waters et al. (75) reported that, in an *in vitro* system, manganese reduced both the number and the viability of alveolar macrophages. Bingham et al. (8) reported a decreased number of alveolar macrophages in the lungs of rats after a subacute exposure to lead sesquioxide (Pb_2O_3) lasting up to 12 months. In a later work the same author (9) showed that the number of macrophages was decreased significantly after one day of exposure to $150 \mu\text{g Pb}_2\text{O}_3/\text{m}^3$ and the number remained low for the duration of exposure (three months). When rats were exposed to nickel oxide (NiO) for two weeks (11), a significant increase in the number of macrophages was seen. The reason for the alterations in the number of macrophages after exposure to different agents is unknown. However, as they are of importance for lung defense against particles, any alteration in the normal number of cells could be taken as an indicator of an unwanted effect.

At one and two days after exposure to MnO_2 the number of macrophages was about the same as in control animals, but it changed to a significant increase at three and seven days after exposure. These results are almost consistent with the findings of Bingham (9). She showed that the decreased number of macrophages seen in animals exposed to Pb_2O_3 for three weeks returned to the level of control animals after three days of breathing filtered air. The author did not, however, report whether an increase was seen again at a later time after exposure. The findings suggest that the initially observed

effect is reversible and, in the case of MnO_2 , accumulative with time after exposure.

La Belle and Brieger (40) stated that there was a correlation between the number of free phagocytic cells and the rate of lung clearance, i.e., a higher number of cells resulted in an increased clearance rate. In their experiment no determinations were made concerning the phagocytic activity of the cells.

Fisher et al. (26) exposed rats to an aerosol of $^{59}\text{Fe}_2\text{O}_3$ particles and measured the lung clearance for up to 50 days. At various times before and after particle exposure Freund's complete adjuvant (FCA) was administered to the rats. The authors found that clearance was increased when the animals were treated with FCA one, two and three days before particle exposure. The clearance was only slightly increased when FCA treatment was given seven days prior to exposure and not affected when FCA treatment was administered either after or 14 days before particle exposure. FCA treatment resulted in a significant increase in alveolar macrophages, as judged by lavage. However, the authors were not able to conclude that a cause and effect relationship existed between the increased clearance and the number of macrophages. The results strongly indicated that the time between FCA treatment and particle exposure was a more important factor than the total number of macrophages with respect to the effect on clearance rate. These findings partly agree with the results of the present experiments.

Fisher concluded also that the number of macrophages was not necessarily proportionally related to the phagocytic activity of inhaled particles; this conclusion also agrees with the results of the present experiments.

Concerning the measurements of macrophages with particles (MWP), the results from the present experiments show that there is a significant increase in the number of MWP one, two and three days after MnO_2 exposure. At the same times, however, there is a decrease in the proportion of MWP which is indicative of a relative decrease in phagocytic capability.

These findings indicate a toxic action of manganese on the macrophages. This conclusion is in accordance with results

reported by Graham et al. (29), who showed that MnO_2 impaired the phagocytic activity of rabbit alveolar macrophages in vitro.

The increase seen in the number of MWP in the present study is explained by the higher total number of macrophages. This increase in the number of phagocytic cells might be a compensatory mechanism for the decreased phagocytic capability.

The decreased proportion of MWP could reflect a mobilization of more immature macrophages with a decreased capability to phagocytize particles in animals exposed to MnO_2 . Bingham et al. (10) have shown that the number of small ($\leq 10 \mu m$) macrophages in lung washings from rats exposed to coal dust was increased over the number in controls, and they suggested that more immature macrophages were found in exposed animals. This phenomenon could not be ascertained in the present study because no cell size measurements were made.

A depressive effect on the phagocytic cell population was seen in animals exposed to *Enterobacter* in conjunction with MnO_2 . The effect seemed to be related to the time of *Enterobacter* exposure. A combined exposure to *Enterobacter* and MnO_2 resulted in a relative decrease in the phagocytic capability of macrophages which was more pronounced than in animals exposed to MnO_2 only. In the combined exposed groups no general pattern of increase in the number of MWP, as seen in the MnO_2 exposed animals, was found.

When data from the clearance studies are compared with data on the number of MWP, it is seen that, although the number of macrophages was increased at one and three days after MnO_2 exposure in the $E_1 + M$ group, a decreased clearance was seen in comparison to MnO_2 exposed animals. However, at the same times, a decreased phagocytosis was seen in the $E_1 + M$ group.

From the present findings it is suggested that, for the full understanding of macrophage clearance capacity, determinations are required of their functional activity in addition to the number of cells. It is thus conceivable to suggest that not only the presence but also the phagocytic function of macrophages is an important factor which determines the efficiency of the

clearance mechanism and that both factors are altered by the action of MnO_2 alone or in combination with *Enterobacter*.

Leukocytes

With reference to the number of leukocytes present at different times after exposure to MnO_2 , an increase was noticed one and three days after exposure. This wave pattern of response was also found in another study (Rylander and Snella, unpublished data), in which animals were exposed to solvents and lipopolysaccharides in a subacute experiment. An explanation for this type of response could not be given, however.

The mechanism behind the leukocyte mobilization into the airways is not entirely clear. One possible mechanism is the mediation of mobilization through macrophages in connection with their uptake of dust particles. When macrophages take up particles, lysosomal hydrolases are released (65) causing, among other things, activation of complement via the indirect pathway. The C 3 b factor then formed is a leukotactic agent, and it could be responsible for inducing the mobilization of leukocytes. The increased number of leukocytes in the airways could thus represent an early-phase inflammatory reaction.

In animals exposed to *Enterobacter* an increase in the number of leukocytes is seen one day after exposure in comparison to unexposed or MnO_2 exposed animals. This reaction has been reported earlier by Rylander et al. (62). The authors found a large increase in the number of leukocytes 24 h after a short-term exposure to Gram-negative bacteria, including *Enterobacter*, or water extracts from different organic dusts. When studying the different bacteria species found in the water extracts, the authors found that endotoxin-producing strains caused the greatest effect.

In comparison to animals exposed to *Enterobacter*, only a combined exposure to *Enterobacter* and MnO_2 resulted in a significant increase in the number of leukocytes two and three days after *Enterobacter* exposure. It thus seems as though the presence of MnO_2 in the macrophages causes a predisposition for a more pronounced leukocyte response to the

bacteria than when the bacteria are given alone.

The preceding discussion demonstrates that a simultaneous exposure to bacteria increases the pulmonary response to MnO_2 and that this effect is dependent upon the time between the exposures. This finding is supported by data from Maigretter et al. (45). In their experiments on mice the highest effect was found when the animals were challenged with *Klebsiella pneumoniae* 5 h after MnO_2 exposure or when they were exposed to influenza virus 48 h before MnO_2 , as compared to when the animals were challenged with the bacteria 1 h after MnO_2 or with virus 24 h before MnO_2 . From the experiments the authors concluded that the longer the period between challenge to the infectious agent and the dust exposure, the higher the level of response. In the present experiments the highest response was seen when bacteria and MnO_2 were given simultaneously.

Although there are discrepancies between Maigretter's study and the present results, the time factor per se was shown to be crucial in both studies. The discrepancies might depend on the type of infectious agent used.

Clearance of viable bacteria

The studies on the clearance of viable bacteria show a significantly greater clearance of bacteria in animals exposed to MnO_2 1 h after exposure to bacteria and a decreased clearance at 3, 5 and 24 h. Immediately after MnO_2 exposure the number of macrophages is decreased and the number of leukocytes is about the same as in unexposed animals. In an experiment reported by Rylander et al. (62), animals exposed to a water extract of cotton dust 24 h prior to the bacterial exposure showed a significantly increased clearance of viable bacteria 1 h after bacterial exposure in comparison to unexposed animals. At that time the number of leukocytes, but not macrophages, was increased in the animals treated with dust extract. The authors concluded that the increase in clearance could be due to an increased number of leukocytes. Whether this increase in clearance was due to the increased number of leukocytes as such — or to a secondary stimulation of macro-

phages — was not investigated. In the present experiments no increase in the number of leukocytes was seen when a faster clearance of viable bacteria was present. Thus, factors other than the number of leukocytes must be important to the increased clearance of viable bacteria observed during the first hour after exposure.

Rylander (57) has shown that, in guinea pigs exposed to *Escherichia coli*, the mechanical elimination of the viable bacteria, mainly via the mucociliary transport system, is responsible for about 50 % of the total clearance during the first hour. It might therefore be suggested that the increased clearance seen during the first hour in the present experiments was primarily due to an increased mechanical clearance that was stimulated by the exposure to MnO_2 . It is possible that the bactericidal clearance during the same time period was in fact decreased, although this effect was masked by the increased mechanical clearance.

The decreased clearance of viable bacteria seen 3, 5 and 24 h after bacterial exposure in MnO_2 exposed animals reflects an effect on the bactericidal elimination mechanism. This finding is supported by the results presented by Rylander (57), who showed that between 1 and 2 h after exposure to viable bacteria mechanical elimination only accounted for 8 % of the total reduction of viable bacteria. At 24 h after MnO_2 exposure, the total number of MWP as well as the number of macrophages and leukocytes was significantly increased in comparison to the values immediately after exposure. On the other hand, the proportion of MWP was lower at 24 h, and this phenomenon indicates a decreased phagocytic capacity of the macrophage population. The results thus suggest that the function of the phagocytic cells, and not the number of the cells, is of primary importance also for the bactericidal elimination mechanism.

In the present study a decreased bactericidal capacity was found in animals exposed to MnO_2 . The contradictory conclusions in the reports by Heine (33) and Lloyd Davies (42) are probably due to differences in experimental methods. The mortality rate of exposed animals and histological changes in tissue reactions are less sensitive indicators of an exposure

effect than the direct measurement of the bactericidal capacity of macrophages that was used in the present study.

Histology

Concerning histological examinations of lung tissue in animals exposed to MnO_2 via inhalation, slight to intense leukocyte infiltration was found in lung tissues from rabbits (36) and mice (42, 45). The exposure time varied between 4 and 20 days. Contradictory findings were reported by Erishmann (21) and Heine (33). In experiments on rabbits and cats, Ehrishmann (21) did not find any pneumonia or pneumoconiotic changes in the lungs of the animals after exposure to MnO_2 dust for up to 15 months. No criteria was, however, given for the histological evaluation. According to Heine (33), no pathological changes in the lungs of guinea pigs were

seen after exposure to ferromanganese dust for up to 200 days.

In the present experiments a large interindividual variation in the degree of leukocyte infiltration was present among MnO_2 exposed and control animals; no difference was found between the groups.

The contradictory experiences from the literature and the present findings suggest that factors such as duration of exposure, concentration of the MnO_2 aerosol, and animal species might be responsible. As extensive alterations in the number of macrophages and leukocytes, as well as their functioning, were present at times when no clear-cut histological findings could be demonstrated, it can also be suggested that data from histological evaluations of lung tissue are poor indices of the acute pulmonary effects of MnO_2 and that, in studies based only on histological evaluations (21), the acute pulmonary effect might have been overlooked.

Conclusions

An evaluation of earlier experiences reported in the literature and the results from the present experiments support the concept that a primary inflammatory reaction can occur in the respiratory tract after exposure to MnO_2 without the presence of pathogenic bacteria. This reaction, which is of a limited duration after an acute exposure, is related to the property of the MnO_2 dust to induce pathological reactions via the cellular elements normally present in the lung. The reaction pattern also explains the ineffectiveness of usual antibiotics used for pneumonia treatment in the acute phase after MnO_2 exposure.

The pulmonary reaction after MnO_2 exposure is more pronounced in lungs challenged with bacteria. In the present experiment the maximum response occurred when the bacteria were administered simultaneously with the MnO_2 particles. For other bacterial species other time relations between the exposures will give a maximum reaction.

Earlier findings have suggested that exposure to MnO_2 causes a decrease in

the resistance toward certain respiratory infections. The present findings that the bactericidal clearance mechanism is decreased after MnO_2 exposure support these observations. Consequently, certain of the earlier observed cases of manganese pneumonia might have had a bacterial genesis, particularly among population groups where exposure to MnO_2 is low and the risk for airborne infection high. The findings thus underline that it is important in future studies on manganese pneumonia to differentiate between bacterially and chemically induced pulmonary reactions.

Based on experiences in the literature and the present findings, the following practical recommendations can be made:

1. Precautions should be taken against exposure to MnO_2 both in the general and work environments.
2. Workers exposed to MnO_2 should be examined for specific respiratory symptoms regularly.
3. For the clarification of unclear cases of pneumonia, the work environment should be investigated for the presence of airborne MnO_2 .

Summary

This work was initiated to study the effects of acute manganese dioxide (MnO_2) exposure on the lung.

A review of the literature showed that information on the pulmonary toxicity of MnO_2 is incomplete and sometimes contradictory, especially with respect to acute pulmonary cell responses, which take place in the lung immediately after exposure via inhalation, and their relation to the development of acute pneumonia. Contradictory findings have also been reported concerning the role of bacteria and their synergistic action with MnO_2 in the development of pulmonary effects.

With reference to results reported in earlier experimental investigations, it was considered important to study the following factors involved in the acute pulmonary action of MnO_2 :

- the pulmonary clearance of MnO_2 ,
- the number of free lung cells present in the airways after an acute exposure to MnO_2 ,
- the phagocytic activity of the alveolar macrophages at different times after exposure to MnO_2 , and
- the effects of an exposure to bacteria on the clearance of MnO_2 , the inflammatory response, and the phagocytic activity of alveolar macrophages.

For the clearance measurements, guinea pigs were exposed to an aerosol of MnO_2 for 24 h; whereafter the amount of MnO_2 was determined in the lungs for up to seven days. Determinations of phagocytic capacity and the presence of inflammation were performed by counting the number of macrophages and polymorphonuclear leukocytes in lung lavage fluid. The phagocytic capacity of alveolar macrophages at different times after expo-

sure was determined by a count of the number of macrophages containing particles.

Clearance of inhaled viable bacteria was measured in animals exposed to an aerosol of *Enterobacter cloacae*. The number of viable bacteria was determined in homogenates of the lungs at different times up to 24 h after exposure.

In the study of the influence of a bacterial exposure on the clearance of MnO_2 , as well as on the inflammatory response of the lung and the phagocytic activity of macrophages, animals were challenged to an aerosol of *E. cloacae* before or after MnO_2 exposure.

The results from the investigation showed that the clearance of MnO_2 from the lungs of guinea pigs was rapid. At seven days after exposure nearly all the MnO_2 had been cleared.

The number of macrophages was significantly decreased immediately after MnO_2 exposure; whereafter a successive increase took place during the following seven days. The increase in the number of leukocytes followed a wave pattern response with peaks at one and three days after MnO_2 exposure.

About 50 % of the macrophages contained phagocytized particles immediately after exposure. The proportion then decreased to 15 % at seven days after exposure.

An increased clearance of viable bacteria took place at 1 h after bacterial exposure in animals preexposed to MnO_2 , indicating an increased mechanical clearance. At 3, 5 and 24 h after the exposure, a significant decrease in the bacterial clearance was present.

The pulmonary reaction after MnO_2 exposure, with regard to the effects on

clearance, number of free lung cells and phagocytic capacity of macrophages, was more pronounced in lungs challenged with *Enterobacter cloacae*.

The clearance of MnO_2 was decreased at one and three days after exposure in animals exposed to *Enterobacter* 24 h before the MnO_2 exposure.

The number of macrophages and leukocytes was increased at two days after exposure to manganese dust in the combined exposed groups in comparison to the groups exposed to either agent alone. The results indicated a synergistic effect. The magnitude of the response was more pronounced in animals challenged with bacteria in immediate connection to MnO_2 exposure.

The phagocytic capacity of the population of alveolar macrophages was decreased in animals exposed to bacteria in combination with MnO_2 .

The depressive effect of MnO_2 on the phagocytic capacity of alveolar macrophages was more pronounced in animals exposed to bacteria in combination with MnO_2 .

The present findings, supported by earlier experiences reported in the literature, suggested that a primary inflammatory reaction of limited duration can occur in the respiratory tract after exposure to MnO_2 .

A more pronounced pulmonary reaction occurred after a combined exposure to MnO_2 and bacteria. The level of this synergistic response was dependent upon the time intervals between exposures.

The bactericidal clearance mechanism was depressed after MnO_2 exposure. This observation supports earlier findings which suggest that exposure to MnO_2 causes a decrease in the resistance towards certain respiratory infections.

It is finally suggested that precautions should be taken against exposure to MnO_2 in the work, as well as the general, environment. Exposed workers should be regularly examined for specific respiratory symptoms. Unclear cases of pneumonia should be investigated with reference to exposure to airborne MnO_2 .

Acknowledgments

This investigation was carried out in the Department of Environmental Hygiene at the University of Gothenburg, Sweden.

I would first like to extend my most sincere thanks to Prof. Ragnar Rylander, my tutor and head of the department, for directing me into the field of inhalation toxicology and the problems involved in the study of pulmonary cell responses to inhaled agents. The completion of the present work would not have been possible without his never failing support, encouragement and critical suggestions to my work.

Special thanks go to Ms. Jane Pettersson, who throughout the work skillfully assisted me with the clearance experiments, providing the samples for chemical analysis and the lung tissue sections for histological evaluation.

For able assistance with the clearance experiments and the counting of numerous cell preparations, my thanks are due to Ms. Margareta Sjöstrand.

In various parts of the laboratory work Ms. Gunilla Arvidsson, Ms. Birgitta Fogelmark, Ms. Eva Johansson, and Ms. Anita Samuelsson assisted me efficiently. I am grateful to all of them for their help.

I am also very grateful to Mr. Dag Söderberg for his intelligent advice and solutions to technical problems occurring throughout the work.

My warm thanks go to Mr. Birger Lind and his staff of the Department of Hygiene at the Karolinska Institute in

Stockholm for providing all the chemical analyses for the clearance experiments. His skillful assistance and critical suggestions on the analytical aspects of my work have been invaluable.

Concerning the technical aspects of aerosol generation and measurements, my thanks are due to Mr. Per-Åke Hellström of the Department of Environmental Hygiene at the National Environment Protection Board in Stockholm for fruitful discussions and advice.

I would like to give special thanks to Ms. Janet Vesterlund for her experienced help with the translation of the manuscript.

The figures were excellently drawn by Ms. Ulla Åhrlin, who also struggled with the assembling of the reference list.

For cooperation in typing the manuscript, my thanks go to Ms. Ingamaj Christenson, Ms. Barbro Engdahl, Ms. Ingrid Lundberg, Ms. Elisabeth Rahl and Ms. Heike Westerberg.

The animal care and technical maintenance was provided by Ms. Svea Johnsson, Ms. Ingrid Larsson and Mr. Uno Svensson, to whom I wish to express my thanks.

Finally I would like to acknowledge the indirect contributions and moral support of many other collaborators and friends, too numerous to name individually.

Financial support was provided by the Swedish Work Environment Fund.

References

1. BAADER, E. W. Manganvergiftungen in Elementefabriken. *Arch. Gewerbepath. Gewerbehyg.* 4 (1932) 101—116.
2. BAADER, E. W. Manganpneumonie. *Ärztl. Sachverständ. Ztg.* (1937): 6, 75—81.
3. BAIR, W. J. and DILLEY, J. V. Pulmonary clearance of $^{59}\text{Fe}_2\text{O}_3$ and $^{51}\text{Cr}_2\text{O}_3$ in rats and dogs exposed to cigarette smoke. In: C. N. DAVIES (ed.), *Inhaled particles and vapours II*. Pergamon Press, London 1967, pp. 251—268.
4. BAKRADSE, S. *Gig. tenda* 1923 no. 10/11. Cited by WASSERMAN and MIHAIL (74).
5. BAUER. (Dissertation). Berlin 22/9 1938. Cited by ELSTAD (22).
6. BELT, T. H. and KING, E. J. Chronic pulmonary disease in South Wales coalminers: III. Experimental studies. D. Tissue reactions produced experimentally by selected dusts from South Wales coalminers. In: *Special report series of the medical research council*, no. 250. London 1945, pp. 29—68.
7. BENACERRAF, B. and SEBESTYEN, M. M. Effect of bacterial endotoxins on the reticuloendothelial system. *Fed. proc.* 16 (1957) 860—867.
8. BINGHAM, E., PFITZER, E. A., BARKLEY, W. and RADFORD, E. P. Alveolar macrophages: Reduced number in rats after prolonged inhalation of Lead Sesquioxide. *Science* 162 (1968) 1297—1299.
9. BINGHAM, E. Trace amounts of lead in the lung. In: D. D. HEMPHILL (ed.), *Trace substances in environmental health III*. University of Missouri, Columbia, Mo. 1969, pp. 83—90.
10. BINGHAM, E., BARKLEY, W., MURTHY, R. and VASSALLO, C. Investigation of alveolar macrophages from rats exposed to coal dust. In: W. H. WALTON (ed.), *Inhaled particles IV*. Pergamon Press, Oxford 1977, pp. 543—548.
11. BINGHAM, E., BARKLEY, W., ZERWAS, M., STEMMER, K. and TAYLOR, P. Responses of alveolar macrophages to metals. *Arch. environ. health* 25 (1972) 406—414.
12. BRAIN, J. D. Free cells in the lungs. *Arch. intern. med.* 126 (1970) 477—487.
13. BREZINA, E. In: *Internationale Übersicht über Gewerbekrankheiten nach den Berichten der Gewerbeinspektionen der Kulturländer über das Jahr 1913* (vol. 8). J. Springer, Berlin 1921. 143 p. Cited by NAS (50).
14. BUBAREV, A. *Gig. bezopnnost patol. tr.* (1931): 6, 85.
15. BUTTNER, H. E. and LENZ, E. Über die Möglichkeit von Manganschäden in Braunschweigbergwerk. *Arch. Gewerbepath. Gewerbehyg.* 7 (1937) 672—684.
16. CAMNER, P., JOHANSSON, A. and LUNDBORG, M. Alveolar macrophages in rabbits exposed to nickel dust: Ultrastructural changes and phagocytose. *Environ. res.* (Accepted for publication).
17. CARAVAN, M. M., COBB, S. and DRINKER, C. K. Chronic manganese poisoning. *Arch. neurol. psychiatr.* 32 (1934) 501—512.
18. CAUVIN, F. *Maroc med.* 22 (1943) 16. Cited by RODIER (55).
19. COUPER, J. On the effects of black oxide of manganese when inhaled into the lungs. *Br. ann. med. pharm.* 1 (1837) 41.
20. DERVILLÉE, P., MORICHAUD-BEAUCHANT, G., CHARPENTIER, M.-J. and DERVILLÉE, E. A propos de la pneumonie manganique. *Arch. mal. prof. med. trav. secur. soc.* 27 (1966) 222—224.
21. EHRISHMANN, O. Untersuchungen über die Aufnahme von Staub durch die Atemwege. *Z. Hyg.* 117 (1935) 662—678.
22. ELSTAD, D. Manganholdig fabrikkrøk som medvirkende årsak ved pneumoni-epidemi i en industribygd. *Nor. mag. laegevidensk.* (1939) 2527—2533.
23. FERIN, J. The effect of particle lung content on clearance pathways. Presented at the 16th Annual Hanford Biology Symposium. Sept. 26—27, 1976, Richland, Washington.
24. FERIN, J. and LEACH, L. J. The effects of selected air pollutants on clearance of titanic oxide particles from the lungs of rats. In: W. H. WALTON (ed.), *Inhaled particles IV*. Pergamon Press, Oxford 1977, pp. 333—340.
25. FERIN, J., URBANKOVA, G. and VLCKOVA, A. Pulmonary clearance and the function of macrophages. *Arch. environ. health* 10 (1965) 790—795.
26. FISHER, M. V., MORROW, P. E. and YUILE, C. L. Effect of Freund's complete adjuvant upon clearance of iron -59 oxide from rat lungs. *J. reticuloendothel. soc.* 13 (1973) 536—556.
27. FREISE, F. W. Gesundheitsschädigungen durch Verarbeitung pflanzlicher Drogen. Beobachtungen aus brasilianischen Gewerbebetrieben. *Arch. Gewerbepath. Gewerbehyg.* 4 (1933) 381—399.
28. GALLEGGO, J. O. *Higiene y patologia del trabajo con manganeso* (Instituto Nacional

- de Prevision Publicatio 445). Instituto Nacional de Prevision, Madrid 1935.
29. GRAHAM, J. A., GARDNER, D. E., WATERS, M. D. and COFFIN, D. L. Effect of trace metals on phagocytosis by alveolar macrophages. *Infect. immun.* 11 (1975): 6, 1278—1283.
 30. GROSS, P., DE TREVILLE, R. T. P., TOLKER, E. B., KASCHAK, M. and BABYAK, M. A. The pulmonary macrophage response to irritants: An attempt at quantitation. *Arch. environ. health* 18 (1969) 174—185.
 31. GUNDEL, M. and FISCHER, H. Untersuchungen über die Aetiologie und Epidemiologie sowie zur Bekämpfung der Lungenentzündungen bei Arbeitern in Thomasschlackenmühlen. *Z. Hyg. Infektionskr.* 120 (1937) 66—86.
 32. HATCH, T. F. and GROSS, P. *Pulmonary deposition and retention of inhaled aerosols*. Academic Press, New York, N.Y. 1964. 192 p.
 33. HEINE, W. Beobachtungen und experimentelle Untersuchungen über Manganvergiftungen und "Manganpneumonien." *Z. Hyg.* 125 (1944) 1—76.
 34. HELLSTRÖM, P. Å. *An aerosol generator for experimental use in inhalation toxicology: Summary of the Swedish report. "Metalldamm för experimentell användning — Generering och fysikaliska data."* Department of Environmental Hygiene, The Karolinska Institute, Stockholm 1977. 22 p.
 35. HOLMA, B. Lung clearance of mono- and di-disperse aerosols determined by profile scanning and whole body counting. *Acta med. scand.* (1967): suppl. 473, 102 p.
 36. JÖTTEN, K. W., REPLOH, H. and HEGEMANN, G. Experimentelle untersuchungen über die Manganpneumonie und ihre Beziehungen zur Thomasschlackenpneumonie. *Arch. Gewerbepath. Gewerbehyg.* 9 (1939) 314—336.
 37. KJELLSTRÖM, T., LIND, B., LINNMAN, L. and NORDBERG, G. A comparative study on methods for cadmium analysis of grain with an application to pollution evaluation. *Environ. res.* 8 (1974) 92—106.
 38. KLOSTERKÖTTER, W. and BUNEMANN, G. Animal experiments on the elimination of inhaled dust. In: C. N. DAVIES (ed.), *Inhaled particles and vapours*. Pergamon Press, London 1961, pp. 327—337.
 39. LA BELLE, C. W. and BRIEGER, H. Synergistic effects of aerosols: II. Effects on rate of clearance from the lung. *Arch. ind. health* 20 (1959) 100—105.
 40. LA BELLE, C. W. and BRIEGER, H. Patterns and mechanisms in the elimination of dust from the lung. In: C. N. DAVIES (ed.), *Inhaled particles and vapours*. Pergamon Press, London 1961, pp. 356—365.
 41. LEVENA, E. N. and ROBACHEVSKY, E. G. Changes in lung tissue with intratracheal injection of manganese. *Gig. sanit.* (1955): 1, 25—28.
 42. LLOYD DAVIES, T. A. Manganese pneumonitis. *Br. j. ind. med.* 3 (1946) 111—135.
 43. LLOYD DAVIES, T. A. and HARDING, H. E. Manganese pneumonitis. *Br. j. ind. med.* 5 (1949) 82.
 44. LUNDBORG, M. and HOLMA, B. In vitro phagocytosis of fungal spores by rabbit lung macrophages. *Sabouraudia* 10 (1972) 152—156.
 45. MAIGRETTTER, R. Z., EHRLICH, R., FENTERS, J. D. and GARDNER, D. E. Potentiating effects of manganese dioxide on experimental respiratory infections. *Environ. res.* 11 (1976) 383—391.
 46. MORROW, P. E. Alveolar clearance of aerosols. *Arch. intern. med.* 131 (1973) 101—108.
 47. MORROW, P. E., GIBB, F. R. and GAZIOGLU, K. The clearance of dust from the lower respiratory tract of man: An experimental study. In: C. N. DAVIES (ed.), *Inhaled particles and vapours II*. Pergamon Press, London 1967, pp. 351—358.
 48. MORROW, P. E., GIBB, F. R. and JOHNSON, L. Clearance of insoluble dust from the lower respiratory tract. *Health phys.* 10 (1964) 543—555.
 49. MYRVIK, O. N., LEAKE, E. S. and FARRISS, B. Studies on pulmonary alveolar macrophages from the normal rabbit: A technique to procure them in a high state of purity. *J. immunol.* 86 (1961) 128—132.
 50. NAS, National Academy of Science. *Manganese review*. NAS Printing Office, Washington D.C. 1973. 191 p.
 51. PANCHERI, G. Industrial atmospheric pollution in Italy. In: *Problems and control of air pollution*. F. S. MALLETT (ed.), Reinhold, New York, N.Y. 1955, p. 263.
 52. PENALVER, R. Manganese poisoning: The 1954 Ramazzini oration. *Ind. med. surg.* 24 (1955) 1—7.
 53. PENALVER, R. Diagnosis and treatment of manganese intoxication. *Arch. ind. health* 16 (1957) 64—66.
 54. RIDDERVOLD, J. and HALVORSEN, K. Bacteriological investigations on pneumonia and pneumococcus carriers in Sauda an isolated industrial community in Norway. *Acta pathol. microbiol. scand.* 20 (1943) 272—298.
 55. RODIER, J. Manganese poisoning in Moroccan miners. *Br. j. ind. med.* 12 (1955) 21—35.
 56. RODIER, J. and RODIER, M. Le manganisme dans le mines marocaines. *Bul. inst. hyg. maroc* 9 (N.S.) (1949) 3—98.
 57. RYLANDER, R. Pulmonary defense mechanisms to airborne bacteria. *Acta physiol. scand.* (1968): suppl. 306, 89 p.
 58. RYLANDER, R. Free lung cell studies — Cigarette smoke inhalation experiments. *Scand. j. resp. dis.* 52 (1971) 121—128.
 59. RYLANDER, R. Influence of infection on pulmonary defense mechanisms. *Ann. n.y. acad. sci.* 221 (1974) 282—289.
 60. RYLANDER, R. Pulmonary cell responses to inhaled cigarette smoke. *Arch. environ. health* 29 (1974) 329—333.
 61. RYLANDER, R. and BERGSTRÖM, R. Particles and SO₂ — Synergistic effects for pulmonary damage. In: *Proceedings of the 3rd international clean air congress*,

- Düsseldorf, 1973. VOI Verlag, Düsseldorf 1973, pp. 423—425.
62. RYLANDER, R., NORDSTRAND, A. and SNELLA, M.-C. Bacterial contamination of organic dusts. *Arch. environ. health* 30 (1975) 137—140.
 63. RYLANDER, R., ÖHRSTRÖM, M., HELLSTRÖM, P. Å. and BERGSTRÖM, R. SO₂ and particles — Synergistic effects on guinea-pigs' lungs. In: W. H. WALTON (ed.), *Inhaled particles III*. Unwin Brothers Ltd., London 1971, pp. 535—541.
 64. SCHOPPER, W. Über Lungenentzündungen bei Brauneisensteinbergarbeitern. *Arch. Hyg. Bakteriol.* 104 (1930) 175—183.
 65. SCHORLEMMER, H. U. and ALLISON, A. C. Effects of activated complement components on enzyme secretion by macrophages. *Immunology* 31 (1976) 781—788.
 66. SHANKER, R., DOGRA, R. K. S., SAHU, A. P. and ZAIDI, S. H. Experimental manganese lymphadenopathy in guinea-pigs. *Arch. toxicol.* 36 (1976) 151—157.
 67. STOCKS, P. The relations between atmospheric pollution in urban and rural localities and mortality from cancer, bronchitis and pneumonia, with particular reference to 3,4 benzopyrene, beryllium, molybdenum, vanadium and arsenic. *Br. j. cancer* 14 (1960): 3, 397—418.
 68. STOKINGER, H. E. Manganese. In: F. A. PATTY (ed.), *Industrial hygiene and toxicology* (vol II). Interscience Publications, New York, N.Y. 1962, pp. 1079—1089.
 69. TANAKA, S. and LIEBEN, J. Manganese poisoning and exposure in Pennsylvania. *Arch. environ. health* 19 (1969) 674—684.
 70. TASK GROUP ON LUNG DYNAMICS. Deposition and retention models for internal dosimetry of the human respiratory tract. *Health phys.* 12 (1966) 173—207.
 71. THIS-EVENSEN, E. Cited by BAADER (2) and ELSTAD (22).
 72. VAN BEUKERING, J. A. Incidence of pneumonia among miners in an iron mine and a manganese mine in South Africa. *Ned. tijdschr. geneesk.* 110 (1966) 473—474.
 73. VIGLIANI, E. Esiste una polmonite de manganese? *Folia med.* (Naples) 23 (1937) 451—458.
 74. WASSERMANN, M. and MIHAIL, G. Recherches concernant la pathologie professionnelle des mineurs des mines de manganese. *Arch. Gewerbepath. Gewerbehyg.* 18 (1961) 632—657.
 75. WATERS, M. D., GARDNER, D. E., ARANYI, C. and COFFIN, D. L. Metal toxicity for rabbit alveolar macrophages in vitro. *Environ. res.* 9 (1975) 32—47.
 76. WRIGHT, B. M. A new dust-feed mechanism. *J. sci. instrum.* 27 (1950) 12—15.
 77. ZAIDI, S. H., DOGRA, R. K. S., SHANKER, R. and CHANDRA, S. V. Experimental infective manganese pneumoconiosis in guinea-pigs. *Environ. res.* 6 (1973) 287—297.

Received for publication: 1977-09-09

Appendix

Table A—1. Number of macrophages (mac.) at different times after manganese dioxide exposure. TRE values represent means from 10 animals at each time. (MnO₂ = manganese dioxide exposure only; E₁ + M = *Enterobacter* 1 day prior to MnO₂; E₀ + M = *Enterobacter* immediately before MnO₂; M + E₀ = *Enterobacter* immediately after MnO₂; TRE = time-related number of macrophages for animals exposed to *Enterobacter* only; N = number of animals; figures in parentheses = standard deviation)

Days after MnO ₂ exposure	MnO ₂		E ₁ + M			E ₀ + M			M + E ₀		
	N	No. of mac.	N	No. of mac.	TRE	N	No. of mac.	TRE	N	No. of mac.	TRE
Controls	59	115 (62)	13	109 (49)	114 (78)	5	183 (60)	114 (78)	22	113 (64)	114 (78)
0	23	46 (31)	9	231 (114)	133 (54)	5	232 (112)	141 (123)	6	39 (41)	
1	19	127 (70)	9	172 (90)	208 (133)	5	608 (293)	133 (54)	11	372 (194)	141 (123)
2	18	114 (62)	6	219 (23)	135 (52)	5	579 (195)	208 (133)	12	481 (184)	133 (54)
3	26	172 (94)	9	120 (65)		4	265 (61)	135 (52)	12	400 (115)	208 (133)
7	13	180 (63)	9	167 (73)		4	206 (52)		6	272 (89)	

Table A—2. Number of leukocytes (leuk.) at different times after MnO₂ exposure. TRE values represent means from 10 animals at each time. (MnO₂ = manganese dioxide exposure only; E₁ + M = *Enterobacter* 1 day prior to MnO₂; E₀ + M = *Enterobacter* immediately before MnO₂; M + E₀ = *Enterobacter* immediately after MnO₂; TRE = time-related number of leukocytes for animals exposed to *Enterobacter* only; N = number of animals; figures in parentheses = standard deviation)

Days after MnO ₂ exposure	MnO ₂		E ₁ + M			E ₀ + M			M + E ₀		
	N	No. of leuk.	N	No. of leuk.	TRE	N	No. of leuk.	TRE	N	No. of leuk.	TRE
Controls	59	74 (50)	13	53 (28)	108 (62)	5	126 (65)	108 (62)	22	67 (45)	108 (62)
0	23	63 (38)	9	310 (134)	371 (226)	5	452 (169)	507 (283)	6	71 (66)	
1	19	124 (79)	9	227 (148)	330 (190)	5	1325 (321)	371 (226)	11	1165 (509)	507 (283)
2	18	98 (56)	6	230 (146)	184 (27)	5	662 (214)	330 (190)	12	1260 (574)	371 (226)
3	26	151 (94)	9	119 (50)		4	244 (82)	184 (27)	12	600 (232)	330 (190)
7	13	93 (37)	9	88 (69)		4	107 (26)		6	269 (57)	