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Lipid peroxidation in workers exposed to manganese

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Objectives The following hypothesis was tested: plasma manganese concentration is associated positively with the product of lipid peroxidation, and lipid peroxidation is associated negatively with the activities of antioxidants in workers exposed to manganese.

Methods The plasma manganese concentration of 22 manganese-exposed workers and 45 referents was determined by graphite furnace atomic absorption spectrophotometry. Malondialdehyde, the product of lipid peroxidation, was determined by high-performance liquid chromatography, and the activities of protective enzymes were measured by ultraviolet-visible spectrophotometry.

Results The activities of superoxide dismutase, glutathione peroxidase, and catalase spread widely among the referents. The activity of superoxide dismutase and the concentrations of malondialdehyde and manganese were significantly higher in the manganese workers than in the referents. The concentration of malondialdehyde in the exposed workers was correlated strongly with the manganese level of plasma.

Conclusions Malondialdehyde can be used as an index of lipid peroxidation induced by manganese exposure.

Key terms occupational health.

Manganese is the most ubiquitous transition metal after iron and tin (1). It is an essential nutrient for humans and animals (2, 3). In addition, manganese is a co-factor for several enzymatic reactions, particularly those for pyruvate kinase, mitochondrial superoxide dismutase, glycosyl transferase, and fatty acid synthesis (4). Manganese and its compounds are used in making steel alloys, dry-cell batteries, electrical coils, welding rods, and so on (4). The neurotoxic effects of manganese are well known and are usually caused by high occupational exposure over long periods of time.

The peroxidation (autooxidation) of lipids exposed to oxygen is not only responsible for the deterioration of foods, but also for damage to tissues in vivo. The deleterious effects are initiated by free radicals produced during peroxide formation from polyunsaturated fatty acid (5). It has been implicated in diverse pathological conditions, including atherosclerosis (6), aging (7), rheumatoid arthritis (8), diabetes mellitus (9), and cancer (10, 11) and also in toxicity induced by certain metals, solvents and drugs (12—17). Numerous reports have shown that divalent metal ions are a contributing factor in the acute toxicity of peroxides in laboratory animals (18). However, the exact mechanism in humans is not known.

In advanced organisms, superoxide dismutase (SOD) is used to accelerate the dismutation of the toxic superoxide radical (O_2^-), produced during oxidative energy processes, to hydrogen peroxide (H_2O_2) and molecular oxygen. Glutathione peroxidase (GPX) is used to protect the membrane lipids from oxidative damage (19). Catalase (CAT), located in organelles, acts as a regulator of H_2O_2 levels and as a specific peroxidase. The lower the catalase activity of erythrocytes, the more effective the action of oxidizing agents (eg, H_2O_2 or X rays).

In the field of occupational hygiene and environmental health, the relationships between lipid peroxide, enzymatic antioxidants, and occupational exposure to manganese have never been reported. In our study, we measured lipid peroxidation in workers exposed to manganese. In as much as malondialdehyde is a stable product of lipid peroxidation (20, 21), we therefore measured malondialdehyde in the blood of a group of workers exposed to manganese and determined the activities of the antioxidants SOD, GPX, CAT as indices of lipid

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Table 1. General characteristics of the exposed workers and referents.

Group	Age (years)		Duration of work (years)		Number of smokers[a]		Number of alcohol consumers[b]		Manganese blood level (μg · l⁻¹)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Exposed workers (N = 22) (manganese smelter)	52	7.0	28	9	15	..	19	..	1.51	0.65
Referents (N = 45) (administrators or unexposed workers)	48	10	25	8	23	..	21	..	0.83	0.20

[a] Smoker: ≥ 1 cigarette equivalent a day.
[b] Alcohol consumer: ≥ 0.1 ounces a day (41).

peroxidation and reactive oxygen metabolites. The purpose of our study was to examine the hypothesis that plasma manganese concentration is associated positively with the product of lipid peroxidation and that lipid peroxidation is associated negatively with the activities of antioxidants in workers exposed to manganese.

Material and methods

Subjects

The study population included 22 manganese-exposed workers and 45 referents from an industrial area in the northern region of Taiwan. The workers had been engaged in the smeltering of manganese for 5 to 30 years. The subjects were selected at the time of the annual physical examinations in January 1995. None of them had a history of liver disorder, renal disorder, heart disease, diabetes ellitus, or other confounding medical complaints. The study had a double blind design for the examiners and subjects. Specimens were discarded when hemolysis occurred. To avoid gender confounding only men were included in the study. Complete data were available for 22 male workers and 45 male referents. The general characteristics of the exposed workers and the referents are summarized in table 1.

Blood collection

Blood was collected by venipuncture into 5-ml evacuated tubes containing EDTA (ethylenediaminetetraacetic acid) solution and 10-ml evacuated tubes containing heparin solution as an anticoagulant. Plastic syringes (Top Surgical Taiwan Corporation, Tokyo, Japan) and 23-gauge needles (Misawa Medical Industry, Tokyo, Japan) were used. The contamination during the sampling stages was controlled as in an earlier study (22).

Table 2. Precision and accuracy of the manganese analysis. (CV = coefficient of variance)[a]

	Mean (μg · l⁻¹)	SD (μg · l⁻¹)	CV (%)
Precision			
Intraassay (N = 8)	2.88	0.12	4.2
Interassay (N = 8)	3.06	0.17	5.6
Accuracy (N = 3)	2.13	0.14	

[a] Accuracy was determined using inorganic manganese in bovine serum (NIST SRM 1598), the certified value being 2.00 μg · l⁻¹.

Manganese in plasma

The manganese concentration of the plasma was determined by pipetting 0.5 ml of plasma (heparinized whole blood) into a test tube, followed by the addition of 0.5 ml of 0.1% Triton-X 100 and mixing. Manganese was measured by an atomic absorption spectrophotometer with a graphite furnace (Perkin-Elmer model 5100PC, Norwalk, Connecticut, United States) (22). Table 2 shows the precision and accuracy of the manganese analysis.

Malondialdehyde in plasma

Blood (EDTA whole blood) was centrifuged, and the supernatant plasma was removed. Care was taken not to contaminate the plasma with platelets. The plasma was stored at 4°C in an ice chest for no longer than 24 h before being frozen (23). The plasma was stored at –70°C for no longer than three weeks prior to the analysis (17). The malondialdehyde concentration in plasma was measured by high-performance liquid chromatography (JASCO model 980-PU, Bio-rad, Japan) with a C_{18} column and an ultraviolet-visible detector (JASCO UV-975, Bio-rad, Japan) (23). The within-run and run-to-run precision of malondialdehyde in plasma was evaluated.

Superoxide dismutase in erythrocytes

Heparinized whole blood (0.5 ml) was centrifuged for 10 min at 3000 revolutions/min and then aspirated off the plasma. The erythrocytes were then washed four times with 3 ml of 0.9% sodium chlorine solution and centrifuged for 10 min at 3000 revolutions/min after each wash. Cold redistilled water was added to the washed erythrocytes to make 2.0 ml of solution, which was then mixed and left to stand at 4°C for 15 min. The SOD

concentration of the erythrocytes was measured by spectrophotometry (Hitachi UV 2000, wavelength 505 nm, Tokyo, Japan) (24).

Glutathione peroxidase in whole blood

The method for determining GPX in whole blood was based on that of Paglia & Valentine (25). Heparinized whole blood (0.05 ml) was diluted with 1 ml of diluting agent and then incubated for 5 min. Thereafter 1 ml of double strength Drabkin's reagent was added. The solution was then mixed well and assayed in the normal manner. GPX catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase and NADPH (nicotinamide adenine dinucleotide phosphate) the oxidized glutathione is immediately converted to the reduced form with the concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm was measured by spectrophotometry.

Catalase in erythrocytes

Heparin-containing venous blood was centrifuged, and the plasma and leukocyte layer were removed. The erythrocyte sediment was then washed three times with isotonic sodium chlorine. The CAT concentration of the erythrocytes was measured by spectrophotometry (Hitachi UV 2000, wavelength 240 nm, Tokyo, Japan) (26).

Data analysis

The statistical analysis was performed with SAS (statistical analysis system) software. The statistical methods included the determination of means and standard deviations; Student's t-test and linear regression were used.

Results

The concentrations or activities of malondialdehyde, SOD, GPX and CAT are shown in table 3. The SOD activity was significantly higher in the exposed workers than in the referents. The difference between the exposed workers and the referents was 422 $U \cdot ml^{-1}$ ($P < 0.01$). The GPX activity was not higher in the exposed workers than in the referents ($P > 0.05$), nor was that of the CAT activity ($P = 0.054$). The malondialdehyde concentration was significantly higher in the exposed workers than in the referents. The mean difference in the malondialdehyde level between these two groups was 0.68 $\mu gmol \cdot l^{-1}$ ($P < 0.001$).

For the workers exposed to manganese, the SOD, GPX and CAT activities did not correlate with the manganese concentration in plasma (table 4), whereas the malondialdehyde concentration correlated strongly with the manganese concentration in plasma ($r = 0.83$, $P < 0.0001$), as shown in figure 1.

Table 3. Manganese blood level and the malondialdehyde, superoxide dismutase, glutathione peroxidase, and catalase of the manganese-exposed workers and referents at the beginning of the study. (Hb = hemoglobin)

Group	Malondialdehyde ($\mu mol \cdot l^{-1}$)			Superoxide dismutase ($U \cdot gHb^{-1}$)			Glutathione peroxidase ($U \cdot l^{-1}$)			Catalase ($kat \cdot gHb^{-1}$)		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Exposed workers (N = 22) (manganese smelter)	1.59	0.55	0.77—2.48	1160.0	638	368—3314	133.0	56.5	67.3—257	181.5	45.0	65.8—272
Referents (N = 45) (administrators or unexposed workers)	0.91	0.19	0.67—1.33	738	521	187—1995	134	62.2	58.9—269	163	31.3	116.8—206
P-value	0.0001			0.009			0.971			0.054		

Table 4. Relationship between the malondialdehyde concentration, the superoxide dismutase, glutathione peroxidase and catalase activity, and plasma manganese in the referents and exposed workers. (Mn = manganese)

	Malondialdehyde		Superoxide dismutase		Glutathione peroxidase		Catalase	
	r	P	r	P	r	P	r	P
Plasma manganese in referents [Mn concentration 0.83 (SD 0.20) $\mu g \cdot l^{-1}$, N = 45]	0.29	>0.05	0.23	>0.05	0.02	>0.05	0.13	>0.05
Plasma manganese in exposed workers [Mn concentration 1.51 (SD 0.65) $\mu g \cdot l^{-1}$, N = 22]	0.83	<0.0001	0.19	>0.05	0.16	>0.05	0.12	>0.05

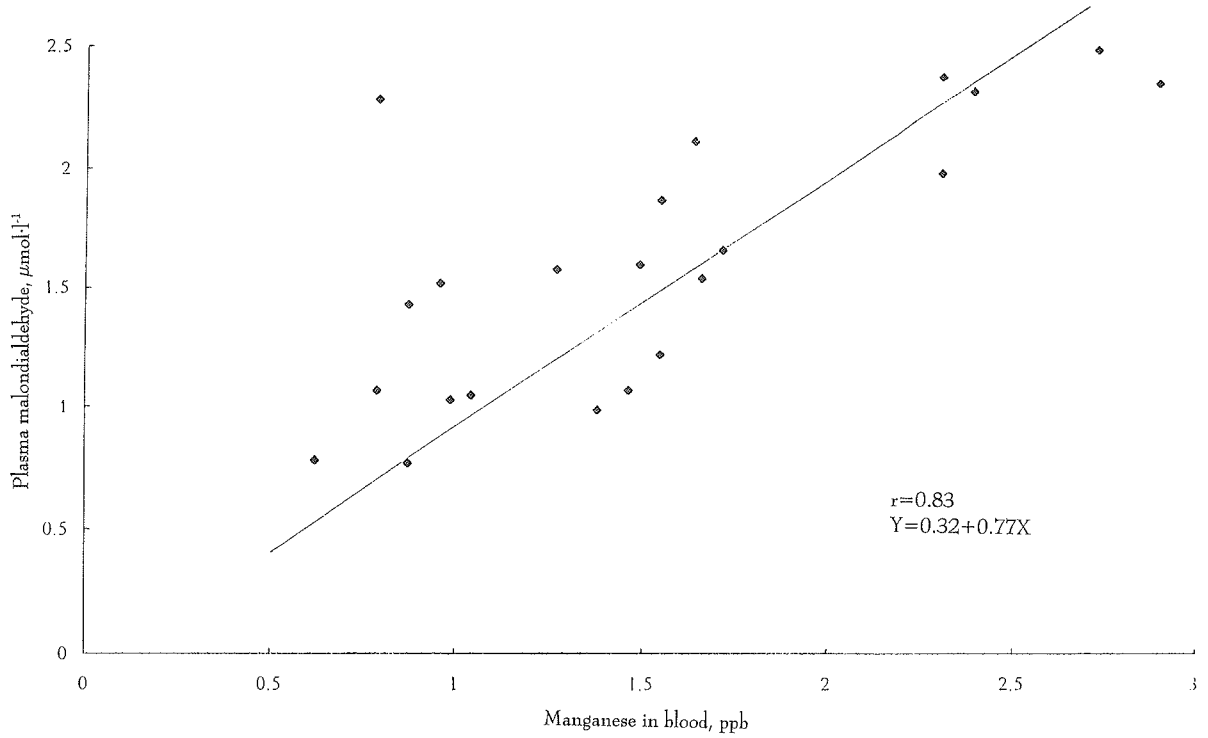

Figure 1. Regression curve for the relationship between manganese in plasma and the plasma malondialdehyde of workers exposed to manganese.

For the referents, the malondialdehyde concentration did not correlate with the manganese concentration of plasma ($r = 0.29$, $P > 0.05$). The SOD, GPX, and CAT activities did not correlate with the manganese plasma level either (table 4). The SOD, GPX, and CAT activities were not intercorrelated ($r < 0.5$, $P > 0.05$) (table 5).

We also examined the correlation between the manganese plasma level and the SOD in erythrocytes, GPX in whole blood, CAT in erythrocytes, and malondialdehyde in plasma and their relation with smoking and drinking (alcohol) behavior. In the reference group, the manganese plasma level of the smokers was similar to that of the nonsmokers. The other parameters were similar for the drinkers and nondrinkers and smokers and nonsmokers, except for GPX. The GPX activity was higher in the nondrinkers than in the drinkers. However, among the smoking and drinking workers that were exposed the manganese plasma level, the SOD, GPX and CAT activities, and the malondialdehyde concentration were not significantly higher.

Table 5. Relationship between superoxide dismutase, glutathione peroxidase and catalase activity in the referents.

	Superoxide dismutase		Glutathione peroxidase		Catalase	
	r	P	r	P	r	P
Superoxide dismutase	—	—	0.04	>0.05	0.32	>0.05
Glutathione peroxidase	0.04	>0.05	—	—	0.20	>0.05
Catalase	0.32	>0.05	0.20	>0.05	—	—

Discussion

The values reported in the literature on the manganese levels in whole blood or serum of workers vary widely (27—28). In healthy unexposed persons, the concentrations of manganese in plasma range from <1 μg · l^{-1} to ≤ 10 μg · l^{-1} in whole blood (29).

Metal ions can result in many oxidation-reduction reactions within the organism. For example, Sunderman et al (30) found increased lipid peroxide in alveolar macrophages of rats administered nickel chloride. An in vitro study has also shown increased lipid peroxidation due to manganese chloride (31). In rats treated with silica, lipid peroxidation gradually increased in the lung (32). In our previous study, we also found that lipid peroxidation was increased in workers exposed to lead (33). Manganese ions can also contribute to the production of free radicals, including oxygen-centered radicals. O_2^- is formed in almost all aerobic cells, one important source being the "respiratory burst" of phagocytic cells when they contact foreign particles of manganese. In aqueous solutions, O_2^- undergoes the so-called dismutation reaction to form hydrogen peroxide and oxygen. The superoxide

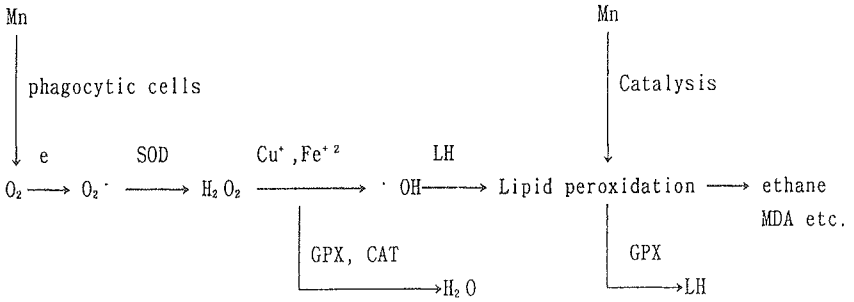

Figure 2. Reaction of lipid peroxidation and antioxidants. (Mn = manganese, O_2 = oxygen, O_2^- = oxygen radical, SOD = superoxide dismutase, H_2O_2 = hydrogen peroxide, Cu^+ = copper ion, Fe^{+2} = iron ion, ·OH = hydroxyl radical, GPX = glutathione peroxidase, CAT = catalase, H_2O = hydrogen oxide, MDA = malondialdehyde, LH = lipid hydroperoxide)

theory of O_2 toxicity has been given an enormous boost by the accumulation of evidence showing that SOD enzymes, which remove O_2^- by accelerating the dismutation reaction, are of great importance in allowing organisms to survive in the presence of O_2 and to tolerate increased O_2 concentrations. O_2^- is a species worth removing in vivo because of the following: (i) SOD are catalysts that have evolved a surface charge arrangement to facilitate the specific use of O_2^- as a substrate and (ii) SOD are important antioxidants, required for the growth of aerobes without excessive DNA (deoxyribonucleic acid) damage in the presence of O_2. O_2^- must be worth removing even at the expense of forming H_2O_2, although SOD in human cells works in conjunction with H_2O_2-removing enzymes such as CAT and GPX (34). In general, one major role of SOD is considered to be cell or tissue protection from damage mediated through the superoxide anion radicals during exposure to xenobiotics. Impairment of the GPX system could have serious consequences with regard to lipid peroxidation in both mice and humans. In biomedical research the study of CAT in the liver, leukocytes, and erythrocytes has received increased attention owing to its role in oxidative metabolism, as well as its protective function, by acting as an H_2O_2 scavenger (35). The overall reaction is shown in figure 2.

With improvements in industrial hygiene and environmental health, manganese intoxication has rarely been reported. However, chronic manganese intoxication in workers has been reported in Taiwan (36). In our study, the manganese concentration of workers exposed to manganese was higher than that of referents, but toxic symptoms were not observed. In animal or human studies (37, 38), manganese is cleared from the blood by the liver within minutes or a few days; only after chronic administration is any substantial increase observed in tissues. In another study (39), there was no significant increase in manganese content even after 30 d of exposure. In one animal experiment, manganese reduced the formation of malondialdehyde in rat brain (40). The conditions in our study were different. The animal experiment concerned acute exposure, whereas our study involved long-term exposure. In the reference group, the manganese level of plasma in smokers was similar to that of nonsmokers; this finding is in agreement with the results of previous studies (35).

In conclusion, lipid peroxidation is one of the primary mechanisms of toxicity for manganese. We believe also that the toxic effect of manganese may cause cellular damage through the catalytic production of free oxygen radicals. The activities of SOD, GPX and CAT had a wide scatter among our referents. Lipid peroxidation and the variation of these enzymes and other antioxidants, for example, vitamin E, vitamin C, and selenium, may have complicated interactions. However, the results indicate that the malondialdehyde concentration in exposed workers is strongly correlated with the manganese concentration. According to these results, malondialdehyde can be used as an index of lipid peroxidation induced by the early biochemical action of manganese.

References

1. Francois B, Olivier G, Josiane A, Francis P, Richard Z. Determination of manganese in biological materials by electrothermal atomic absorption spectrometry: a review. Clin Chem 1988;34:227—34.
2. David B, Rodger LS, Ralston NVC. Manganese content of the cellular components of blood. Clin Chem 1990;36:450—2.
3. Keen CL, Lonnerdal B, Hurley L. Manganese. In: Frieden E, editor. Biochemistry of the essential ultratrace elements. New York (NY): Plenum, 1984:89—132.
4. Klaassen CD, Amdur MO, Doull J. Casarett and Doull's toxicology: the basic science of poisons. 3rd ed. New York (NY): Macmillam Publishing Company, 1986:592.
5. Murray RK, Granner DK, Mayes PA, Rodwell VW. Harper's biochemistry. 21st ed. Norwalk (CT): Prentice-Hall, 1988: 138—9.
6. Ledwozyw A, Michalak J, Stepien A, Kadziolka A. The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis. Clin Chim Acta 1986;155:275—84.
7. Pryor WA. Free radical involvement in chronic diseases and aging: the toxicity of lipid hydroperoxides and their decomposition products [review]. In: Finley JW, Schwass DW, editors. Xenobiotic metabolism: nutritional effects. Washington (DC): American Chemical Society, 1985:77—96.
8. Humad S, Zarling EJ, Skosey JL. Lipid peroxidation in rheumatoid arthritis: measurement of pentane in breath samples by gas chromatography. Clin Res 1985;33:919A.
9. Nishigake I, Hagihara M, Tsunekawa H, Maseki M, Yagi K.

Lipid peroxide levels of serum lipoprotein fractions of diabetic patients. Biochem Med 1981;25:373—8.
10. Halliwell B, Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 1984;219:1—14.
11. Halliwell B. Oxygen radicals: a common sense look at their nature and medical importance. Med Biol 1984;62:71—7.
12. Sunderman FW Jr. Metals and lipid peroxidation (review). Acta Pharmacol Toxicol 1986;59 (suppl 7):248—55.
13. Knight JA, Pieper RK, Smith SE, Crockett HH. Increased urinary lipoperoxides in drug abusers. Ann Clin Lab Sci 1988;18:374—7.
14. Wendel A, Feurstein S, Konz KH. Acute paracetamol intoxication of starved mice leads to lipid peroxidation in vivo. Biochem Pharmacol 1979;28:2051—5.
15. Burk RF, Lane JM. Ethane production and liver necrosis in rats after administration of drugs and other chemicals. Toxicol Appl Pharmacol 1979;50:467—78.
16. Yasaka S, Ohya I, Matsumoto J, Shiramizu T, Sasaguri Y. Acceleration of lipid peroxidation in human paraquat poisoning. Arch Intern Med 1981;141:1169—71.
17. Uysal M, Bulur H, Erdine DS, Demiroglu C. Erythrocyte and plasma lipid peroxides in chronic alcoholic patients. Drug Alcohol Depend 1986;18:385—8.
18. Knight JA, Voorhees RP. Peroxidation of linolenic acid catalized by transition metal ions. Ann Clin Lab Sci 1990;20:347—52.
19. Kantola M, Sarranen M, Vanha PT. Selenium and glutathione peroxidase in seminal plasma of men and bulls. J Reprod Fertil 1988;83:785—94.
20. Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malonyldialdehyde) in biochemical systems. Anal Biochem 1966;16:359—64.
21. Porter NA. Prostaglandin endoperoxides. In: Prior WA, editor. Free radicals in biology. New York (NY): Academic Press, 1980:261—94.
22. Lin SY, Yang MH, Yiin SJ, Lin TH, Shih TS. Biological monitoring of occupational manganese exposure. J Occup Safety Health 1994;2(3):57—70.
23. Wong SHY, Knight JA, Hopfer SM, Zaharria O, Leach CN Jr, Sunderman FW Jr. Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. Clin Chem 1987;33:214—20.
24. Randox: RANSOD, Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, UK, 1995.
25. Plagia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158—65.
26. Aebi HE. Catalase in vitro. Methods Enzymol 1984;105:121—6.
27. Järvisalo J, Olkinuora M, Tossavainen A, Virtamo M, Ristola P, Aitio A. Urinary and blood manganese as indicators of manganese exposure in manual arc welding of mild steel. In: Brown SS, Savory J, editors. Chemical toxicology and clinical chemistry of metals. London, New York: Academic Press, 1983:123—6.
28. Chandra SV, Shukla GS, Srivastava RS. An exploratory study of manganese exposure to welders. Clin Toxicol 1981;18(4):407—16.
29. Tsalev DL, Zaprianov ZK. Atomic absorption spectrometry in occupational and environmental health practice; vol I (Analytical aspects and health significance). Boca Raton (FL): CRC Press, 1984:153—7.
30. Sunderman FW, Hopfer SM, Lin SM, Plowman MC, Stojanovic T, Wong SHY, et al. Toxicity to alveolar macrophages in rats following parenteral injection of nickel chloride. Toxicol Appl Pharmacol 1989;100:107—18.
31. Knight JA, Voorhees RP. Peroxidation of linolenic acid catalysis by transition metal ions. Anal Clin Lab Sci 1990;20:347—52.
32. Zsoldos T, Tigyi A, Montsko T, Puppi A. Lipid peroxidation in the membrane damaging effect of silica-containing dust on rat lungs. Exp Pathol 1983;23(3):73—7.
33. Yiin SJ, Lin TH. Lipid peroxidation in workers exposed to lead. Arch Environ Health 1994;49:256—9.
34. Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol 1990;186:1—17.
35. Pleban PA, Pearson HK. Determination of Mn in whole blood and serum. Clin Chem 1979;25:1915.
36. Huang CC, Chu NS, Lu CS, Wang JD, Tsai JL, Tzeng JL, et al. Chronic manganese intoxication. Arch Neurol 1989;46:1104—6.
37. Klaassen CD. Biliary excretion of manganese in rats, rabbits and dogs. Toxicol Appl Pharmacol 1974;29:458—68.
38. Scheuhammer AM, Cherian MG. The distribution and excretion of manganese: the effects of manganese dose, L-Dopa, and pretreatment with zinc. Toxicol Appl Pharmacol 1982;65:203—13.
39. Scheuhammer AM, Cherian MG. Influence of chronic $MnCl_2$ and EDTA treatment on tissue levels and urinary excretion of trace metals in rats. Arch Environ Contam Toxicol 1982a;11:515—20.
40. Shukla GS, Chandra SV. Manganese toxicity: lipid peroxidation in rat brain. Acta Pharmacol Toxicol 1981;48(2):95—100.
41. Rothman KJ, Keller AZ. The effect of joint exposure to alcohol and tobacco on risk of cancer of the mouth and pharynx. J Chronic Dis 1972;23:711—6.

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