Indices of Lead-Exposure in Blood and Urine of Lead-Exposed Workers and Concentrations of Major and Trace Elements and Activities of SOD, GSH-Px and Catalase in Their Blood

MOMOKO CHIBA, ATSUKO SHINOHARA, KAZUKO MATSUSHITA*, HIROMI WATANABE and YUTAKA INABA

*Department of Epidemiology and Environmental Health
and *Division of Rheumatology, Department of Internal
Medicine, Juntendo University School of Medicine, Tokyo
113*

CHIBA, M., SHINOHARA, A., MATSUSHITA, K., WATANABE, H. and INABA, Y. *Indices of Lead-Exposure in Blood and Urine of Lead-Exposed Workers and Concentrations of Major and Trace Elements and Activities of SOD, GSH-Px and Catalase in Their Blood.* Tohoku J. Exp. Med., 1996, 178 (1), 49–62 — Seventy male factory workers were studied. The lead concentrations in their blood (Pb-B) were 16.55 ± 11.53 $\mu\text{g}/100$ ml (range 1.5 to 50.2 $\mu\text{g}/100$ ml). The subjects were divided into three groups according to Pb-B (in $\mu\text{g}/100$ ml): group A, $\text{Pb-B} \leq 10$ ($n=22$); group B, $10 < \text{Pb-B} \leq 20$ ($n=30$); group C, $\text{Pb-B} > 20$ ($n=18$). The mean $\pm$ S.D. in each group was 5.57 ± 2.53 , 15.02 ± 2.75 , and 32.52 ± 9.49 $\mu\text{g}/100$ ml, respectively. Pb in plasma was 0.011 ± 0.010 , 0.017 ± 0.033 , and 0.021 ± 0.021 $\mu\text{g}/\text{liter}$, and Pb in the RBC was 0.281 ± 0.246 , 0.701 ± 0.325 , and 1.626 ± 0.861 $\mu\text{g}/\text{g}$ Hb, respectively. In addition to Pb concentration, the concentrations of 34 elements in the plasma or in the RBC were determined. Se concentrations in RBC in each group were 0.618 ± 0.139 , 0.670 ± 0.207 , and 0.728 ± 0.200 $\mu\text{g}/\text{g}$ Hb, and the mean values were significantly different between groups A and C ($p < 0.05$). For Se concentrations in plasma, the mean $\pm$ S.D. in each group was 0.132 ± 0.035 , 0.130 ± 0.031 , and 0.126 ± 0.021 $\mu\text{g}/\text{ml}$, respectively, and there was no significant difference between groups. On the other hand, when the activities of total SOD, Mn-SOD, Cu, Zn-SOD, and catalase in the plasma and the activities of GSH-Px both in the plasma and in the RBC were assayed, some differences were found. The activities in GSH-Px in RBC were 17.19 ± 5.03 , 17.59 ± 3.95 , and 15.25 ± 3.18 $\mu\text{mol}/\text{g}$ Hb/min, and those in plasma were 0.069 ± 0.032 , 0.081 ± 0.023 , and 0.080 ± 0.028 $\mu\text{mol}/\text{ml}/\text{min}$. In group C, GSH-Px activity was lower in the RBC and higher in the plasma than those in group A, and it was observed that the Se concentration was higher in RBC, and that there was no remarkable change in the plasma. Catalase activity in group C was 3.58 ± 0.81 $\text{mgH}_2\text{O}_2/\text{ml}/30$ min, which was significantly higher than that in group A (2.81 ± 0.90 $\text{mgH}_2\text{O}_2/\text{ml}/30$ min). Further investigation is necessary in order to explain the above results. The regular indices used for evaluating lead exposure, showed significant correlations

Received August 4, 1995; revision accepted for publication September 15, 1995.

This paper was presented at the Sendai Symposium : Medico-Biological Application of Plasma Ionization Source Mass Spectrometry, August 4, 1995, Sendai.

with Pb-B: $r = -0.786$ vs δ -Aminolevulinic acid (ALA) dehydratase activity in blood, $r = 0.927$ vs. inhibition rate, and $r = 0.339$ vs. ALA in urine. ——— lead in blood; δ -aminolevulinic acid in urine; trace element concentration in blood; porphyrins; GSH-Px in plasma and in RBC

Lead (Pb) has been used by human beings for over two thousand years, and is one of the most commonly used metals. In Japan, workers who have occupational lead exposure are required to undergo a health examination twice a year. The medical examination for lead-workers is divided into 2 steps. The first step, which all lead-exposed workers receive, includes determining the lead concentration in blood (Pb-B), the δ -aminolevulinic acid concentration in urine (ALA-U), personal occupational history, and self-aware and objective symptoms. The physicians suggest that those who have marked effects of lead in the first step, go on to the second step of the health examination. The second step includes determining the amounts of protoporphyrin in blood, an examination of the blood in relation to anemia, a neuro-internal check, an investigation of working conditions, and the other items in relation to self-aware or objective symptoms. In 1992 in Japan, about 13,000 lead-exposed workers received the special medical examination, and about 2% of them were found to have disorders due to lead (Health and Welfare Statistics Association 1994).

As mentioned above, Pb-B in most lead-exposed workers is determined at least once a year. However, there is no information of the other trace elements in the blood of lead-exposed workers. Recently a highly sensitive analytical instrument, the plasma-mass spectrometer, has been developed that permits multielement analyses at sub-ppb levels. We attempted to observe the effects of lead-exposure on the concentrations of various elements in the blood by using a microwave induced plasma—mass spectrometer (MIP-MS).

Furthermore, there have been few reports concerning the effects of lead on the activities of metalloenzymes in blood except for reports on ALA dehydratase and the other enzymes involved in porphyrin synthesis. We analyzed the activities of glutathione peroxidase (GSH-Px) which is a seleno-enzyme, manganese superoxide dismutase (Mn-SOD), copper, zinc-superoxide dismutase (Cu, Zn-SOD), catalase which contains iron, in addition to ALA dehydratase which is known as a zinc-enzyme and is considered as the most sensitive indicator of exposure to lead.

The results of this study may be useful for preventive medicine, if we can identify the subclinical changes in the essential elements or enzyme activities.

MATERIALS AND METHODS

Examinees

Workers in a steel rope factory were studied. They agreed to participate in this study. About 70 workers were engaged in jobs related to lead. There are two lead furnaces in the plant, although the surfaces of the furnaces are covered

with small cubes of manmade materials to prevent the lead from contaminating the environment. Four Friday mornings in the fall of 1993, blood and urine samples from 63 of approximately 70 lead-exposed workers and an additional 7 control male workers were collected at the clinic in this factory for periodic health examinations. Blood samples were drawn from the cubital vein, and put into a glass tube which contained sodium heparin as an anticoagulant. A spot urine sample was collected in a plastic bottle.

Determination of lead in whole blood (Pb-B)

An aliquot of whole blood (0.1 ml) was added to 0.9 ml 1% Triton x-100 solution. Ten μ l of the 10 times diluted blood was injected into the graphite furnace of an atomic absorption spectrometer (Z-8100; Hitachi, Tokyo). The operating conditions were: wavelength at 283.3 nm, drying at 80 to 120°C for 30 sec, charring at 600°C for 30 sec, atomizing at 2,000°C for 5 sec, and cleaning at 2,400°C for 4 sec, flow rate of argon gas at 200 ml/min except during atomizing when it was 30 ml/min. The standard addition method was adopted to calculate Pb concentration in the blood. The detection limit was 1 ng/ml.

Determination of ALA in urine

The method of extraction by organic solvent after developing color (Wada et al. 1969) was adopted. Namely, 1.0 ml urine sample and 1 ml 20% acetic acid were mixed, n-butanol (4 ml) was then added, and the mixture was vigorously shaken. After separation of phases, 250 μ l was removed from the bottom layer of each sample with a pipet and transferred to 2 glass tubes. Seven-hundred and fifty μ l of a solution of 1 volume ethyl acetoacetate and 20 volumes 0.5 M sodium phosphate buffer, pH 6.8, was added to one tube, and 750 μ l sodium phosphate buffer only was added to the other tube, which was prepared as the blank. All tubes were put in a boiling water bath for 10 min. After cooling, 1 ml Ehrlich reagent was added to each tube, and then the contents were mixed well. After 10 min at room temperature, 2 ml chloroform was added to all tubes, and then the tubes were shaken vigorously. Absorbance of the chloroform layer was read at 555 nm. The composition of the Ehrlich reagent was 64 ml glacial acetic acid, 20 ml 70% perchloric acid, 20 ml concentrated hydrochloric acid, 5 ml 0.2 M mercuric chloride, and 2 g p-dimethylaminobenzaldehyde. ALA concentration was calculated from a standard calibration curve. However, the recovery rate depended upon urine samples. Therefore, we prepared one more tube for each sample to obtain the individual recovery rate; this tube contained 1.0 ml urine, 50 μ l ALA standard solution (200 μ g/ml), and 950 μ l 20% acetic acid solution. ALA-U was determined as described above from the absorbance and individual recovery rate. ALA-U values were expressed as mg/g creatinine or the mg/liter urine as its specific gravity, 1.024.

Determination of some parameters in urine samples

Sugar, protein, and pH values in urine samples were checked with test papers. Creatinine concentration was determined by Jaffe's method (Bonsnes and Taussky 1945). Specific gravity was determined by an Elma gravity photometer.

Determination of zinc protoporphyrin (ZP) and protoporphyrin (PP) in whole blood

An aliquot of whole blood (50 μ l) was dropped into a glass tube containing 2.5 ml N, N-dimethylformamide. The tube was then vigorously shaken for 10 min. After centrifuging at 2,500 rpm for 5 min, 20 μ l supernatant was applied to a high performance liquid chromatography (HPLC, 6A; Shimadzu, Kyoto) equipped with a C18 column (Fine Pak, 4.6×150 mm; Japan Spectroscopic Co. Ltd. Tokyo) and a precolumn (Biofine, 4.6×10 mm; Japan Spectroscopic Co. Ltd.). As a mobile phase, acetonitrile: acetic acid: 0.2 M ammonium acetate (90 : 5 : 5) was used at a flow rate of 2 ml/min. The optimal wave lengths of the fluorometric detector (RF-535; Shimadzu) were Ex 410 nm and Em 590 nm for ZP, and Ex 405 nm and Em 630 nm for PP. Detection limits of this method were 20 pg for ZP and 80 pg for PP. The recovery rates of ZP and PP were $78.2 \pm 8.1\%$ and $95.2 \pm 7.2\%$ (mean $\pm$ S.D., $n=5$), respectively.

Calculation of free erythrocyte protoporphyrin (FEP)

FEP amount (g) = PP amount + ZP amount/1.1, where 1.1 is the molecular weight ratio, ZP/PP.

Determination of ALA dehydratase activity in whole blood

Two kinds of ALA dehydratase activities were assayed, native activity (A) and recovered activity (B). For native activity, 40 μ l whole blood was put into a glass tube containing 360 μ l demineralized water. Then 100 μ l substrate A solution, which consisted of 25 mM ALA in 250 mM sodium phosphate buffer (pH 6.8), was added. After incubation for 30 min in a 37°C water bath, the reaction was stopped by adding 0.5 ml 1 M trichloroacetic acid containing 70 mM mercuric chloride. After centrifuging at 3,000 rpm for 5 min, the supernatant (0.5 ml) was placed in a glass tube and to this was added an equal volume Ehrlich reagent. The absorption at 555 nm was then read between 5 and 25 min after addition of Ehrlich reagent. The constituents of the Ehrlich reagent were 68 ml glacial acetic acid, 32 ml 70% perchloric acid, 4 ml concentrated hydrochloric acid, and 2 g *p*-dimethylaminobenzaldehyde. For recovered activity, the procedure was the same as that used to assay the native activity, except that the substrate solution contained 50 mM dithiothreitol and 1 mM zinc in addition to 25 mM ALA.

One unit of ALA dehydratase activity was defined as one μ mol por-

phobilinogen (PBG) formed per 1 ml erythrocytes for 30 min incubation. In practice the calculation is determined by (absorption at 555 nm—blank value) $\times$ $78.125 \times 1/Ht$, where 78.125 is from dilution rate and the molar absorption coefficient of PBG ($\epsilon = 6.2 \times 10^4$).

Calculation of the inhibition rate of ALA dehydratase

As it is known that ALA dehydratase is a sulfhydryl and zinc enzyme, the recovered activity B is higher than the native activity A. The inhibition rate is expressed by $(1-A/B) \times 100\%$.

Assay of total superoxide dismutase (SOD) activity in plasma

Eighty μ l 0.1 M potassium phosphate-sodium tetraboric acid buffer containing 0.5 mM EDTA (pH 8.2), 80 μ l 0.5 mM hypoxanthine solution, 40 μ l mixture of 8.8 mM hydroxylamine-O-sulfonic acid and 10 mM hydroxylamine hydrochloride, 80 μ l distilled water, and 40 μ l diluted plasma were preincubated at 37°C for 5 min. After addition of 80 μ l diluted xanthine oxidase solution, incubation was performed at 37°C for 30 min. The reaction was stopped by adding 0.8 ml of a mixture of 30 μ M N-1-naphthylethylenediamine, 3 mM sulfanilic acid, and 25% acetic acid. After leaving the mixture at room temperature for 30 min, the absorbance at 550 nm was read with a spectrophotometer (U-1000; Hitachi, Tokyo).

Calculation of total SOD showed the following: the absorbance of the incubation mixture at 550 nm without the plasma sample was regarded as 0% inhibition. Two kinds of the blank values, the blank of the standards and the blank of the samples, were also obtained from the incubation mixture without xanthine oxidase. Superoxide anion produced from hypoxanthine and xanthine oxidase was consumed by plasma SOD. Different amounts of plasma were used, and then the plasma concentration corresponding to 50% inhibition of SOD activity (ID_{50}) was estimated. The activity was expressed as NU/ml plasma, where NU is the activity unit of nitrite method and is defined as showing 50% inhibition of the enzyme activity in the 3 ml incubation mixture. This method was a modified technique of Oyanagui's method (Oyanagui 1984).

Determination of Mn-SOD and Cu, Zn-SOD in plasma

When total SOD was assayed, 80 μ l distilled water was added to the preincubation mixture as described above. When Mn-SOD was assayed, 80 μ l 5 mM potassium cyanide (KCN) was added instead of distilled water, because KCN inactivates Cu, Zn-SOD, but has no effect on Mn-SOD at this concentration. The other procedure was the same as the total SOD assay method. This was done according to the method of Oyanagui (1984).

The activity of Mn-SOD was calculated from the percent inhibition at a plasma concentration equal to ID_{50} . The value of Cu, Zn-SOD was obtained by

difference between total SOD and Mn-SOD.

Assay of catalase activity in plasma

Ten μl plasma was added to the reaction mixture which consisted of 170 μl 1/15 M sodium phosphate buffer (pH 6.8), 100 μl 45 mM hydrogen peroxide (H_2O_2), and 30 μl distilled water. As a blank, 30 μl 10 mM NaN_3 was added instead of distilled water. After 30 min incubation at 37°C, 100 μl of the incubation mixture was pipetted into 1 ml of a reaction-stopping medium, which consisted of 5% titanium (IV) sulfate solution containing 13.5% sulfuric acid. This reaction-stopping mixture developed color soon after receiving the incubation mixture. After leaving the mixture at room temperature for 15 min, the absorbance at 410 nm was read. Calibration was performed by the standard substance, H_2O_2 . Catalase activity was expressed as mg H_2O_2 consumed/30 min/ml plasma.

Assay of glutathione peroxidase in plasma or in erythrocytes

H_2O_2 was used as the substrate. The plasma sample was preincubated at 25°C with a mixture containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM reduced glutathione, 1 mM NaN_3 , 0.2 mM NADPH, and 1 European unit/ml glutathione reductase (all final concentrations). The reaction was then started by adding H_2O_2 at 250 μM concentration to the medium. When GSH-Px in erythrocytes was assayed, hemolyzate diluted 40 times with the same kind of buffer was used instead of plasma. The oxidized glutathione formed was immediately converted into reduced glutathione by glutathione reductase and NADPH. GSH-Px activity was then assayed by following the time-dependent decrease in absorbance at 340 nm of NADPH ($\epsilon = 5.6 \times 10^3$) and expressed as $\mu\text{mol}/\text{min}/\text{ml}$ plasma, or $\mu\text{mol}/\text{min}/\text{g}$ Hb.

Determination of various element concentrations in plasma and erythrocytes

Plasma and erythrocyte samples were decomposed by a simplified wet digestion method. To each 0.1 ml sample was added 2 ml concentrated nitric acid and 0.1 ml perchloric acid, and then the samples were heated at 120 to 130°C for the first 2 hr, and then at 200 to 220°C for 1 hr. The residue was dissolved in 1.0 ml 0.5% nitric acid. This solution was diluted with 0.5% nitric acid by an appropriate amount depending upon the analytical instruments used. The concentrations of Ag, As, Bi, Cd, Co, Cr, Cs, Cu, Fe, Ga, Ge, Hg, In, Mn, Mo, Ni, Pb, Pd, Pt, Rb, Sb, Sn, Sr, Te, Th, Tl, V, U, Zn, and Zr were determined by MIP-MS (P-7000; Hitachi). The operating conditions are shown in Table 1. Eu was used as an internal standard. Oyster tissue (NBS SRM 1566a) was used as the standard reference material. Detection limits were ppt levels, from Ca 0.4 pg/ml to Ba 250 pg/ml. The details of MIP-MS technique have been described by Shinohara et al. (1994).

The concentrations of Ca, Mg, K, and Na were determined by a flame atomic

TABLE 1. *Operating conditions for MIP-MS*

Rfpower	: 1.3 kW
Carrier gas flow (N ₂)	: 1.3 liter/min
Sampling cone	: Pt-made
Temperature of cooling chamber	: 4°C
Scanning conditions	
Dwell time	: 50 msec
Number of sweeps	: 3 times
Sample flow	: 0.24 ml/min
Element measured:	
⁵¹ V, ⁵² Cr, ⁵⁵ Mn, ⁵⁷ Fe, ⁵⁹ Co, ⁶⁰ Ni, ⁶³ Cu, ⁶⁸ Zn, ⁷¹ Ga, ⁷² Ge, ⁷⁴ As, ⁸⁵ Rb,	
⁸⁸ Sr, ⁹⁰ Zr, ⁹⁵ Mo, ¹⁰⁵ Pd, ¹⁰⁷ Ag, ¹¹¹ Cd, ¹¹⁵ In, ¹¹⁸ Sn, ¹²¹ Sb, ¹²⁸ Te,	
¹³³ Cs, ¹⁹⁵ Pt, ²⁰² Hg, ²⁰⁵ Tl, ²⁰⁸ Pb, ²⁰⁹ Bi, ²³² Th, ²³⁸ U	
Internal standard: ¹⁵³ Eu	

absorption spectrometer (Z-6000; Hitachi), using acetylene-air flame. Samples were aspirated at 5.5 ml/min. Detection limits were 10 ng/ml for Mg, 50 ng/ml for Na, and 100 ng/ml for K.

Phosphorus concentration was determined by a colorimetric method; 600 μ l sample solution, 200 μ l 2 M sulfuric acid, and 200 μ l 7.8 mM ammonium molybdate solution were mixed, then 160 μ l of a mixture containing 5% ascorbic acid and 2 mM antimony potassium tartrate, and 840 μ l distilled water were added. After 10 min, the absorption at 880 nm was read. Phosphorus concentration was obtained from a standard calibration curve. The detection limit was 0.05 μ g/ml.

Selenium concentrations were independently determined, because some organic selenocompounds which have low boiling temperatures were lost during the common digestion procedure. After wet ashing, residues were dissolved in 10% HCl, then determined by fluorometry. Determination of Se was done according to the fluorometric method of Watkinson (1966), with some modifications: 0.1 ml plasma or 0.1 g hemolyzate, 2 ml concentrated nitric acid, and 0.25 ml perchloric acid were kept at 130~140°C. After 8 hr 0.25 ml perchloric acid was added again, and the temperature was increased to 200°C and kept for 1 hr. This digested residue was dissolved in 0.5 ml 10% HCl. To each tube was added 0.5 ml 20% ammonium sulfamate and then the tubes were put in a boiling water bath for 10 min. After cooling, the pH was adjusted at 1~1.5 by 10% NaOH. To each tube was added 0.5 ml 0.2 M EDTA-2Na and 1 ml 2,3-diaminonaphthalene solution. All tubes were incubated in a 50°C water bath for 30 min. After cooling, 2.0 ml cyclohexane was added. All tubes were shaken for 5 min. Fluorescence in the cyclohexane layer was determined at 378 nm for Ex wavelength and at 520 nm for Em wavelength (FP-770 spectrofluorometer; Japan Spectroscopic Co., Ltd.).

RESULTS

Lead concentration in blood

Table 2(A) shows the regular parameters relating to lead exposure in lead-workers and the controls. Regarding Pb-B, the mean $\pm$ s.d. of 63 lead workers was 17.8 ± 11.5 $\mu\text{g}/100$ ml (range 1.5 to 50.2 $\mu\text{g}/100$ ml), and for 7 control workers it was 5.7 ± 4.3 $\mu\text{g}/100$ ml (range 1.5 to 13.6 $\mu\text{g}/100$ ml). There was a significant difference between the two groups ($p < 0.01$). However, the minimum values in both groups were the same. This fact implies that subjects categorized as lead-workers contained few lead-exposed subjects. The ambient lead concentrations of 22 points in the working area were from < 0.01 to 0.07 mg/m^3 . These values were considered to indicate a relatively low lead exposure. Therefore, all examinees were divided into three groups according to Pb-B, namely $\text{Pb-B} \leq 10$ $\mu\text{g}/100$ ml ($n = 22$), $10 < \text{Pb-B} \leq 20$ $\mu\text{g}/100$ ml ($n = 30$), and $\text{Pb-B} > 20$ $\mu\text{g}/100$ ml ($n = 18$). All analyzed parameters were redistributed according to these three groups. The recalculated results are shown in Table 2(B).

ALA excretion in urine

The values of ALA-U showed significant differences between each group when all subjects were classified into the 3 groups, although there was no difference

TABLE 2. Regular parameters related to lead exposure

(A) Lead-exposed workers and controls

	Lead-exposed workers ($n = 63$)	Controls ($n = 7$)
Pb-B ($\mu\text{g}/100$ ml)	$17.8 \pm 11.5^{**}$	5.7 ± 4.3
ALA-U (mg/liter)	2.65 ± 1.25	2.39 ± 0.74
PP ($\mu\text{g}/100$ ml)	$9.1 \pm 18.2^*$	3.0 ± 0.7
ZP ($\mu\text{g}/100$ ml)	$33.3 \pm 27.0^{**}$	20.1 ± 7.3
FEP ($\mu\text{g}/100$ ml)	$42.4 \pm 43.0^{**}$	23.1 ± 7.5

* $p < 0.05$; ** $p < 0.01$.

(B) Three groups divided according to Pb-B

	Pb-B ($\mu\text{g}/100$ ml)		
	≤ 10 ($n = 22$)	$10 < \text{Pb-B} \leq 20$ ($n = 30$)	$20 < $ ($n = 18$)
Pb-B ($\mu\text{g}/100$ ml)	5.57 ± 2.53	$15.02 \pm 2.75^{**}$	$32.52 \pm 9.49^*$
ALA-U (mg/liter)	2.12 ± 0.82	$2.73 \pm 1.15^*$	$3.03 \pm 1.55^*$
PP ($\mu\text{g}/100$ ml)	4.3 ± 2.8	6.5 ± 7.4	16.9 ± 32.3
ZP ($\mu\text{g}/100$ ml)	23.8 ± 7.6	24.7 ± 9.2	$54.0 \pm 43.3^*$
FEP ($\mu\text{g}/100$ ml)	28.1 ± 9.5	31.2 ± 13.5	$70.9 \pm 72.6^{**}$

* $p < 0.05$ vs. $\text{Pb-B} < 10$ group; ** $p < 0.01$ vs. $\text{Pb-B} < 10$ group.

Fig. 1. Relationship between lead in the blood (Pb-B) and ALA excretion in the urine (ALA-U).

when they were grouped into lead-workers (ALA-U ranged from 0.75 to 6.98 mg/liter) and the controls (ranged from 1.37 to 3.63 mg/liter). Fig. 1 shows the relationship between ALA-U and Pb-B. The correlation coefficient was $r = 0.339$ ($p < 0.05$).

ZP, PP, and FEP concentrations in whole blood

As shown in Table 2(B), ZP and FEP concentrations showed significant

Fig. 2. Relationship between lead in the blood (Pb-B) and (A) zinc protoporphyrin (ZP), (B) protoporphyrin (PP), and (C) free erythrocyte protoporphyrin (FEP).

differences between the group with Pb-B ≤ 10 $\mu\text{g}/100$ ml and the group with Pb-B > 20 $\mu\text{g}/100$ ml. PP did not show any significant difference between groups, although the mean values tended to increase with increasing Pb-B. Fig. 2 shows the relationships between ZP and Pb-B, PP and Pb-B, and FEP and Pb-B. The correlation coefficients were $r=0.649$ ($p<0.001$), $r=0.452$ ($p<0.02$), and $r=0.599$ ($p<0.001$), respectively.

ALA dehydratase activity in whole blood and its inhibition rate

When subjects were classified into lead-worker and the control groups, their mean values $\pm$ s.d. were 0.48 ± 0.20 unit in lead workers, and 0.74 ± 0.11 unit in the

Fig. 3. Relationship between lead in the blood (Pb-B) and (A) ALA dehydratase activity in whole blood and (B) the inhibition rate.

TABLE 3. *Enzyme activity in whole blood, plasma, and in erythrocytes*

Enzyme	Pb-B ≤ 10 ($n=22$)	$10 < \text{Pb-B} \leq 20$ ($n=30$)	$20 < \text{Pb-B}$ ($n=18$)
Whole blood			
ALA dehydratase ($\mu\text{mol PBG}/\text{ml erythrocytes}/30$ min)	0.715 ± 0.148	$0.486 \pm 0.136^{**}$	$0.276 \pm 0.099^{**}$
Inhibition rates of ALA dehydratase (%)	23.31 ± 8.68	$47.83 \pm 7.27^{**}$	$71.04 \pm 9.62^{**}$
Plasma			
Total SOD (NU/ml)	22.49 ± 4.44	24.60 ± 11.36	24.03 ± 6.13
Cu, Zn-SOD (NU/ml)	11.84 ± 3.35	14.31 ± 11.30	12.32 ± 4.34
Mn-SOD (NU/ml)	10.66 ± 2.58	10.30 ± 3.47	11.71 ± 2.83
Catalase (mg $\text{H}_2\text{O}_2/\text{ml}/30$ min)	2.81 ± 0.89	3.07 ± 0.94	$3.58 \pm 0.81^{**}$
GSH-Px ($\mu\text{mol}/\text{ml}/\text{min}$)	0.069 ± 0.032	0.081 ± 0.023	0.080 ± 0.028
Erythrocytes			
Total SOD (NU/mg Hb)	10.28 ± 0.90	11.53 ± 4.49	10.92 ± 2.12
GSH-Px ($\mu\text{mol}/\text{g Hb}/\text{min}$)	17.19 ± 5.03	17.59 ± 3.95	15.25 ± 3.18

* $p < 0.05$ vs. Pb-B < 10 group; ** $p < 0.01$ vs. Pb-B < 10 group.
Pb-B, $\mu\text{g}/100$ ml.

TABLE 4. *Element concentrations in plasma and in erythrocytes*

	Concentrations in plasma ($\mu\text{g/ml}$)			Concentrations in erythrocyte ($\mu\text{g/g Hb}$)		
	Pb-B ≤ 10 ($n=22$)	10 < Pb-B ≤ 20 ($n=30$)	20 < Pb-B ($n=18$)	Pb-B ≤ 10 ($n=22$)	10 < Pb-B ≤ 20 ($n=30$)	20 < Pb-B ($n=18$)
As	0.021 $\pm$ 0.010	0.017 $\pm$ 0.010	0.020 $\pm$ 0.014	1.644 $\pm$ 0.273	1.964 $\pm$ 1.653	1.651 $\pm$ 0.279
Ca	97.55 $\pm$ 3.23	97.16 $\pm$ 4.05	99.87 $\pm$ 6.68	—	—	—
Cd	0.005 $\pm$ 0.006	0.003 $\pm$ 0.007	0.004 $\pm$ 0.005	0.067 $\pm$ 0.049	0.145 $\pm$ 0.459	0.062 $\pm$ 0.028
Co	ND	ND	ND	0.013 $\pm$ 0.011	0.047 $\pm$ 0.200	0.010 $\pm$ 0.009
Cr	ND	ND	ND	ND	0.030 $\pm$ 0.152	ND
Cu	1.010 $\pm$ 0.280	1.041 $\pm$ 0.205	1.110 $\pm$ 0.186	2.320 $\pm$ 0.504	2.569 $\pm$ 0.703	2.429 $\pm$ 0.487
Fe	3.175 $\pm$ 2.433	3.835 $\pm$ 6.706	2.185 $\pm$ 0.580	3638 $\pm$ 191	3597 $\pm$ 113	3573 $\pm$ 130
Ge	0.005 $\pm$ 0.004	0.006 $\pm$ 0.006	0.005 $\pm$ 0.004	0.161 $\pm$ 0.034	0.220 $\pm$ 0.327	0.157 $\pm$ 0.038
K	139 $\pm$ 9	143 $\pm$ 9	147 $\pm$ 14*	10319 $\pm$ 558	10427 $\pm$ 527	10484 $\pm$ 513
Mg	19.71 $\pm$ 1.42	19.41 $\pm$ 1.37	19.88 $\pm$ 1.49	187.7 $\pm$ 18.4	183.0 $\pm$ 15.4	176.3 $\pm$ 16.1*
Mn	ND	ND	ND	ND	ND	ND
Mo	0.001 $\pm$ 0.003	0.001 $\pm$ 0.005	0.001 $\pm$ 0.002	0.012 $\pm$ 0.031	0.056 $\pm$ 0.232	0.022 $\pm$ 0.027
Na	3150 $\pm$ 58	3139 $\pm$ 66	3160 $\pm$ 59	1184 $\pm$ 164	1285 $\pm$ 325	1270 $\pm$ 358
Ni	ND	0.006 $\pm$ 0.019	0.005 $\pm$ 0.018	0.003 $\pm$ 0.041	0.145 $\pm$ 0.804	ND
P	124 $\pm$ 18	126 $\pm$ 13	128 $\pm$ 13	1795 $\pm$ 143	1879 $\pm$ 227	1829 $\pm$ 155
Pb	0.011 $\pm$ 0.010	0.017 $\pm$ 0.033	0.021 $\pm$ 0.021	0.281 $\pm$ 0.246	0.701 $\pm$ 0.325**	1.626 $\pm$ 0.861**
Rb	0.181 $\pm$ 0.200	0.146 $\pm$ 0.034	0.153 $\pm$ 0.031	11.86 $\pm$ 3.46	13.33 $\pm$ 2.84	13.03 $\pm$ 3.36
Se	0.132 $\pm$ 0.035	0.130 $\pm$ 0.031	0.126 $\pm$ 0.021	0.618 $\pm$ 0.139	0.670 $\pm$ 0.207	0.728 $\pm$ 0.200*
Sn	0.010 $\pm$ 0.007	0.010 $\pm$ 0.007	0.010 $\pm$ 0.005	0.100 $\pm$ 0.338	0.103 $\pm$ 0.308	0.067 $\pm$ 0.070
Sr	0.023 $\pm$ 0.031	0.013 $\pm$ 0.007	0.014 $\pm$ 0.007	0.116 $\pm$ 0.067	0.129 $\pm$ 0.045	0.120 $\pm$ 0.049
V	0.015 $\pm$ 0.003	0.014 $\pm$ 0.003	0.014 $\pm$ 0.003	0.012 $\pm$ 0.009	0.045 $\pm$ 0.184	0.012 $\pm$ 0.008
Zn	1.440 $\pm$ 1.880	1.065 $\pm$ 0.264	1.220 $\pm$ 0.735	52.57 $\pm$ 16.60	53.49 $\pm$ 12.34	48.64 $\pm$ 12.00
Zr	0.015 $\pm$ 0.024	0.018 $\pm$ 0.022	0.023 $\pm$ 0.025	0.094 $\pm$ 0.042	0.124 $\pm$ 0.047	0.104 $\pm$ 0.038

* $p < 0.05$ vs. Pb-B < 10 group; ** $p < 0.01$ vs. Pb-B < 10 group.Pb-B, $\mu\text{g}/100\text{ ml}$ ND, under detection limit; —, not determined.

Concentrations of In and Te were under detection limit.

controls. These two groups showed highly significant differences. Fig. 3 shows the relationship between Pb-B and ALA dehydratase activity, and Pb-B and the inhibition rate. The correlation coefficients are $r = -0.786$ and $r = 0.927$, respectively.

Total SOD, Mn-SOD, Cu, Zn-SOD, catalase, and GSH-Px activities in plasma or in erythrocytes

As shown in Table 3, total, Mn- and Cu, Zn-SOD activities in plasma did not show a clear correlation with lead-exposure. Catalase activity in plasma markedly increased with an increase in Pb-B; 2.81 ± 0.90 unit (mg H_2O_2 /ml plasma/30 min) in the low Pb-B group, 3.07 ± 0.94 unit in the middle group, and 3.58 ± 0.81 unit in high Pb-B group. There were statistically significant differences between the low and the high Pb-B groups. GSH-Px activities in the plasma did not change, although, those in erythrocytes slightly decreased with increasing Pb-B.

Major and trace element concentrations in plasma and erythrocytes

Table 4 shows various element concentrations in the plasma and erythrocytes in the three groups. Each element concentration in plasma is expressed as $\mu\text{g}/\text{ml}$ plasma, while those in erythrocytes are expressed as $\mu\text{g}/\text{g}$ Hb, because erythrocytes were hemolyzed during strage at -80°C until analysis. Most Pb in the blood existed was present in the erythrocytes. A small amount of lead was found in plasma which increased with increasing Pb concentration in erythrocytes, although there were not statistically significant difference in plasma Pb between three groups. Therefore the ratio of Pb in plasma to Pb in erythrocytes was decreased with increasing Pb concentration in erythrocytes.

It was noticed that Se concentrations in erythrocytes increased with increasing Pb concentration, and showed a significant difference between the low Pb group (0.618 ± 0.139 $\mu\text{g}/\text{g}$ Hb) and the high Pb group (0.728 ± 0.200 $\mu\text{g}/\text{g}$ Hb) in erythrocytes. The Se concentration in plasma showed reversed behavior. It appears that the changing level of Se observed in these subjects did not effect GSH-Px in the plasma or in the erythrocytes.

Another element that was significantly different in erythrocytes in the Pb-B groups was Mg (187.7 ± 18.4 $\mu\text{g}/\text{g}$ Hb in low Pb-B group and 176.3 ± 16.1 $\mu\text{g}/\text{g}$ Hb in high Pb-B group).

In plasma samples, only the K concentration showed a significant difference; 139 ± 9 $\mu\text{g}/\text{ml}$ in the low Pb-B group and 147 ± 14 $\mu\text{g}/\text{ml}$ in the high Pb-B group.

DISCUSSION

The effects of lead absorbed in the human body on the activities of enzymes in the blood and on the concentrations of various elements were studied in 70 male subjects working at the same plant. Their periods of employment ranged from less than 1 year to over 30 years. The lead burden in these workers was mainly

from occupational lead exposure. Some of them were working near furnaces containing melted lead. Lead concentrations in the working environments were under $70 \mu\text{g}/\text{m}^3$, and the maximum lead concentration in the workers' blood was $50.2 \mu\text{g}/100 \text{ ml}$ whole blood, the mean $\pm$ s.d. of all 63 workers was $17.8 \pm 11.5 \mu\text{g}/100 \text{ ml}$. Therefore, the lead burden of this group was not considered to be severe. All subjects were divided into three groups according to their Pb-B, $\leq 10 \mu\text{g}/100 \text{ ml}$ ($n=22$), > 10 and $\leq 20 \mu\text{g}/100 \text{ ml}$ ($n=30$), and $> 20 \mu\text{g}/100 \text{ ml}$ ($n=18$). The average values of the three groups were compared.

Since the 1960s there have been many reports on the effects of lead on erythropoiesis. In a mass screening test, ALA-U was clearly higher in workers with over $40 \mu\text{g}/100 \text{ ml}$ Pb-B. For this reason many clinical investigators came to use ALA-U as one of the indices of lead-exposure, and the Ministry of Labor in Japan changed from using coproporphyrin in the urine (CP-U) to ALA-U as an index of lead-exposure. One reason was that determination of CP-U was not easy and was not specific. In the determination of ALA-U, analytical techniques were gradually simplified; extraction with organic solvent was replaced with three columns packed with resin. Recently, HPLC-fluorometry has been developed, which is much more sensitive and specific. Therefore, relationship between ALA-U and Pb-B has been changing; namely, ALA-U shows a marked increase when Pb-B is over $15 \mu\text{g}/100 \text{ ml}$ (Okayama 1992). Our results agreed with Okayama's report. ALA-U was significantly different in each group. This observation suggested to us how to read the results. The relationship between ALA-U and Pb-B depends upon the accuracy, specificity, and sensitivity of the method, and especially on the mechanisms of the determining procedure of ALA-U. It is important to pay attention to blank values and the recovery rates in the individual samples.

It is well known that ALA dehydratase activity is the most sensitive index of lead exposure. The inhibition rate emphasizes the specificity, and decreases individual variation of ALA dehydratase activity (Chiba et al. 1976). It is considered that ALA dehydratase is too sensitive to use as an index of lead exposure for a mass screening program.

PP concentration in whole blood was not significantly different between groups because of large s.d. values. Generally, ZP concentrations were much higher than PP concentrations in whole blood as shown in Table 2. Furthermore the intensity of fluorescence of ZP was much stronger than that of PP. The value of FEP depends largely upon ZP concentration.

In the past 20 years, working environments have improved considerably. Furthermore, workers have been better understanding their own jobs and their environments; they are paying more attention to which job and which environments are associated with health hazards. In recent years, there has been little severe lead poisoning caused by occupational lead exposure, although cases of occupational diseases were not rare some decades ago. Our own interests have

been widening from contaminated working environments to contamination in general living environments. We have to pay attention to low level exposure to toxic substances. We need to determine what indices we can use to detect health hazards of low level exposures. We tried to analyze some kinds of enzyme activities, and found that plasma catalase activity was influenced by lead exposure, and that SOD activity was not influenced by lead in our subjects. GSH-Px activity changed slightly. If the lead burden were stronger, GSH-Px might change according to the extent of lead exposure.

As to the concentrations of major and trace elements in the blood, Se, Mg, and K were considered to be influenced by lead. However, the Se changes do not appear to have any remarkable health effects. In the present study we can determine various kinds of elements in the body, while these analyses were considered nearly impossible a few decades ago. Advances like this are essential for preventive medicine.

References

- 1) Bonsnes, R.W. & Taussky, H.H. (1945) On the colorimetric determination of creatinine by the Jaffe reaction. *J. Biol. Chem.*, **158**, 581-591.
 - 2) Chiba, M., Tashiro, A., Nishima, T. & Kikuchi, M. (1976) Availability of activity ratio obtained from activity of 5-aminolevulinate hydro-lyase in human blood before and after heat treatment to evaluate lead exposure. *Ind. Health*, **14**, 67-74.
 - 3) Health and Welfare Statistics Association (1994) Present status of industrial hygiene. *Kousei no Shihyou*, **41**, 379-383. (in Japanese)
 - 4) Okayama, A. (1992) New fluorometric method for determining δ -aminolevulinic acid in urine — The meaning and practical application —. *Roudouisei Kanri*, **3**, 45-51. (in Japanese)
 - 5) Oyanagui, Y. (1984) Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. *Anal. Biochem.*, **142**, 290-296.
 - 6) Shinohara, A., Chiba, M. & Inaba, Y. (1994) Analysis of trace elements in biological materials by microwave induced plasma-mass spectrometry. *Jpn. J. Hygiene*, **49**, 924-934. (in Japanese)
 - 7) Wada, O., Toyokawa, K., Urata, G., Yano, Y. & Nakao, K. (1969) A simple method for the quantitative analysis of urinary delta-aminolevulinic acid to evaluate lead absorption. *Br. J. Ind. Med.*, **26**, 240-243.
 - 8) Watkinson, J.H. (1966) Fluorometric determination of selenium in biological materials with 2,3-diaminonaphthalene. *Anal. Chem.*, **38**, 92-97.
-