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Effects of exposure to welding fume: an experimental study in sheep

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Effects of exposure to welding fume: an experimental study in sheep. P-E. Näslund, S. Andreasson, R. Bergström, L. Smith, B. Risberg.
ABSTRACT: Welding fume contains various metals and pulmonary effects from their inhalation are largely unknown. We have studied the effects of exposure to welding fume in sheep. The animals were exposed to either a bolus dose of welding fume solution or to five weeks daily exposure. Lung physiology parameters were studied and biopsies taken. Magnetopneumography was used to register the longterm exposure. Acutely exposed animals had elevated pulmonary arterial pressure. Arterial oxygen tension was reduced after fume instillation. These animals had accumulation of iron (Fe), magnesium (Mg) and manganese (Mn) in the lungs. Mn was elevated 40 times. Longterm exposed sheep increased the iron oxide accumulation significantly in lungs as seen with the magnetopneumographic technique. Following long term exposure, Mn was the metal most heavily retained in the lungs. Metals like Mn, Fe and Mg retained in the lungs can possibly give negative health effects. Besides this, the metals could be used for quantitation of welding fume exposure.
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Epidemiological studies of manual metal arc welders have demonstrated that welders frequently accumulate iron oxide in the lungs following exposure to welding fume. There is a correlation between exposure time and accumulation of iron oxide [1–4]. Pulmonary problems, *eg.* symptoms of chronic phlegm production, are more frequent in welders with long exposure [4, 5].

Table 1. – Percentage of metals in fume from welding electrode ESAB OK 48.00 in black iron

Substance	%
Iron	14.10
Manganese	4.00
Titanium	0.56
Magnesium	0.17
Nickel	<0.005
Chromium	<0.005
Zinc	0.02
Copper	0.02
Aluminium	0.50

The welding fume is a mixture of various gases and solid particles (mainly metal oxides) (table 1), depending on the kind of electrode used and the welded material. Following inhalation the fates of the metals are largely unknown, except that of iron oxide which accumulates in the lung tissue. Retention of particles and metals in the lung could lead to reactions in the interstitium. Particles cleared from the lung by lymph or blood circulation could have remote effects in other organs. To study these aspects of inhalation of welding fume, experimental studies in animals are needed.

It was the aim of the present study in sheep to clarify how the lung handles various metals in the welding fume and if welding fume may damage the lung.

Material and methods

Animals

Twenty sheep with a mean weight of 33 kg (range 16–60 kg) were used in the present experiments. The animals were divided into 2 groups; 7 sheep were acutely exposed to welding fume; 13 were longterm exposed to welding fume. Two animals in the first and 4 in the second group were controls.

Acutely exposed group. In these animals a lung lymph fistula was prepared as described by Staub *et al.* [6]. The animals were anaesthetized with Ketamine (500 mg *im.*) and Thiopental sodium (20 mg·kg^{-1} bw *iv.*), intubated and ventilated with air in an Engström respirator. Anaesthesia was maintained during surgery by continuous infusion of Ketamine. A continuous positive

end expiratory pressure (PEEP) of 5 cmH_2O was applied. Briefly, the caudal mediastinal lymph node was ligated below the inferior pulmonary ligament through a right sided thoracotomy in the ninth intercostal space. All lymph contributaries to the node from diaphragm and thoracic wall were severed. Through another right sided thoracotomy in the fifth intercostal space the efferent lymph duct from the lymph node was cannulated with a heparin glutaraldehyde treated silastic catheter (OD 1.19). After surgery the animals recuperated for 3–4 days before the experiment.

On the day of experiment there was a flow of clear lung lymph in 4 of the 7 sheep. Three catheters had clotted. During a short Ketamine anaesthesia (500 mg *im.*) catheters were placed in the superior caval vein and a thermistor equipped artery catheter was put into the aorta through the carotid artery for measurement of the extravascular lung water (EVLW) and cardiac output by thermal dye dilution technique, using Edward's lung water computer 9310 (Edwards Laboratory, Santa Ana, CA). A thermistor tipped balloon catheter was floated into the pulmonary artery. Lymph flow (Q_L) was measured in 15 min intervals and samples for protein concentration in plasma and lymph were taken at 30 min intervals. The total protein concentration ratio in lymph and plasma (L/P) was calculated. Leucocyte and platelet count, haemoglobin (Hb) and haematocrit (Hct) were analyzed using standard methods. Partial pressure of oxygen (Po_2), carbon dioxide (Pco_2) and pH were measured using an automized blood gas analyser. During the experiment the animals were studied awake, standing in a cage with free access to water. After a baseline period of 1 h all animals were anaesthetized with Ketamine (100 mg *iv.*). During anaesthesia a rigid bronchoscope was inserted into the trachea, a suspension of welding fume (see below) was instilled into the right main bronchus. Following exposure the animals were monitored for 4 h. Two animals served as controls and were given 50 ml of normal saline in the bronchial tree as above. These 2 animals were followed for 1 h.

Samples from plasma and lymph were taken hourly for atomic absorption spectrometric measurement of metal content. Finally, biopsies from all lungs were taken for atomic absorption spectrometric analysis.

Longterm exposed group. The longterm exposed group consisted of 13 sheep. Nine of these were chosen for exposure to welding fume. The remaining four sheep served as controls. One of the nine animals was excluded because of foreign magnetic material in the thorax detected by measurement before exposure. Two of the exposed animals died after 4 days exposure to 64±9.8 $mg \cdot m^{-3}$, the highest value of all the animals. The degree of exposure was estimated by magnetopneumography (see below).

All animals were tracheotomized in Ketalar anaesthesia with insertion of a cuffed tracheostomy tube with an inner exchangeable cannula (Shiley). The inner cannula was changed twice daily. The sheep were exposed to welding fume for 3 h, 5 days per week to simulate a normal exposure to welding fume for a welder. This was carried out for 5 wks. Following the 5 wk exposure period the animals were subjected to surgery with insertion of a chronic lung lymph fistula as described above. This was successfully performed in two animals. These two animals were then exposed to welding fume for another week.

During the first 5 wk period venous blood samples were taken weekly for analysis of metal composition. All animals were investigated in the magnetopneumograph (MPG - see below) to study accumulation of iron oxide in the lungs. Six of the longterm exposed sheep were MPG-measured with 1–2 wks interval. Finally biopsies (5 experimental and 2 control sheep) were taken from lung, liver, kidney and ribs for atomic absorption spectrometric analysis and for histology.

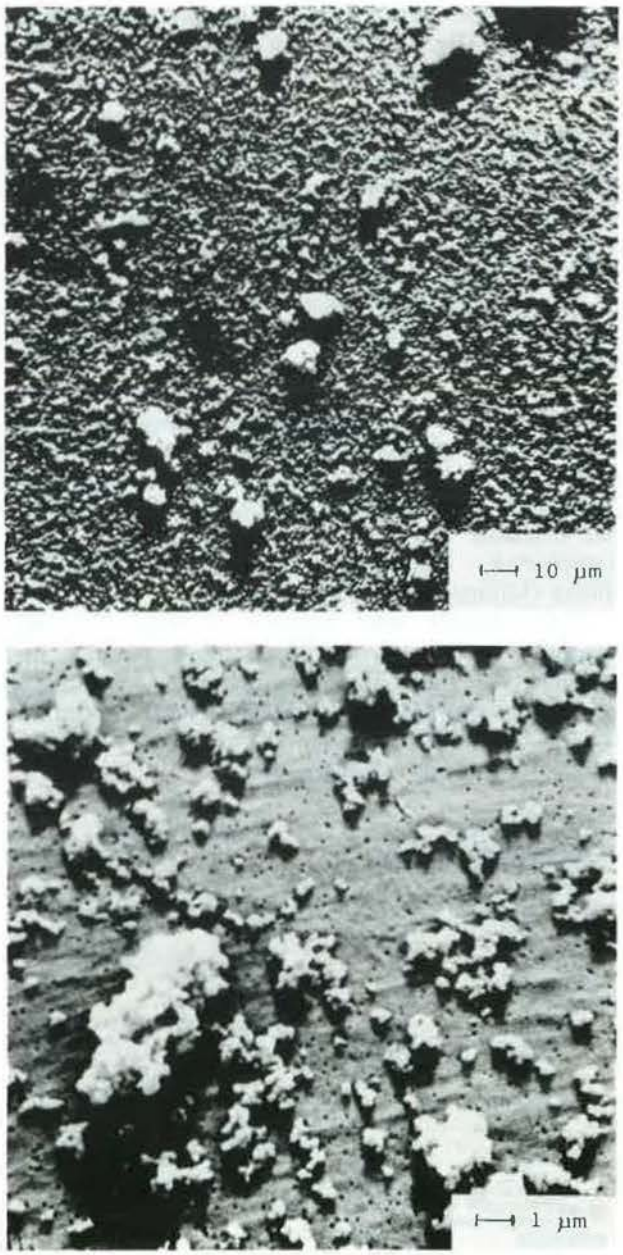

Fig. 1. – Welding fume particles. Scanning electron microscopy. ×500 and ×1000.

Table 2. – Results of analysis of welding fume (OK 48.00) with scanning electron microscopy

Particle size µm	Amount %	Mean diameter µm	Weight %
<1	92.3	0.5	4
1–5	7.5	2.5	40
>5	0.2	10	56

Generation of welding fume

Fume was produced by manual metal arc welding in black iron using an ESAB electrode OK 48.00 (ESAB, Göteborg, Sweden) to an amount of dust of about 2 g·min^{-1}. The particles tend to conglomerate and thus vary in size (fig. 1). The mean diameter of the particles was <1.0 µm (table 2).

Acute exposure. The welding fume particles were collected on a filter paper (Munktell filter OOR, diameter 240 mm). The sheep were given 0.5 g of fume suspended in 50 ml of saline. This solution was installed in the right main bronchus by a rigid bronchoscope. The fume amount of 0.5 g was calculated from a welding fume exposure of 5–10 mg·m^{-3} for three effective working hours. This was supposed to be an average welders exposure to welding fume.

Longterm exposure. The animals were exposed to welding fume through a mixing chamber in to which welding fume was sucked from a bench, where welding was performed. Figure 2 shows the chamber. The volume of the chamber was 0.74 m^3. The fume was collected continuously. Sampling was done with preweighed filters (Millipore with 0.8 µm pores).

Eight animals were exposed to fume 3 h daily (monday–friday) during 25–33 days through their tracheal tubes (table 3). The welding fume was manually generated every 6th minute for 20 s. The electrodes were ESAB OK 48.00 of 4 mm diameter and the welded material was plates of black iron.

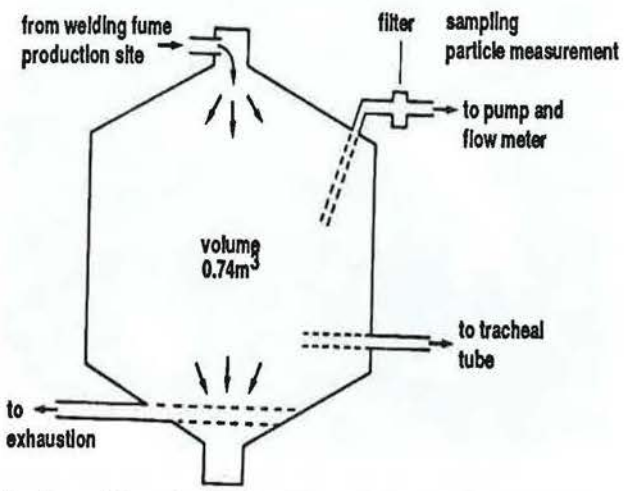

Fig. 2. – Schematic drawing of the mixing chamber.

Table 3. – Days of exposure, exposure time per day and exposure concentration per day for 6 animals exposed to welding fume for 5 weeks or more

Animal no.	Days of exposure	Exposure time per day-min	Exposure concentration per day-mg·m^{-3}
1	29	176±23	47.5±22.6
2	25	176±20.9	51.9±20.9
4	25	174±30	33.1±17.9
6	33	176±26	31.1±14.9
9	25	181±3	32.1±12.4
10	25	181±3	33.9±13.2

The particle size was determined by an optical particle counter (Royco model 225) and, in combination with the sampling procedure in the chamber, with a particle meter (Sibata P5) at concentrations of 2.4, 18.8, 20.5 and 37.4 mg·m^{-3}. Particle measurement was also performed from the tracheal tube. A sample of the fume was analysed with scanning electron microscopy.

Atomic absorption spectrometry

Blood and lymph were diluted 1:10 with distilled water and analysed. The concentrations of iron (Fe), magnesium (Mg), zinc (Zn) and copper (Cu) were determined by flame atomic absorption spectrometry. Manganese (Mn), titanium (Ti), chromium (Cr), nickel (Ni) and aluminium (Al) were determined by flameless atomic absorption spectrometry. Standard addition was used throughout the analysis.

Biopsies from various organs containing 1 g of tissue were dissolved in 5 ml of concentrated HNO_3 and 2 ml of concentrated H_2SO_4 and diluted to 50 ml. The metal content was measured with the same technique as above. The instrument used was a PU-9000 atomic absorption spectrometer (Pye Unicam, Philips).

Magnetopneumography

This technique measures the amount of magnetizable particles, in this case magnetite (Fe_3O_4), with an accuracy of less than 0.5 mg magnetite. The magnetic moment was measured in µA·m^2 (microampere × square metre). The technique involves a brief application of a strong magnetic field across the chest to align the dipoles within the magnetizable particles (magnetization) and to rotate the magnetized particles into a common alignment. The remanent field, which can be measured with a sensitive external magnetometer, decays as the magnetic particles becomes randomly orientated. The strength of the remanent field at the time the magnetizing field is turned off can be related to the amount of magnetic dust in the field of view of the magnetometer, provided that the spatial distribution of the dipoles in relation to the detector and the relation between magnetic moment and dust mass are known. The magnetic moment was calculated by a computer connected to the measuring device [7–9].

Biochemical analysis

The total protein concentration was analysed in lymph and plasma using the Biuret method and the lymph to plasma ratio for total protein (L/P) was calculated.

Histological examination

Biopsies of lungs from one unexposed, one acutely and two longterm exposed sheep were taken for routine histology. The sections were stained with haematoxylin-eosin. Some sections were stained for iron oxide with prussian blue.

Statistics

Data are presented as means±SEM. Significance calculations were made using Student's t-test.

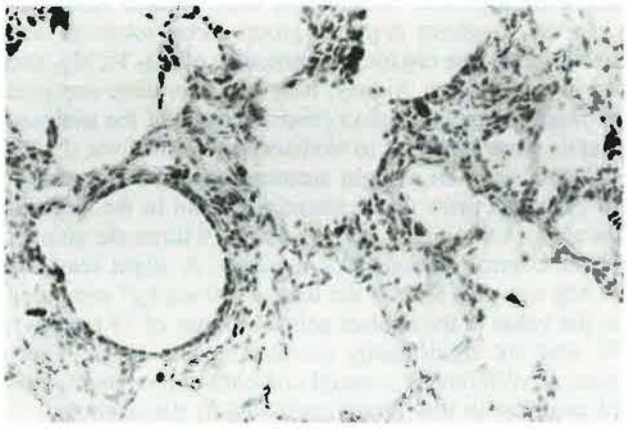

Fig. 3. – Section of lung biopsy from a sheep exposed to an instillation of suspended welding fume particles. Iron particles are seen in the bronchi and to lesser extent in the alveoli. Prussian blue staining.

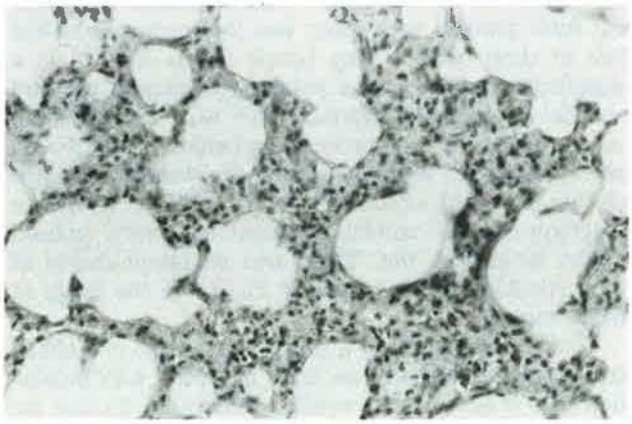

Fig. 4. – Lung biopsy from longterm exposed sheep. The picture is characterized by a fibrosing pneumonitis with heavy infiltration of fibroblasts. Emphysema is seen.

Results

Histology

The lungs from unexposed sheep had a normal morphology with well preserved interstitial structures. In one acutely exposed animal, iron particles were confined in the bronchi but to a lesser extent in the alveoli. No iron was found in the interstitium (fig. 3). The two longterm exposed animals investigated had a histological picture of fibrosing pneumonitis with iron particles demonstrable in the interstitium. Only a few inflammatory cells were seen and the picture was dominated by fibroblasts. A slight emphysema was found (fig. 4).

Haemodynamic, respiratory and lymph data

In the acutely exposed group the circulatory parameters remained stable during the baseline period. Mean aortic ($\bar{P}sa$) and mean pulmonary ($\bar{P}pa$) arterial pressure were 96±7 and 17±1 mmHg, respectively. Cardiac output was 132±19 $ml \cdot min^{-1} \cdot kg^{-1}$ bw and EVLW 8.2±0.9 $ml \cdot kg^{-1}$ bw. Q_L was 1.8±0.3 ml·30 min^{-1} and L/P 0.75±0.05 in the 4 sheep with a functioning lymph catheter. The number of leucocytes and platelets were 4.4±0.6 and $653 \pm 48 \times 10^9 \cdot l^{-1}$, respectively. Hct was 26±2% during baseline. Po_2 and Pco_2 were within normal limits.

After instillation of the welding fume suspension $\bar{P}pa$ increased and was 26±2 mmHg after 30 min, which was significantly increased compared to baseline ($p<0.05$). The pressure then successively decreased but was elevated throughout the experiment compared to baseline. $\bar{P}sa$ did not change during the experiment. Cardiac output and EVLW did not change during the first hour and remained stable in the 4 animals where they were followed for 3 h (table 3). There were no significant changes in Q_L and L/P. The number of leucocytes successively increased and was $7.1 \pm 1.2 \times 10^9 \cdot l^{-1}$ ($p<0.05$) at the end of the experiment whereas the number of platelets remained unchanged. Hct increased successively and

Table 4. – Percentage amount of particles of different sizes in exposure chamber at different times after generation of welding fume

Min after welding	1.0	2.5	4.0	6.0
n	20	19	19	18
Dg >0.5 µm	100	100	100	100
Dg >1.5 µm	68	24	16	23
Dg >2.0 µm	46	5.2	5.9	9.6
Dg >3.0 µm	20	0.8	2.0	3.2
Dg >5.0 µm	0.7	0.2	0.6	0.9

The aerosol concentration was 20.5 $mg \cdot m^{-3}$. Dg: geometric diameter according to Royco measuring device; n: number of observations.

was 29±2% ($p<0.05$) after 4 h. Po_2 decreased ($p<0.05$) after fume instillation to the lowest value, 7.3±1 kPa after 60 min and Po_2 remained low throughout the experiment. Pco_2 increased ($p<0.05$) during the first 30 min but then successively decreased and was no longer different from baseline between 60–180 min, but was decreased compared to baseline ($p<0.05$) after 240 min. There were no changes in $\bar{P}sa$, $\bar{P}pa$, Q_L or L/P after instillation of normal saline into the bronchi.

The longterm exposed sheep were not monitored with haemodynamic or respiratory parameters. Lymph flow and protein composition were not altered in the two sheep that were exposed for 1 wk after surgery.

Exposure

The mean fume concentration in the mixing chamber for each animal in the longterm exposed group is shown in table 3.

Analysis of the particle amount and size in the exposure chamber and in the tracheal tube were similar. The amount of welding fume particles up to 6 min after fume generation is demonstrated in table 4. At the particle size measurement the agglomerations were measured as individual particles.

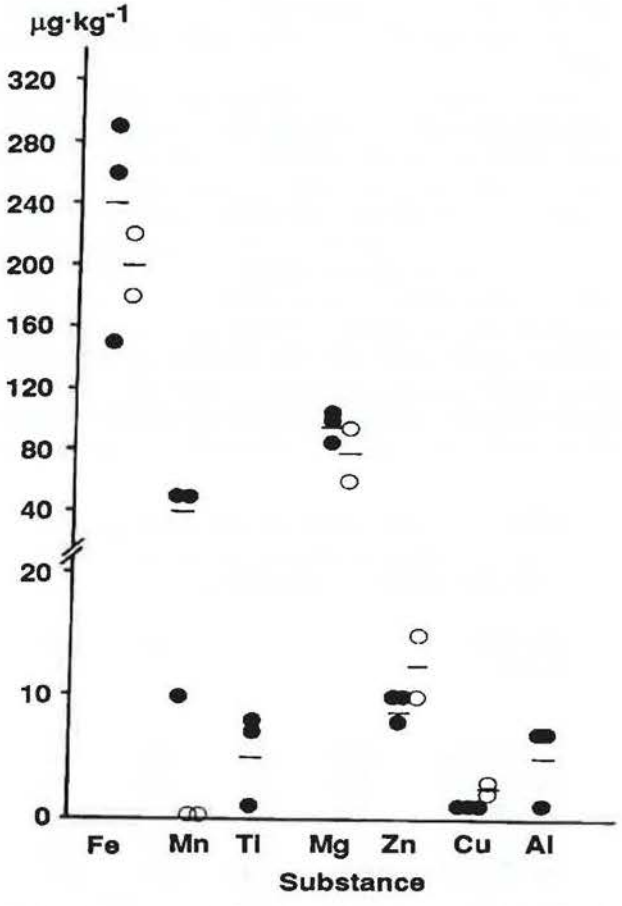

Fig. 5. – Metal content in lung tissue in acutely exposed animals and controls. ●: exposed; ○: controls. Mean value is denoted. Ti and Al were not registered in the control animals.

Magnetopneumography

Animals in the acute exposure group were not investigated with this technique. All sheep in the longterm exposed group were measured before exposure. This group were then measured several times during the exposure period. The initial control mean value was 0.8 $\mu A \cdot m^2$ (range 0.0–1.8). After 25 days of exposure the mean value was 3.4 $\mu A \cdot m^2$ (range 2.7–3.7).

Atomic absorption spectrometry

In the acutely exposed animals, lung tissue from three animals was analysed for metal content. The analysis was too expensive to allow analysis of lung tissue from all acutely exposed sheep. A slight accumulation of Fe and Mg was seen. Mn was increased to approximately 40 times the level of unexposed animals. Ti and Al were increased in exposed lungs but the Zn level was not elevated (fig. 5). Zn, Cu, Mn and Mg were analysed in blood and lymph. Fe was analysed only in lymph. All of these metals were elevated in lymph during the 4 h registration period. No changes were seen in blood.

In the longterm exposed group metal retention was found in various organs. The amounts of Cu, Ti, Mg, and Zn in the skeleton, kidney, lung and liver were analysed in exposed animals and in controls. None of the analysed metals were elevated in skeleton or in the liver. In the kidney there was a slight accumulation of Mn and Mg. In the lungs there was a retention of Mn in the exposed animals, (1.8 $mg \cdot kg^{-1}$), approximately 9 times the amount in the control animals (0.2 $mg \cdot kg^{-1}$). A slight retention of Mg was also seen in the lungs (110 $mg \cdot kg^{-1}$ compared to the value in the control animals' lungs of 75 $mg \cdot kg^{-1}$). Fe was not significantly elevated in any organ. There were no differences in metal content in blood and lymph (2 animals) in this group compared to the controls.

Discussion

In the present study, we exposed sheep either acutely or longterm to welding fume. Acute instillation of a welding fume particle suspension into the tracheo-bronchial tree in sheep with a lung lymph fistula resulted in a significant increase in the pulmonary vascular pressure and development of hypoxia. This was not seen after saline instillation. A pronounced retention of Mn, Fe, Ti and Al was seen in the lungs and Mn was also retained despite increased interstitial clearance by the lymph. In longterm exposed animals the dominant metal retained in the lungs was Mn. There was an accumulation of magnetizable particles (mainly Fe_3O_4) in the lungs of these animals.

To evaluate effects of a single heavy dose of welding fume particles we used an acute approach with instillation of a bolus dose of welding fume particles into the bronchial tree. The dose was chosen to correspond to a possible 1 day exposure for a metal arc welder. With this approach, only the solid particles in the welding fume

were instilled. The various gases in the welding fume were thus omitted. It is, however, generally considered that the metal particles (Fe) are responsible for X-ray changes developing in subjects exposed to welding fume [1–4, 10]. How the lung handles the other metal components in welding fume is not known. This technique enabled us to study interstitial clearance of welding fume particles in sheep with a lung lymph fistula.

Acute exposure in this way may be deleterious to lung function with elevation of pulmonary arterial pressure and hypoxia. There were no changes in lung water so, apparently, the elevated pulmonary pressure did not produce any detectable oedema. This was further supported by unchanged lymph flow and L/P ratio for total proteins. The atomic absorption analysis showed that pulmonary deposition of Fe was less pronounced probably because of the large burden of body iron. Mn seemed to be the metal proportionally most heavily deposited in the lungs. The high levels of Mn in lymph could reflect this.

Particles are deposited and cleared in the lung depending on size. Particles of respirable size are more easily deposited in the alveoli. Large particles are retained in the major airways and mechanically cleared by the mucus escalator [8]. The welding fume contains mainly small particles (99.8% <2.5 μm). The data indicated that most metals were cleared rapidly from the lung except for Fe, Ti, Al and Mn. Analysis of metals other than Fe in the tissues could thus be of interest when evaluating exposure to welding fume, and could have some merits over Fe for this purpose. The large body burden of iron makes it difficult to recognize relatively small changes of the total Fe level.

The longterm exposure model does bear more resemblance to the situation of the welder. One problem with this approach is the unpredictability of the individual exposed dose. The nasal cavity of the sheep has a high capacity of clearing particles. In the present study we avoided this by having the animals tracheotomized. The dose of exposure was thus easier to define. The irritation caused by the tracheostoma with heavy mucous production could, however, be one source of error. We did not monitor haemodynamics or respiratory parameters in the longterm exposed animals. Thus, it could not be evaluated if chronic exposure had any deleterious effects on these functions. Longterm exposure to welding fume has, in man, given symptoms of simple chronic bronchitis. If lung fibrosis, renal damage and possibly other diseases can be accredited is under discussion [11–14].

The most pronounced metal retention of the various organs studied was found in the lungs. In the longterm exposed animals Fe, Mn, Mg and Zn were retained to a higher degree than in the control animals. The differences were not statistically significant. Following deposition, the metals were transported into the interstitium and further, by lymph, into the blood and distributed to various organs. If this represented a selective retention in these organs cannot be determined. The exposure might have been too short to give a homogenous distribution in different organs, as in the acutely exposed animals Mn was highly retained in the lungs. The amount of Fe measured with atomic absorption spectrometry fluctuated more than the other metals in the different organs. This was true especially for the organs with high blood content (lung, liver and kidney). The endogenous Fe seemed to dominate so much that a relatively small elevation of exogenous Fe only had a minor additive effect. The amount of exogenous Fe, measured with the MPG-technique, increased about 4 times in the lungs after 5 wks exposure to welding fume. After approximately 10–15 days the level of magnetizable Fe reached a plateau. The level was about 10 times lower than that seen in metal arc welders after several years of exposure. The histological sections from the longterm exposed sheep demonstrated a fibrosing pneumonitis as a response to the inhalation of a chronic irritant. Such chronic injury with fibroblast infiltration could herald pulmonary fibrosis. A slight emphysema was found. This picture is similar to the picture found in rats by LIEMITSU *et al.* [15].

Can these findings be translated to human conditions? In diseased welders we have found high levels of metals in different organs (P-E. Näslund, unpublished data). In lungs, livers and kidneys, iron-oxide was found 3–40 times more than in a control subject. Mn was found in lungs and Cr and Ni in the kidneys. Zn was accumulated in the liver of the diseased welders. In the lungs of these welders there was also signs of an early emphysematous reaction. These human findings fit in very well within the results of the sheep study and we thus believe that it is possible to compare the findings in the sheep with human conditions.

In conclusion this study demonstrated a high retention of Mn in the lungs and of other metals in different organs in the body which might be related to diseases in these organs after exposure to welding fume.

We have investigated sheep exposed to either a bolus dose of welding fume particles or to a five week welding fume exposure. This seems to be the first investigation where retention in different organs of several metals from welding fume have been studied.

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Effets de l'exposition aux fumées de soudure. Une étude expérimentale chez le mouton. P-E Näslund, S. Andreasson, R. Bergström, L. Smith, B. Risberg.

RÉSUMÉ: Les fumées de soudure contiennent divers métaux, et les effets pulmonaires de leur inhalation sont encore mal connus. Vingt moutons ont été exposés aux fumées de soudure, Un groupe a été exposé de façon aiguë à l'instillation bronchique d'une suspension de fumée de soudure. Chez ces animaux, l'on a provoqué une fistule lymphatique pulmonaire. Après instillation aiguë de fumée de soudure, l'hémodynamique, l'eau pulmonaire, les décomptes cellulaires, les gaz du sang, et le débit lymphatique et sa composition, ont été siuvis. Les animaux ont été observés pendant 4 heures. L'analyse spectrométrique d'absorption atomique a été réalisée sur le plasma, la lymphe et les biopsies pulmonaires. Le second groupe de moutons a inhalé des fumées de soudure pendant 5 à 6 semaines. Ces animaux ont été soumis à une analyse magnétopneumographique externe de l'accumulation d'oxyde de fer dans les poumons. A la fin de l'expérience, des biopsies des organes ont été prélevées pour analyse spectrométrique d'absorption atomique et pour examen histologique. Les animaux soumis à une exposition aiguë avaient une pression artérielle pulmonaire augmentée. L'eau extra-vasculaire pulmonaire n'est pas modifiée. Il n'y a eu aucune modification du débit lymphatique ni du rapport des protéines lymphatiques/protéines plasmatiques pour les protéines totales. La pression artérielle d'oxygène est réduite après instillation de fumée, Ces animaux ont une accumulation de fer et de magnésium dans les poumons. Mn est augmenté 40 fois. Ces métaux font l'objet d'une clearance par la lymphe pulmonaire. Les moutons exposés à long terme développment une accumulation significative d'oxyde de fer dans les poumons, démontrée par la technique magnétopneumographique. Après exposition à long terme, Mn est le métal dont la rétention pulmonaire est la plus importante. Le poumon a la possibilité de réaliser une clearance rapide de différents métaux provenant de la fumée de soudure et présents dans les tissus pulmonaires. Certains métaux, comme Fe, Mn et Mg, sont toutefois retenus dans les poumons et pourraient donc y entraîner des effets défavourables pour la santé. Ces métaux pourraient également être utilisés pour quantifier l'exposition aux fumées de soudure.
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