EVALUATION OF LEAD MOBILIZATION TEST WITH INTRAVENOUS INFUSION OF CaEDTA IN WORKERS OCCUPATIONALLY EXPOSED TO LEAD

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(Received August 22, 1975)

Twenty-three workers who had occupationally been exposed to lead were examined for blood and urinary lead contents (bPb, uPb), urinary delta-aminolevulinic acid and coproporphyrin contents (δ -ALA, Copro), erythrocyte δ -ALA dehydratase activities (ALA-D), hemoglobin concentration (Hb), and 2-hr and 24-hr urinary lead excretions since the start of 1-hr intravenous infusion of 20 mg CaEDTA per kilogram of body weight (uPb_{2-hr mob.}, uPb_{24-hr mob.}). The blood lead value ($\mu\text{g}/100\text{ g}$) ranged from 4 to 73 with an average of 24.

By factor analysis, the parameters were classified to three groups. The first group consisted of uPb_{24-hr mob.}, uPb_{2-hr mob.}, bPb, uPb and ALA-D; the second group, δ -ALA and Copro; and the third group, Hb.

There were found highly significant linear relationships between all pairs of bPb, uPb, ALA-D, uPb_{2-hr mob.} and uPb_{24-hr mob.} on a log scale. δ -ALA, Copro and Hb had less in extent, but significant correlations with five parameters mentioned above. bPb, uPb, Hb and uPb_{2-hr mob.} had the closest correlations with uPb_{24-hr mob.}; ALA-D, with bPb; and δ -ALA, Copro.

The criteria for the mobilization yield were estimated from the viewpoint of acceptable limit in lead absorption.

Practical usefulness of "2-hr method (uPb_{2-hr mob.})" was indicated as a simplified diagnostic mobilization test.

Interrelationships of parameters indicative of lead absorption have been extensively investigated in lead workers.^{1,2)} But the lead mobilization test with the intravenous administration of CaEDTA (Calcium Disodium Ethylene-diamine Tetraacetate) was seldom involved for the study of interrelationships of the parameters. If the mobilization test is one of the best means of estimating the active body burden of lead as disclosed experimentally by Teisinger *et al.*,³⁾ it should be of worth to examine the relations of the mobilization test to other parameters indicative of lead absorption. In this respect, the author found only two reports which revealed significant correlations of the mobilization yield of lead following intravenous administration of CaEDTA with urinary contents of lead and coproporphyrin⁴⁾ and with fluorescent erythrocytes⁵⁾ in lead-intoxicated people.

The present author analysed the interrelationships of the cardinal parameters including the mobilization yield in people free from occupational exposure to lead in the previous report, and found significant correlations between them though small in extent.⁶⁾ Through the procedure of factor analysis, the yield of mobilization was classified side by side with blood lead content to a group which had to be related to the body burden of lead.⁷⁾

In this report, lead workers were selected for the subjects and the author intended to analyse the relationships between the results of lead mobilization test and other cardinal parameters of lead absorption.

SUBJECTS AND METHODS

Subjects

The subjects studied were twenty-three male workers who had been exposed to lead occupationally for varying lengths of years. Occupation, age, interval of lead exposure and blood lead levels are as listed in Table 1. None of them had ever suffered from hematological, hepatic or renal diseases.

Table 1. Subjects examined.

Occupation	Numbers	Age (yr)	Lead exposure (yr)	Blood lead ($\mu\text{g}/100\text{ g}$)
Brass founder	1	57	16	73
Plastic worker	1	21	3	57
Stereotype founder	4	22, 23, 25, 37	4, 4, 4, 19	7, 39, 44, 52
Lead mill worker	2	42, 51	3, 21	29, 46
Stereotype examiner	2	43, 52	22, 28	11, 35
Glass maker	1	43	5	35
Battery maker	1	31	13	23
Typesetter	5	39, 52, 54, 55, 58	25, 30, 38, 39, 43	5, 7, 8, 10, 15
Type founder	1	36	19	13
X-ray technician ¹	5	22, 24, 25, 38, 41	3, 5, 5, 16, 18	4, 7, 7, 7, 8

¹The X-ray technicians handled a mass of lead with bare hands for routine roentgenographic examinations.

Collection of samples

Urine was collected for 24 hr to measure the urinary contents of lead, delta-amino-levalulinic acid and coproporphyrin (uPb, δ -ALA and Copro, respectively). Intravenous infusion of CaEDTA was given to each subject in a dosage of 20 mg per kg body weight in 250 ml of 5% glucose solution for 1 hr. Since the start of the infusion, urine was collected for 2 hr and the following 22 hr to analyse the 2-hr and 24-hr urinary contents of lead (uPb_{2-hr mob.} and uPb_{24-hr mob.}, respectively). Blood samples were collected on the day before the administration of CaEDTA to measure the blood lead

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content (bPb), erythrocyte delta-aminolevulinic acid dehydratase activities (ALA-D) and hemoglobin concentration (Hb).

Analytical methods

Urinary lead was determined by atomic absorption spectrophotometry (Perkin-Elmer 403), using a procedure of wet ashing, chelation by ammonium pyrrolidine dithiocarbamate and extraction to water-saturated isobutylmethylketone. BPb was also measured by atomic absorption spectrophotometry; ALA-D, by the method of Weissberg *et al.*;⁸⁾ δ -ALA, by the method of Wada *et al.*;⁹⁾ Copro, by fluorescence spectrophotometry (Hitachi, MPF-2A); Hb, by the cyanmethemoglobin method; creatinine in urine, by Jaffe's reaction; specific gravity of urine, by refractometry.

Statistical analysis

Factor analysis was performed by the use of Hitachi 8700, the University of Tokyo. Factor loadings are extracted by multiplying eigenvectors by the square root of corresponding eigenvalues through the method of principal component analysis.¹⁰⁾

RESULTS

1. Classification of parameters indicative of lead absorption by factor analysis

The means and standard deviations of the eight parameters are listed in Table 2. The values are transformed into logarithm in all the parameters except for Hb to normalize the distribution.

As shown in Fig. 1 a and b, the 8 parameters can be classified to 3 groups by

Table 2. Means and standard deviations of parameters indicative of lead absorption.

Parameters	Mean	SD
1) log bPb: $\mu\text{g}/100\text{ g}$	1.209 (24)	0.393
2) log uPb: $\mu\text{g}/24\text{ hrs}$	1.479 (61)	0.526
$\mu\text{g}/1$, SG 1.020	1.381 (49)	0.540
3) log ALA-D: u.	1.421 (31)	0.262
4) log δ -ALA: $\text{mg}/24\text{ hrs}$	0.553 (4.2)	0.250
$\text{mg}/1$, SG 1.020	0.455 (3.5)	0.256
5) log Copro: $\mu\text{g}/24\text{ hr}$	2.048 (135)	0.262
$\mu\text{g}/1$, SG 1.020	1.957 (116)	0.289
6) Hb: g/dL	14.9	1.0
7) log uPb _{2-hr mob.} : $\mu\text{g}/2\text{ hrs}$	1.669 (109)	0.562
$\mu\text{g}/1$, SG 1.020	2.518 (596)	0.500
8) log uPb _{24-hr mob.} : $\mu\text{g}/24\text{ hrs}$	2.462 (599)	0.529
$\mu\text{g}/1$, SG 1.020	2.322 (410)	0.517

The means of original values before the conversion to logarithm are shown in the parentheses.

SD means standard deviation; SG, specific gravity of urine.

Fig. 1. Factor analysis of parameters indicative of lead absorption. The (a) and (b) show a plain consisting of the first factor (FC 1) and the second factor (FC 2). Urinary contents of uPb, δ -ALA, Copro, uPb_{2-hr mob.} and uPb_{24-hr mob.} are expressed in the corrected concentration by urinary creatinine content and the corrected concentration to a specific gravity of urine in (a) and (b), respectively. The proportion of the first principal component to the total variance is 68 and 70% in (a) and (b), respectively. 81% of the information is involved in the two components in (a); and 82%, in (b). The eigenvalue is beyond 1.0 and 0.9 up to the second component in (a) and (b), respectively.

the procedure of factor analysis. The first group consists of uPb_{24-hr mob.}, uPb_{2-hr mob.}, bPb, uPb and ALA-D. The second group contains δ -ALA and Copro. Hb has a character intermediate between the two groups.

2. Relations of mobilization yield to blood and urinary contents of lead and ALA-D

As obvious from Table 3, there exist highly significant correlations in all the pairs of bPb, uPb, ALA-D, uPb_{2-hr mob.} and uPb_{24-hr mob.} ($p < 0.001$). The relationships between all the pairs of those 5 parameters are assumed to be linear on a log scale in the range of the present values (Fig. 2).

3. Relations of mobilization yield to δ -ALA, Copro and Hb

The yield of mobilization has less in extent, but significant correlations with δ -ALA, Copro and Hb ($0.001 < p < 0.05$) than with bPb, uPb and ALA-D, as shown in Table 3.

The relations of the mobilization yield and bPb to δ -ALA and Copro are plotted in

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Table 3. Correlation matrices of parameters indicative of lead absorption.

1)	2)	3)	4)	5)	6)	7)	8)	Correlation coefficient
1.000	0.782†††	0.909†††	0.291	0.387	0.431†	0.895†††	0.917†††	1) log bPb
0.828†††	1.000	0.799†††	0.581††	0.470†	0.411	0.864†††	0.886†††	2) log uPb
0.909†††	0.798†††	1.000	0.450†	0.579††	0.467†	0.830†††	0.892†††	3) -log ALA-D
0.444†	0.627††	0.504†	1.000	0.628††	0.396	0.447†	0.518†	4) log δ-ALA
0.524†	0.515†	0.616††	0.679†††	1.000	0.356	0.514†	0.588††	5) log Copro
0.431†	0.444†	0.467†	0.432†	0.414†	0.100	0.519†	0.525†	6) -Hb
0.905†††	0.900†††	0.812†††	0.467†	0.518†	0.457†	1.000	0.952†††	7) log uPb _{2-hr mob.}
0.919†††	0.931†††	0.866†††	0.617††	0.616††	0.483†	0.923†††	1.000	8) log uPb _{24-hr mob.}

Urinary contents of uPb, δ-ALA, Copro, uPb_{2-hr mob.} and uPb_{24-hr mob.} are expressed in the corrected concentration by urinary creatinine content and the corrected concentration to a specific gravity of urine above and below the diagonal, respectively. A dagger, double dagger and triple dagger indicate statistical significance at 5, 1 and 0.1% level, respectively. The sample size is 23.

Fig. 3. There is not qualitatively remarkable difference between the mobilization yield and bPb in the relationships with uPb, ALA-D, δ-ALA, Copro and Hb (Figs. 2, 3 and 4).

Hb is assumed to have straight relations with the logarithm of uPb_{24-hr mob.} and bPb (Fig. 4).

4. Relations of blood and urinary contents of lead, ALA-D, δ-ALA, Copro and Hb to yield of mobilization

On the correlation matrices (Table 3), uPb_{24-hr mob.} is conspicuous because the pair of parameters including the uPb_{24-hr mob.} shows the closest correlation coefficient in case bPb, uPb, Hb and uPb_{2-hr mob.} are a counterpart of the uPb_{24-hr mob.}, or the nextly or thirdly closest correlation in cases of ALA-D, δ-ALA and Copro, among the series of pairs concerned. For instance, the correlation coefficient of 0.917 is obtained for the pair of bPb vs. uPb_{24-hr mob.}, and this is the highest among all the correlation coefficients calculated on bPb and each of other parameters.

5. Criteria for the mobilization yield from the viewpoint of acceptable limit in lead absorption

For the differentiation of acceptable and excessive lead exposure, such levels in the biochemical indicators as 80 µg/dl (bPb), 150 µg/l (uPb), 20 mg/l (δ-ALA) and 500 to 800 µg/l (Copro) were proposed as critical by Lane *et al.*¹¹⁾ and Zielhuis.¹⁾ The Japanese Ministry of Labour¹²⁾ prescribed as the criteria for the diagnosis of occupational lead poisoning to be legally compensated: bPb, 60 µg/dl; uPb, 150 µg/l; δ-ALA, 6 mg/l; Copro, 150 µg/l; Hb, 12.5 g/dl for man, 11.0 g/dl for woman.

Fig. 2. Interrelationships of mobilization yield, blood and urinary contents of lead and ALA-D. Solid lines show fitted regression lines of Y-axis on X-axis; and broken lines, X-axis on Y-axis. Triple dagger indicates statistical significance at 0.1% level.

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Fig. 3. Relations of mobilization yield and blood lead content to δ -ALA and Copro. A dagger and double dagger indicate statistical significance at 5 and 1% level, respectively.

Fig. 4. Relations of mobilization yield and blood lead content to Hb. Solid lines show fitted regression lines of Y-axis on X-axis; Broken lines, X-axis on Y-axis. A dagger indicates statistical significance at 5% level.

In order to apply results of the mobilization test to the diagnosis, the data have to be coordinated with the other biochemical indicators. In this connection, the similarity of the mobilization yield to bPb in terms of the classification due to the factor analysis and the comparative relationship to the other parameters provides the logical basis for the coordination.

The corresponding levels of uPb_{24-hr mob.} and uPb_{2-hr mob.} to each of bPb; 60 $\mu\text{g}/100\text{ g}$, and uPb; 150 $\mu\text{g}/\text{l}$ were obtained from the regression equations, and the figures are shown with the lower 90% and 95% prediction limits in Table 4.

Table 4. Acceptable limits of lead mobilization test: corresponding level of mobilization yield to acceptable limits of blood and urinary lead levels and the lower 90% and 95% prediction limits.

Acceptable limits		uPb _{24-hr mob.}		uPb _{2-hr mob.}	
		$\mu\text{g}/24\text{ hr}$	$\mu\text{g}/\text{l}$, SG 1.020	$\mu\text{g}/2\text{ hr}$	$\mu\text{g}/\text{l}$, SG 1.020
bPb, 60 $\mu\text{g}/100\text{ g}$	Corresponding level	1476	1023	242	1487
	Lower 90% (95%) prediction limit	611 (508)	422 (351)	75 (59)	592 (488)
uPb, 150 $\mu\text{g}/\text{l}$	Corresponding level	1431	1028	254	1460
	Lower 90% (95%) prediction limit	506 (407)	401 (329)	84 (66)	523 (422)

DISCUSSION

In contrast to the present results in workers who had occupationally been exposed to lead, the ALA-D and urinary lead content were classified not to the group consisting of the mobilization yield and blood lead content but to the group consisting of δ -ALA and Copro in the previous factor analysis for the occupationally non-exposed people.⁷⁾ This contrast may suggest the qualitative difference of human responses to lead between occupationally non-exposed people and workers occupationally exposed to lead. ALA-D and urinary lead are considered as having strongly reacted to lead in the level of sub-clinical state of occupational lead absorption.

The close relations of the mobilization yield to various cardinal parameters of lead absorption, disclosed by the present study, confirmed that the lead mobilization test with intravenous administration of CaEDTA was an useful method to assess the extent of human absorption of lead.

In Table 5, the acceptable limits of urinary lead contents following intravenous administration of CaEDTA in workers occupationally exposed to lead are compared with values given by various authors.¹³⁻¹⁹⁾ Even in consideration of the difference of the dosis of CaEDTA, the lower level of 90% prediction interval for individual values is lower in the present quantitative analysis than the acceptable values estimated qualitatively by other authors. Further clinical and quantitative studies should be made in detail

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Table 5. Comparison of values given by various authors as the acceptable limits of lead mobilization test with intravenous administration of CaEDTA in workers occupationally exposed to lead up to time of examination.

Author	No. of cases	Dosis of CaEDTA	Acceptable limits mg, lead/24 hrs
Leckie & Tompsett ¹³⁾ (UK, 1958)	8 ^{A)}	2 g. inf.	1.5 ^{B)}
Albahary <i>et al.</i> ¹⁴⁾ (French, 1961)	11 ^{C)}	0.5–1 g inf.	1.2 ^{B)}
Teisinger ¹⁵⁾ (Czechoslovakia, 1964)	50 ^{D)}	?	0.8 to 1.0 ^{E)}
Teisinger ¹⁶⁾ (Czechoslovakia, 1971)	50 ^{F)}	2 g. i. v.	2.0 ^{B)}
Araki <i>et al.</i> ¹⁷⁾ (Japan, 1974)	23 ^{G)}	20 mg/kg inf.	1.4 to 1.5 ^{H)} 0.5 to 0.6 ^{I)}

There are further reports by Zambrazo *et al.*¹⁸⁾ and Brieger & Rieders.¹⁹⁾

Notes:

- A) uPb: 50 to 330 $\mu\text{g}/24\text{ hr}$, Copro: 40 to 1000 $\mu\text{g}/\text{l}$
- B) the lowest value
- C) manifested lead poisoning with uPb: 55 to 235 $\mu\text{g}/\text{l}$
- D) incomplete positive laboratory tests without subjective complaints
- E) maximum permissible value
- F) bPb: 60 $\mu\text{g}/100\text{ g}$ or more
- G) bPb, uPb, ALA-D, δ -ALA, Copro & Hb, examined
- H) fitted level to bPb 60 $\mu\text{g}/100\text{ g}$ & uPb 150 $\mu\text{g}/\text{l}$
- I) lower 90% prediction limit

especially in subjects suffering from clinical lead poisoning.

The usefulness of 2-hr method as a simplified diagnostic mobilization test, as suggested by the previous report,⁶⁾ was supported by the following findings. The correlation between the 2-hr method and the 24-hr method was highly significant (Table 3). The correlations of the 2-hr method with other parameters were nearly equal, though somewhat lower, to those of the 24-hr method (Table 3). And both the parameters were classified to the same group through factor analysis. If 2-hr urine samples are properly collected, the 2-hr method which was proposed for the first time by Yamada²⁰⁾ may be of practical use because it requires the least time interval among the simplified methods so far reported.^{21–24)}

The criteria for ALA-D activity in lead absorption were mentioned in some papers from the viewpoint of corresponding level to the acceptable limits of other parameters.^{8, 25, 26)} The present author estimated the corresponding level of the ALA-D to the mobilization yield of 1.5 mg/24 hr at 12.9 u. (0.387 $\mu\text{mole}/\text{ml}$ RBC/hr; 32% of the mean value in the occupationally non-exposed men) from the regression equation in the process of the present study. The details will be mentioned in other paper together with 90% and 95% prediction limits.

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ACKNOWLEDGEMENT

The author wishes to express his thanks to Dr. K. Ushio, vice director of Tokyo Rosai (Labour Accident) Hospital, for his interest and kind help, and Prof. H. Katsunuma, University of Tokyo, and Prof. T. Suzuki, Tohoku University, for their encouragement and valuable advice. Thanks are also due to Miss M. Sakata and Miss H. Watanabe for their technical assistance. This paper was presented at the 47th Annual Meeting of Japan Association of Industrial Health, 1974.

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