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Biochemical Diagnosis of Occupational Exposure to Lead Toxicity

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The heavy metal, lead, is a major environmental pollutant and its toxicity continues to cause health problems in many occupations. Lead and its compounds are widely used in industries, automobile assembly plants, autobody repair and servicing stations (Ludwig et al. 1965; Grandjean 1979; David et al. 1982). Studies have shown that workers to be at risk in lead refineries, smelting plants, automobile assembly plants, repair shops and servicing stations, petrol bunks, battery manufacture, ship building and other industries (Ludwig et al. 1965; Clausen and Rastogi 1977; WHO 1977; Grandjean 1979; Grandjean and Fischbein 1980; Guerin 1981; Lilis et al. 1981). Although major symptoms of lead poisoning are uncommon at blood concentrations below 40 µg/100 ml, some workers appear to be inordinately sensitive to the metal and develop symptoms even at lower blood concentrations (Lamola and Yamane 1974; Tomokuni and Ogata 1976; Raghavan et al. 1980). Hence, blood lead (Pb-B) levels are often used for biological monitoring of exposure and risk of poisoning. However, blood lead levels in subjects without occupational exposure varies with age, sex (males have higher levels than females), drinking and smoking habits (Quinn 1985) and with area of living.

Lead has been shown to interfere with the biosynthesis of heme in a number of in vitro systems and in experimental animals as well as in human beings. Several steps of the heme biosynthetic chain are subject to the toxic effects of lead. ALA- dehydratase and Ferrochelatase, in particular, are two enzymes which are strongly inhibited by lead, leading to decreased heme synthesis, a constituent of hemoglobin (Chisolm 1974; Sassa et al. 1975; Shemin 1975). The inhibition of ALA dehydratase in the red blood cells by lead is generally recognised as the most sensitive index of the individuals exposure to this environmental chemical. A significant decrease in the enzyme's activity in erythrocytes occurs even at blood levels in the so called upper "normal ranges" (20-40 µg/ 100 ml blood) (Millar et al. 1970; Granick et al. 1973).

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Earlier reports show that the determination of blood lead content (Pb-B), zinc protoporphyrin levels and erythrocyte Aminolevulinic acid dehydratase (ALA.D) are widely used as biological indicators for lead toxicity (Beritic et al. 1977; Grandjean 1979). Hence, the aim of the present study was to screen for occupational exposure to lead in the workers of three different occupations and correlate their blood lead levels with erythrocyte ALA.D and total blood porphyrin content as biochemical indicators of lead exposure.

MATERIALS AND METHODS

We studied 107 male subjects (age group 17 to 55 years) from three different occupations like autobody workshop workers, professional automobile drivers and petrolbunk workers. They were screened for possible exposure to lead and detailed information regarding their age, experience and alcoholic intake was obtained.

Blood samples (5 ml) were collected into heparinized containers and stored at $4^{o}C$. Erythrocyte ALA.D activity was measured in triplicates, in 20 µl aliquots, within 6 hr of blood collection, according to the method of Granick et al. (1973). The results were expressed as n moles of porphobilinogen formed per ml erythrocytes per hour at $37^{o}C$. Total blood porphyrin content was estimated according to the method of Heller et al. (1971) by extracting the porphyrins in to 1.5 N Hcl and taking absorption at A_{380}, A_{407} and A_{430}. The following formula was used to calculate the erythrocyte porphyrin concentration.

$$\frac{[2\ (A_{407}) - (A_{380} + A_{430})] \times 1.28 \times \text{ml HCl Soln.} \times 100}{\text{Hematocrit} \times \text{ml whole blood}} = \mu g/100\ ml\ \text{of RBC}$$

The factor 1.28 was derived from the absorptivity of pure protoporphyrin in the solvent system employed in the assay.

Blood lead was measured with the Perkin Elmer 4000 Atomic Absorption spectrophotometer with graphite furnace and deuterium back ground correction (Giri et al. 1983). The semilogarithmic transformation was employed for the study of correlation between Pb-B to erythrocyte ALA.D and total porphyrin levels (Bortoli et al. 1986). The equation used to perform least square regression analysis was $\hat{y} = \hat{a} + \hat{b}x$.

RESULTS AND DISCUSSION

Potential health hazards from lead was evident in many small automobile workshops and other work places due to excessive usage of petrol and petroleum by products (Guerin 1981) and inhalation of automobile exhaust is a considerable source for the body burden of lead (Grandjean and Fischbein 1980). Hence, the subjects of the present study were the workers of automobile work shops (i.e. mini garages), petrol bunk and professional automobile drivers. Since there are reports that lead exposed workers who consume alcohol

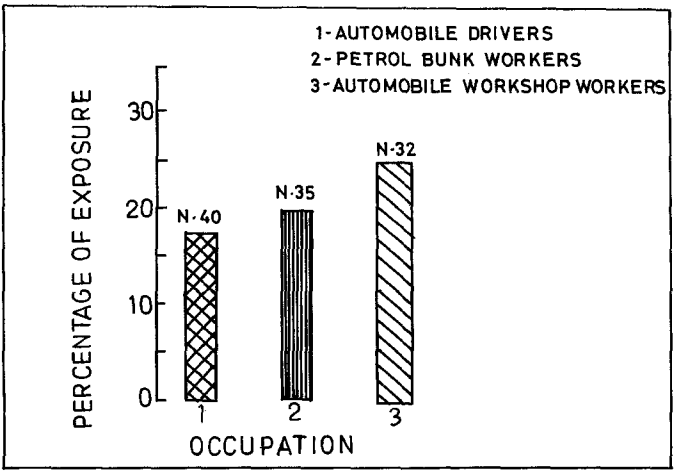

Fig 1: Percentage of exposure resulting in high blood lead content in different occupational workers.

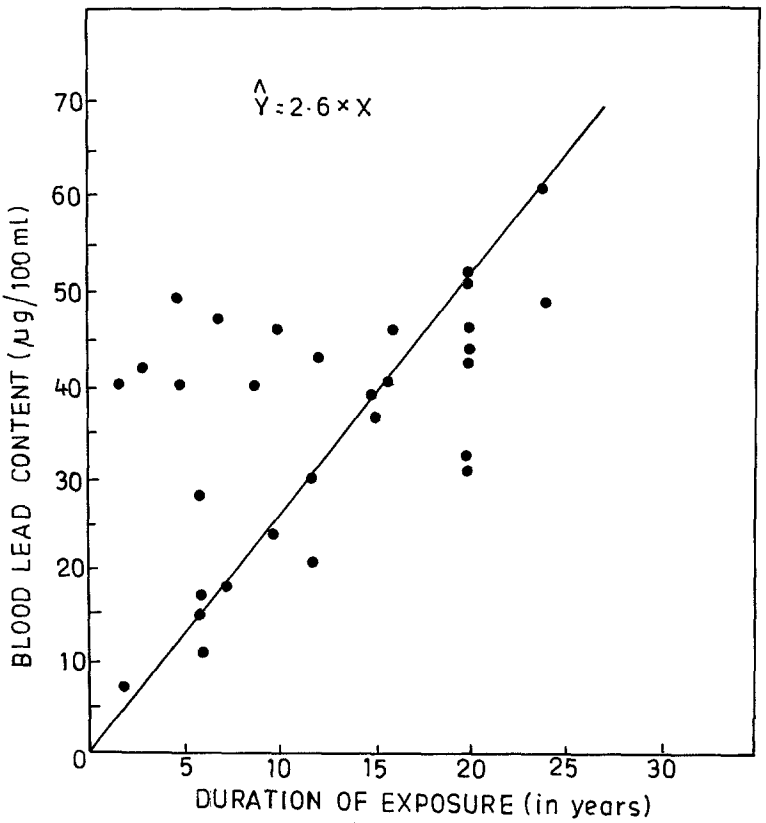

Fig 2: Relationship between the duration of exposure and blood lead content.

regularly were found to have a definitely higher incidence of lead poisoning than those with lower liquor consumption (Cramer 1966). Hence, information regarding their age, number of years of experience, drinking and smoking habits were obtained to account for the effect on physiological variations of the measured parameters.

Fig. 1. shows the results of the percentage of occupational exposure to lead in the workers of three different categories studied. Our results show that automobile workshop workers had higher incidence of exposure than petrol bunk workers followed by automobile drivers. Similar studies on the occupational exposure to lead show that the workers of small autobody repair shops and autobody workers in an automobile assembly plant were found to be at risk for lead toxicity (David et al. 1982; Lilis et al. 1981). Further, we have observed that there was positive relationship between the number of years of exposure to that of increased blood lead levels (Fig. 2).

Determination of ALA dehydratase activity in erythrocytes is sensitive, and is widely used as a biological indicator of exposure to environmental lead even at low levels (Granick et al. 1973). The activity of ALA dehydratase in the blood of humans changes in various hematological diseases, hereditary tyrosinemia, porphyrias, liver cirrhosis, alcoholism and poisoning with heavy metals such as lead. In the present findings, the logarithm of ALA.D activity of erythrocytes has been shown to decrease linearly with an increase in blood lead concentration (Fig. 3). The inhibition of the erythrocyte ALA.D is due to the interaction of lead with the sulfhydryl groups present at the active site of the enzyme (Sassa 1978).

The interaction of lead with the heme biosynthetic pathway illustrates how this pathway can be used as an indicator of exposure to toxic agents. Earlier reports show that lead inhibits ferrochelatase activity resulting in the accumulation of porphyrins in the blood (Sassa et al. 1975), and there by decreasing heme biosynthesis (Chisolm 1974). Similarly, our results regarding the relationship between the blood lead content and total porphyrin levels show a positive correlation between blood lead (Pb-B) and porphyrin levels (Fig. 4). An increase in erythrocyte protoporphyrin levels observed in chronic lead poisoning has been attributed to the interaction of lead with the sulfhydryl groups of ferrochelatase, resulting in inhibition of the enzyme. Hence, in the present findings the accumulation of blood porphyrins in the workers with high blood lead content may be due to the effect of lead on ferrochelatase activity.

In conclusion, we have shown the erythrocyte ALA.D activity in specific cases of workers whose blood lead levels $\geq$ 40 μg/ 100 ml in relation to their age and number of years of experience (Table 1). We observed that (Average mean) 47% inhibition of ALA.D activity in workers with $\geq$ 14 years of exposure and $\geq$ 46 μg/ 100 ml blood lead content. Present findings demonstrate that in

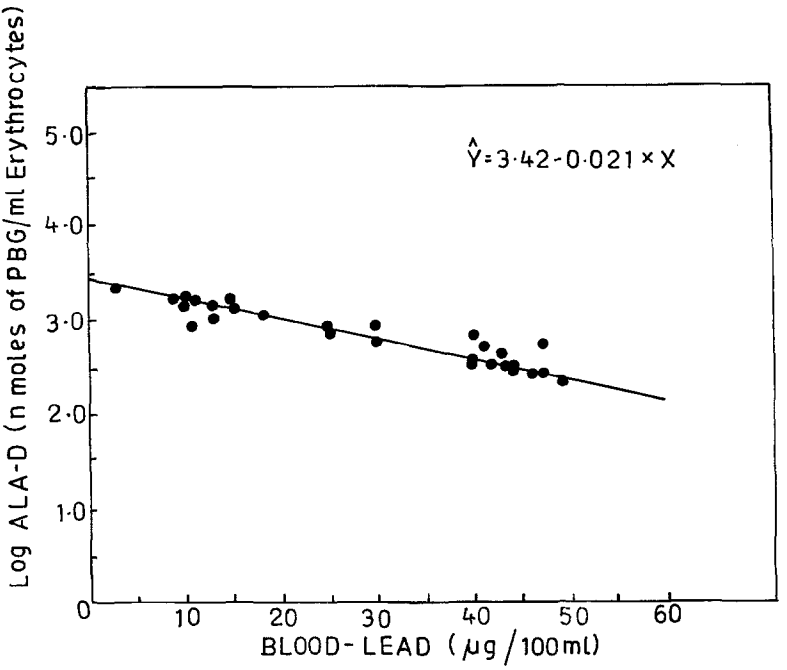

Fig 3: Correlation between log ALA.D activity and blood lead level.

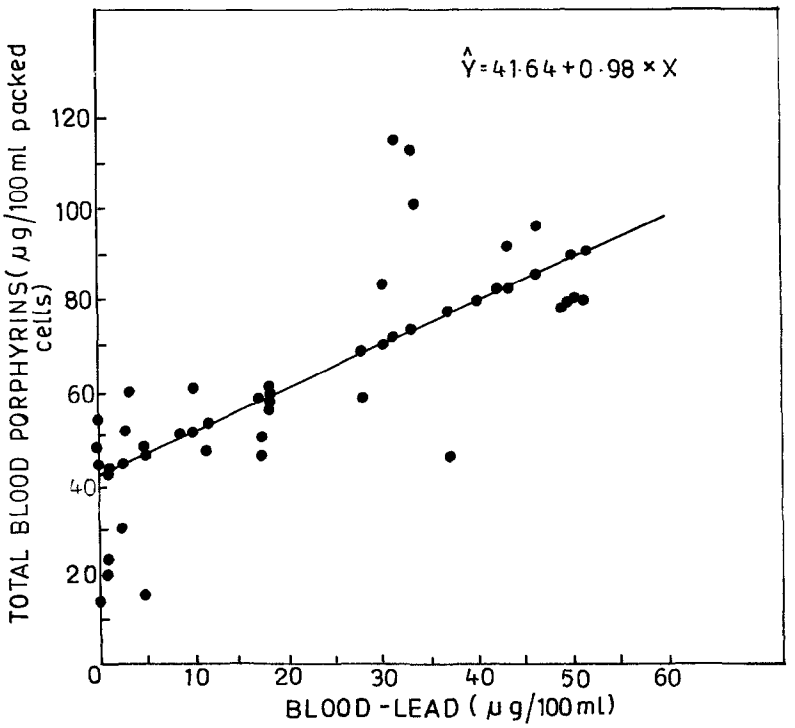

Fig 4: Correlation between blood lead content and total porphyrin levels.

monitoring for occupational exposure to lead, estimation of ALA dehydratase activity and blood porphyrin levels in conjunction with measurements of blood lead levels, may be more useful than the measurement of blood lead levels alone in assessing the toxicity of biochemical lead poisoning.

Table 1. Erythrocyte ALA.D activity in specific cases of blood lead levels ($\geq$ 40 µg/100 ml) in relation to age and number of years of experience.

S.No.	Age (Years)	Experience (Years)	Blood lead content (µg/100 ml)	ALA.D. activity[a] n moles PBG* formed per ml RBC/hr.	% inhibition[+]
1.	21	2	40.0	649.5 ± 13.5	7.22
2.	28	3	42.4	390.0 ± 1.0	44.29
3.	17	5	40.0	906.0 ± 12.0	-
4.	20	5	49.8	300.0 ± 6.0	57.15
5.	31	6	41.0	637.5 ± 7.5	8.93
6.	21	7	46.6	378.30± 22.24	45.95
7.	31	7	47.5	538.5 ± 7.0	23.08
8.	37	9	40.0	690.0 ± 2.5	1.43
9.	25	10	46.0	832.05± 20.29	-
10.	28	12	43.2	447.5 ± 3.8	36.08
11.	33	14	44.7	366.0 ± 21.90	47.72
12.	43	16	46.8	253.4 ± 6.0	63.8
13.	40	20	43.2	420.0 ± 3.0	40.0
14.	33	20	46.4	309.0 ± 8.0	55.86
15.	38	20	44.0	375.0 ± 6.0	46.43
16.	40	20	51.3	621.3 ± 5.0	11.25
17.	47	24	49.5	240.0 ± 2.0	65.72

* PBG = Porphobilinogen

a The normal values of human Blood ALA.D = 700 - 1800 n moles of PBG/mlRBC

\+ The lower limit of 700 n moles of PBG/ml RBC is taken for calculating the percentage of inhibition.

\- Normal.

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