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Biological monitoring of workers with past lead exposure

Biochemical findings

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CORSI G, BARTOLUCCI GB. Biological monitoring of workers with past lead exposure: Biochemical findings. *Scand j work environ health* 8 (1982) 260–266. In 56 subjects with lead exposure that had terminated at least 3 a previously and in a reference group of nonexposed subjects, the behavior of several biochemical indicators of dose and effect were studied. The mean values for blood lead (PbB) (1.4 ± 0.4 μmol/l), chelatable lead (PbUEDTA) (3.4 ± 1.5 μmol/24 h) and free erythrocyte protoporphyrin (FEP) (62 ± 25.2 μg/100 ml red blood cells) were found to be significantly higher in the subjects with past lead exposure than in the referents. The cut-off levels (mean + 2 SD calculated for the reference group) of PbB, PbUEDTA and FEP were exceeded in 35.7, 67.9, and 28.6 % of the exposed subjects, respectively. A normalization of lead doses and indicators of effect was reached only when the exposure had not exceeded 2 a. The time elapsed after termination of exposure did not significantly reduce the PbB level and PbUEDTA excretion, whereas these parameters correlated significantly with the length of exposure. A close correlation was found between the PbB and the PbUEDTA. Due to poor sensitivity at PbUEDTA values of less than 5.3 μmol/24 h (91 % of the cases), PbB and FEP were not useful for subjects whose exposure had terminated more than 3 a earlier. In these cases, only PbUEDTA was capable of revealing elevated active lead deposits.

Key terms: blood lead, chelatable lead, free erythrocyte protoporphyrin, length of exposure, time elapsed since exposure, validity of biochemical tests.

Biological monitoring of workers with past lead exposure has shown the presence of active deposits of the metal and alterations in several biological indicators of effect on heme synthesis (2, 3, 4, 7, 8, 12, 14, 15, 17, 18, 19, 21, 22). In some cases, the values reported were similar to those that, according to different authors (5, 11, 24, 25, 26, 27), indicate critical biological effects and consequences both at the subclinical and clinical levels.

With this in mind we examined, in relation to the effects on porphyrin metabolism, subjects whose exposure had terminated at different times. This study also allowed us to evaluate the influence of length of exposure and time elapsed since exposure on the dose indicators and to examine the behavior of several dose and effect indicators when a lead equilibrium has been reached in the various compartments.

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Subjects and methods

Selection of subjects

The clinical records of workers admitted to the Institute of Occupational Medicine of the University of Padua in the last 27 a were considered, and workers presenting symptoms of poisoning and biochemical signs of exposure to high lead concentrations were selected. Among the subjects traced, a further selection based upon the following criteria was effected: certainty regarding the termination of exposure (eg, exclusion of subjects who, although distant from direct exposure, still worked in the same plant); exposure terminated at least 3 a earlier; no treatment with chelat-

ing compounds [calcium disodium ethylenediamine tetra-acetate ($CaNa_2EDTA$)] in the last year; absence of conditions recognized as capable of influencing lead or porphyrin metabolism; and limited alcohol (less than 100 g/24 h) and tobacco (less than 20 g/24 h) intake.

Fifty-six subjects agreed to participate in the research project. Table 1 reports the main biochemical parameters at the time of the subjects' first hospitalization and the respective "normal" values.

All the subjects were males and lived in the province of Padua, the vast majority residing in an agricultural area. Concerning lead exposure, the subjects were employed as follows: 22 lead founders, 15 workers in the battery industry, 6 glazers in the ceramics industry, 5 men employed in the mixing of lead stearate in a plastics factory, 7 workers involved in different lead-exposed tasks. Further data for the series are shown in table 2.

The reference group consisted of 20 subjects without occupational lead exposure; this group was matched with the series for age (table 2), sex, alcohol and tobacco intake, and area of residence. The subjects with past lead exposure and the referents underwent the same examinations, performed during the same time.

Biochemical analysis

Urinary δ-aminolevulinic acid (ALAU), urinary coproporphyrin (CPU), and creatinine levels were determined in urine samples collected in the morning. Blood lead (PbB), free erythrocyte protoporphyrin (FEP), and hematocrit values were determined in samples of heparinized venous blood. On the occasion of the blood sampling, 10 ml of a 10 % $CaNa_2EDTA$ solution was given intravenously; the chelatable lead (PbUEDTA) level was determined in urine samples collected over the following 24 h.

The Fernandez method (10) was used for PbB; the PbUEDTA was determined by acidification with nitric acid, appropriate dilution, and pyrrolidin dithiocarbamic acid ammonium salt/methyl isobutyl ketone system extraction. In both tests the dosage was done with atomic absorption spectrophotometry using a graphite HGA 76B furnace and a background corrector.

Table 1. Values (mean, standard deviation and range) of several biochemical parameters at the time of the first hospitalization (56 selected subjects[a]).

Biochemical parameters	Values at first hospitalization				"Normal" values[b]
	N	Mean	SD	Range	
Blood lead (μmol/l)	15	4.8	1.6	2.9– 8.7	< 1.9
Urinary lead (μmol/24 h)	20	1.2	0.4	0.7– 2.1	< 0.5
Chelatable lead (μmol/24 h)	30	25.9	17.9	7.1– 69.5	< 2.4
Erythrocyte protoporphyrin (μg/100 ml red blood cells)	56	780.0	668.0	172.0–2,056.0	< 60.0
Urinary coproporphyrin (μg/24 h)	56	1,726.0	1,339.0	180.0–6,005.0	< 150.0
Urinary δ-aminolevulinic acid (mg/24 h)	13	27.0	19.0	6.0– 79.0	< 6.0

[a] Several biochemical parameters were determined for smaller numbers of cases.
[b] "Normal" values used at the Occupational Medicine Institute, University of Padua.

Table 2. Age of the referents and the age, length of exposure, and time elapsed since exposure of subjects with past lead exposure.

	Referents' age (a) (N = 20)	Subjects with past lead exposure (N = 56)		
		Age (a)	Length of exposure (months)	Time elapsed since exposure (months)
Median	55	57	68.4	146.4
Mean	52.5	53.5	102.8	147.3
SD	12.9	13.3	114.0	75.6
Range	23–75	23–72	1–480	36–324

The method of Schwartz et al (20) was followed for CPU, and the Piomelli method (13) for FEP. In both cases spectrophotofluorometer readings were done.

The method proposed by Davis & Andelman (9), employing the ALA-duo column Kit, was used for ALAU.

Creatinine was determined in urine with the Jaffé method without deproteinization. The CPU and ALAU values were corrected for the creatinine concentration.

Results

Table 3 reports the means, standard deviations, and ranges of the biochemical parameters of subjects with past lead exposure and the corresponding values of the referents. Student's t-test demonstrated a significant difference for PbB, PbUEDTA, and FEP and no significant difference for CPU and ALAU between the two groups.

The percentage of subjects with past exposure who had biochemical parameters with values exceeding the respective cut-off levels (means + 2 SD calculated for the referents) were 35.7 for PbB, 67.9 for PbUEDTA, 28.6 for FEP, 14.3 for CPU, and 7.1 for ALAU.

There was no relation between the biochemical parameters observed in the present research and the values of respective parameters revealed at the time of the first hospitalization. None of the regression correlation coefficients followed were significant. In order to evaluate the extent to which the dose indicators are influenced by the length of exposure and the time elapsed since exposure, the multiple regression technique was used. The most suitable equation for both PbB and PbUEDTA was $\log y = a + b \log x_1 - b \log x_2$, where y is PbB or PbUEDTA, x_1 the length of exposure, and x_2 the time elapsed since exposure. A high significance ($p < 0.001$) was found for the two regressions; the multiple correlation coefficient (r) was 0.56 for PbB and 0.72 for PbUEDTA. Both PbB and PbUEDTA are influenced only by the length of exposure with predictive values of 32.7 and 53 %, respectively. In view of the poor predictive value of the time elapsed since exposure (3.5 % for PbB and 5.5 % for PbUEDTA), and its low significance ($p < 0.2$ and < 0.1, respectively), this parameter was not considered in the successive analyses of the data. Fig 1 shows the regression lines of PbB, PbUEDTA, and FEP on length of exposure.

Table 3. Values (mean, standard deviation and range) and significance (Student's t-test) of several biochemical parameters for subjects with past lead exposure and referents.

Biochemical parameters	Subjects with past lead exposure			Referents			p-Value
	Mean	SD	Range	Mean	SD	Range	
Blood lead (μmol/l)	1.4	0.4	0.5– 2.1	1.0	0.2	0.5– 1.4	< 0.001
Chelatable lead (μmol/24 h)	3.4	1.5	0.9– 6.7	1.5	0.4	0.7– 2.4	< 0.001
Free erythrocyte protoporphyrin (μg/100 ml red blood cells)	62.0	25.2	25.0–130.0	42.7	12.0	25.0–65.0	< 0.005
Urinary coproporphyrin (μg/g creatinine)	68.3	52.1	21.0–283.0	52.4	21.1	22.0–92.0	NS
Urinary δ-aminolevulinic acid (mg/g creatinine)	2.7	1.0	1.2– 5.8	2.4	1.0	1.0– 4.8	NS

Table 4. Relationship between length of exposure and percentage of subjects with past lead exposure exceeding the cut-off levels of several biochemical parameters.[a]

Exposure (months)			Number of subjects	Percentage with blood lead > 1.5 (μmol/l)	Percentage with chelatable lead > 2.4 (μmol/24 h)	Percentage with free erythrocyte protoporphyrin > 66.7 (μg/100 ml red blood cells)
Range	Mean	SD				
≤ 6	3	2	10	0	0	0
7– 24	18	5	8	12.5	37.5	12.5
25–120	68	27	21	42.9	85.7	28.6
121–240	177	38	10	50.0	100	25.0
≥241	341	93	7	66.7	100	100

[a] Cut-off levels are means + 2 SD calculated for the referents (see table 3).

When the values for 18 subjects with lengths of exposure of less than 2 a were excluded, the correlation was significant for PbUEDTA ($r = 0.36$, $p < 0.05$) and FEP ($r = 0.43$, $p < 0.01$), while it was not significant for PbB ($r = 0.21$). CPU and ALAU did not correlate with the length of exposure. A comparison between the length of exposure and the percentage of subjects that exceeded the cut-off levels of PbB, PbUEDTA, and FEP is shown in table 4.

The regression function of PbB on PbUEDTA that best fit the data was $\log y = a + b \log x$. The FEP correlated both with PbB and PbUEDTA; for both, the best regression function was $\log y = a + b\ x$. The corresponding regression lines are shown in fig 2. The correlations between the indicators of dose (PbB and PbUEDTA) and the other indicators of effect considered (CPU and ALAU) were not statistically significant. The validity of PbB for the prediction of PbUEDTA was tested at different PbUEDTA levels (table 5); it was slight for the values ranging from 2.4 μmol/24 h (497 μg = PbUEDTA cut-off level) to 5.3 μmol/24 h (1,100 μg). Of the subjects 51.6 % showed normal PbB values in this range.

The FEP validity at different PbB levels was low due to the low sensitivity, which improved when PbUEDTA was chosen as the parameter of dose (table 6).

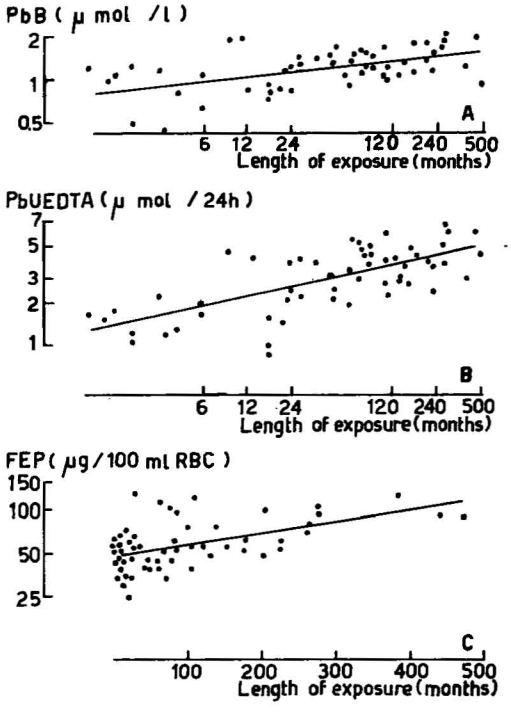

Fig 1. Relationship between several biochemical parameters and length of exposure [A: blood lead (PbB) (log scale) on length of exposure (log scale), $r = 0.57$, $p < 0.001$; B: chelatable lead (PbUEDTA) (log scale) on length of exposure (log scale), $r = 0.73$, $p < 0.001$; C: free erythrocyte protoporphyrin (FEP) (log scale) on length of exposure, $r = 0.53$, $p < 0.001$]

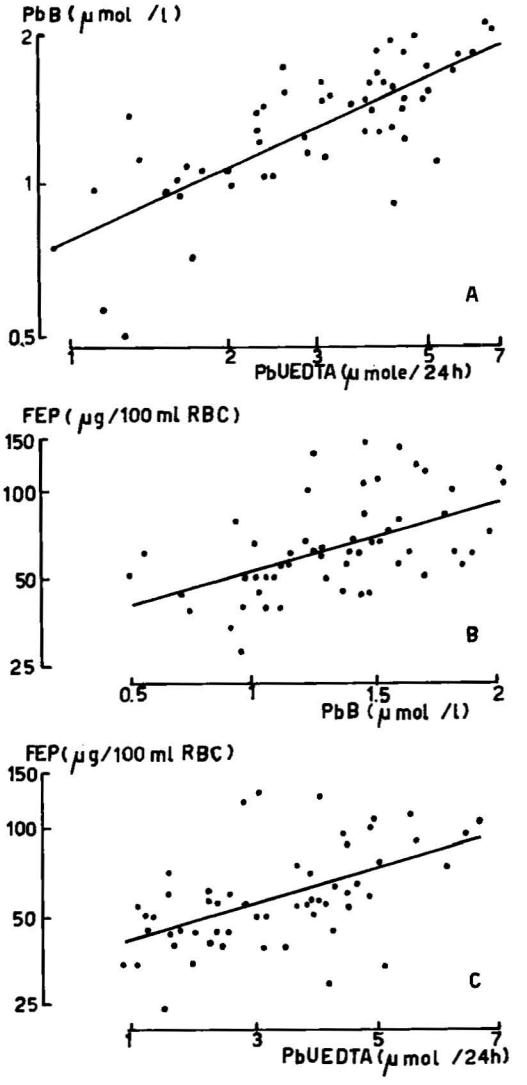

Fig 2. Relationship between several biochemical parameters. [A: blood lead (PbB) (log scale) on chelatable lead (PbUEDTA) (log scale), $r = 0.76$, $p < 0.001$; B: free erythrocyte protoporphyrin (FEP) (log scale) on PbB, $r = 0.51$, $p < 0.001$; C: FEP (log scale) on PbUEDTA, $r = 0.55$, $p < 0.001$]

Table 5. Validity (sensitivity + specificity) of blood lead (PbB) for prediction of chelatable lead (PbUEDTA) at different levels (PbB cut-off level same as in table 4).

PbUEDTA (µmol/24 h)	PbB		
	Sensitivity	Specificity	Validity
≥ 1.4	0.38	1.00	1.38
≥ 2.4	0.53	1.00	1.53
≥ 3.4	0.59	0.89	1.48
≥ 4.3	0.71	0.78	1.49
≥ 5.3	1.00	0.72	1.72

Table 6. Validity (sensitivity + specificity) of free erythrocyte protoporphyrin (FEP) for prediction of blood lead (PbB) and chelatable lead (PbUEDTA) at different levels (FEP cut-off level same as in table 4).

	FEP		
	Sensitivity	Specificity	Validity
PbB (µmol/l)			
≥ 0.7	0.29	1.00	1.29
≥ 1.2	0.38	0.94	1.32
≥ 1.7	0.50	0.78	1.28
PbUEDTA (µmol/24 h)			
≥ 1.4	0.32	1.00	1.32
≥ 2.4	0.41	0.95	1.36
≥ 3.4	0.46	0.89	1.35
≥ 4.3	0.71	0.86	1.57
≥ 5.3	1.00	0.78	1.78

Discussion

The PbB, PbUEDTA, and FEP values of the exposed group were significantly different from those of the reference group, but both the mean and maximal values of these parameters were only slightly altered, unlike findings reported for similar series by other authors (2, 14). The CPU or ALAU increases found in a few cases could not be attributed to lead, as normal PbB and PbUEDTA levels were found in these subjects.

According to several authors (5, 24, 25, 26, 27) values similar to those found by us would not result in clinical signs of lead poisoning. We must therefore conclude that it is not likely that permanent moderate active lead deposits following a time lapse of at least 3 a after exposure have clinical consequences.

The decrease in PbB and PbUEDTA due to period of time elapsed after termination of exposure was slight and not statistically significant. We believe that this result was due to the fact that our subjects had not been exposed for at least 3 a (in 91 % of cases for at least 5 a).

There was, on the other hand, a significant correlation between the length of exposure and PbB, PbUEDTA, and FEP. Moreover, all 10 subjects with a length of exposure of less than six months presented normal values for the various biochemical parameters, whereas, of the eight with length of exposure ranging from 6 to 24 months, six had normal values and two presented the most serious clinical picture of poisoning observed in the entire series. On the other hand, three subjects had lengths of exposure of 30, 48 and 60 months and presented PbUEDTA values within the normal range; these subjects had had a dishomogeneous interrupted exposure. We therefore believe that, only if chelating therapy is undertaken within 2 a of the beginning of exposure to high concentrations, can the lead deposit be consistently reduced.

It is evident that the presence of numerous normal values for length of exposure of less than 2 a considerably influences the regression. For a length of exposure of more than 2 a only, PbUEDTA and FEP, unlike PbB, still retain a signifcant correlation. As confirmed by recent research (1), this finding may be due to the fact that PbB is influenced more by daily lead intake than lead released from the skeleton, even in subjects with previous exposure.

Few data are available in the literature on the relation between PbB and PbUEDTA in subjects with past lead exposure or poisoning; our correlation coefficient of 0.76 is similar to those of Chisolm et al (8) and those of Přerovská & Teisinger (calculated by us) (14). The discrepancy with the data of Alessio et al ($r = 0.54$) (3) may be explained by the different lead concentrations in the various compartments in relation to the different periods of times elapsed since termination of exposure rather than, as other authors (3) suggest, by the different metabolism of adolescents and adults. In fact, the groups of Chisolm et al, Přerovská & Teisinger, and our own include subjects of considerably different ages who had not been exposed for at least 3 a, whereas several subjects in

the group of Alessio et al had been exposed up to a year before.

In spite of the close correlation between PbB and PbUEDTA, the poor predictive value of PbB for PbUEDTA values of under 3.1 μmol/24 h (1,100 μg) (found in the vast majority of cases examined by us) leads us to conclude that PbB can no longer be considered useful for subjects with past lead exposure. The FEP on the PbB regression of exposed subjects is exponential (6, 16, 22, 23); this phenomenon is also confirmed for previous exposure by our data. The low predictive value of the FEP on the PbB observed by us has also been pointed out by Baloh (6) and Alessio et al (2, 4).

On the other hand, the correlation coefficients between PbUEDTA and FEP, high for Alessio et al (2, 4), was low for us. This variation may depend upon the different ranges of PbUEDTA considered in the respective series. Our data show a good validity for FEP as a dose indicator for PbUEDTA values higher than 5.3 μmol/24 h (1,100 μg). Moreover, since, in our series, only 8.9% of the subjects had a PbUEDTA value of more than 5.3 μmol/24 h, we believe that FEP is not adequate for the prediction of the biologically effective body burden in subjects with exposure that terminated at least 3 a earlier. In these cases, for the parameters considered in this report, only PbUEDTA is capable of revealing elevated active lead deposits.

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