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Hemoglobin, Serum Iron, and Zinc Protoporphyrin in Lead-Exposed Workers

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In a previous study of secondary lead smelter workers (males), a significant prevalence of low hemoglobin levels (less than 14 g/100 ml) was found; a statistically significant negative correlation between hemoglobin and zinc protoporphyrin was also detected. In the present study serum iron (Fe) levels and total iron binding capacity (TIBC) were included in the investigation of 111 secondary lead smelter workers and 37 nonexposed controls.

The distribution and mean values of serum iron and TIBC were found to be in the normal range in the lead exposed workers; there was no significant difference when compared to the control population. There was no significant correlation between blood lead or zinc protoporphyrin and serum iron, TIBC and Fe/TIBC. A statistically significant negative correlation between hemoglobin and blood lead levels was found; the correlation between hemoglobin and zinc protoporphyrin reached a much higher level of significance.

The results support the view that anemia (low hemoglobin levels) in lead exposed male workers is related to the heme synthesis inhibiting effects of lead, as reflected by elevated zinc protoporphyrin levels, and is not due to iron deficiency.

Introduction

A clinical cross-sectional study of 158 male secondary lead smelter workers (*1*) demonstrated relatively high prevalence of low hemoglobin (< 14 g/100 ml) levels. Almost half (47%) of the examined workers were found to have low hemoglobin levels. This finding was already present in 40% of the subjects with short (less than 1 year) exposure. Rapid build-up of lead body burden, as evidenced by a relatively high proportion of workers with elevated blood lead and zinc protoporphyrin levels after less than 1 year of exposure, was thought to be related to the high prevalence of anemia.

An interesting finding was the significant negative correlation between hemoglobin and zinc protoporphyrin ($r = 0.25; p < 0.01$); almost half of the group with zinc protoporphyrin (ZPP) levels between 200 and 500 μg/100 ml had hemoglobin levels less than 14 g/100 ml; the proportion rose to 72% for those with ZPP levels in excess of 500 μg/100 ml.

Although anemia has long been accepted as one of the effects of lead toxicity, the general opinion has been that it is to be expected only at relatively high blood lead levels, i.e., in excess of 80 μg/100 ml. Nevertheless, in the group of secondary lead smelter workers studied, hemoglobin levels of less than 14 g/100 ml were found in a sizable proportion (40%) of individuals with blood lead concentrations lower than 80 μg/100 ml. One of the distinctive characteristics of this group, beside the rapid build-up of toxic lead body burden, was the frequent use of chelation therapy. Therefore it was difficult to assess the relative applicability of two possible explanations: first, that the rapid build-up of toxic levels of lead would result in a significant number of cases of anemia even before the blood lead level reaches the 80 μg/100 ml level or, secondly, that the "homogenizing" effect of chelation therapy, applied repeatedly, without cessation of toxic exposure, may have temporarily reduced some high blood lead levels, without eliminating the

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toxic effects of those levels, i.e., existing anemia.

Since iron deficiency has been shown to enhance lead retention and lead toxicity in experimental studies (*2*), and negative correlations between serum iron and blood lead had been found in both children (*3*) and healthy nonexposed male adults (*4*), although not in another experimental study on male volunteers (*5*), the possibility of iron deficiency contributing to the relatively high prevalence of anemia in the group of secondary lead smelter workers studied was considered. It was decided to explore the problem of anemia in lead-exposed workers further, to assess the possible role of iron deficiency.

Population and Methods

A clinical field survey of 111 workers employed in another secondary lead smelter was undertaken to investigate this problem; 55 additional workers from two steel plants in the same area were concomitantly examined as controls. After thorough analysis of their occupational histories, it was found that 37 were completely free of history of lead exposure, while 18 had had minimal exposure. It was decided to restrict the control group to those 37 without history of lead exposure.

The laboratory tests included, in particular, blood lead determinations by atomic absorption, complete blood counts, serum iron and total iron binding capacity, zinc protoporphyrin (hematofluorometer), δ-aminolevulinic acid and coproporphyrin in urine, as well as many other biochemical parameters.

There was long duration of lead exposure in many of the examined workers; 42 workers had an exposure of over 10 years and 31 had worked for more than 20 years (Table 1).

The age distribution of the lead-exposed workers and control subjects were comparable, except for a higher proportion of younger (less than 30 years) individuals among the exposed workers (Table 2). All the lead-exposed workers except one were males; there were 4 females in the control group. 67 (52%) of the lead-exposed workers were white, and 42 (38%) were black. The proportion of blacks was lower in the control group (16%).

The statistical significance of differences between mean values was assessed by one-tailed *t*-test.

A χ^2 test was used to evaluate the significance of differences in distribution of serum iron and total iron binding capacity values. Least-square fitted linear correlation was used in the assessment of relationships between indicators of lead absorption (blood lead, zinc protoporphyrin) and hemoglobin, serum iron, total iron binding capacity (TIBC), and the ratio of Fe/TIBC.

Table 1. Duration of lead exposure.

Duration of exposure, yr	Number examined	Portion of total, %
< 1	23	21
1–3	14	13
3.1–10	31	28
10.1–20	11	10
20.1–30	31	28

Table 2. Age distribution of lead-exposed workers and controls.

	Lead-exposed (109)		Controls (37)	
Age, yr	Number	%	Number	%
< 30	26	24	3	8
31–50	52	48	23	62
< 50	31	28	11	30

Results

Blood lead levels were measured by atomic absorption spectrophotometry (Perkin-Elmer Model 303 combined with a Varian-Techtron carbon rod atomizer, Model 63). Values exceeded 80 μg/100 ml in one case and were in the range of 60–79 μg/100 ml in 20 cases. Thus, 21% of the workers had blood lead levels of 60 μg/100 ml or higher at the time of the examination. None of the control workers had a blood lead level in excess of 50 μg/100 ml; 94% had less than 40 μg/100 ml (Table 3).

Table 3. Blood lead levels in secondary lead smelter workers.

	Lead exposed		Controls	
Blood lead levels, μg/100 ml	Number of workers	%	Number of workers	%
< 40	17	17	34	94
40–59	61	61	2	6
60–79	20	20	—	
> 80	1	1	—	

Zinc protoporphyrin levels were determined by a hematofluorometer. ZPP was found to be less than 100 μg/100 ml in 36 (32.7%) of the workers; 31% had ZPP levels in excess of 200 μg/100 ml. In the control group, the majority of ZPP values were lower than 50 μg/100 ml, all were less than 100 μg/100 ml (Table 4). ZPP levels correlated well with blood lead levels (Fig. 1).

Low hemoglobin levels (less than 14 g/100 ml) were found in 20 (18.3%) of the lead-exposed workers; there was only one individual with a hemoglobin level of less than 13 g/100 ml. In the control group, 4 (11.4%) workers had hemoglobin levels of

Table 4. Zinc protoporphyrin levels in lead smelter workers and controls.

ZPP, μg/100 ml	Lead exposed: Number of workers	Lead exposed: %	Controls: Number of workers	Controls: %
< 100	36	32.7	36	97.3
100–200	40	36.7	1	2.7
201–500	31	28.2	—	—
> 500	3	2.7	—	—
Total	110			

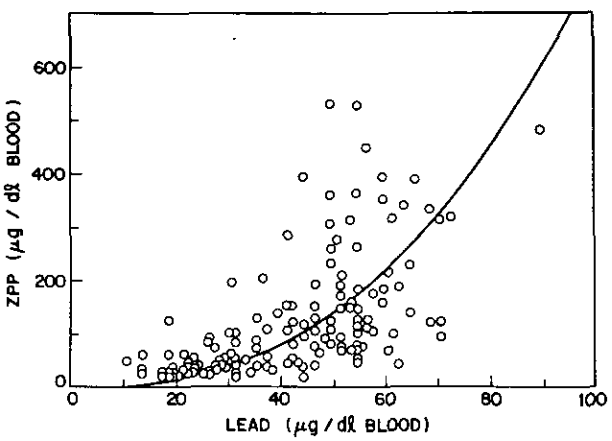

FIGURE 1. Zinc protoporphyrin levels of secondary lead smelter workers plotted against their blood lead levels. Data fitted to a power function by the least-square method had an exponent of 2.5.

less than 14 g/100 ml; none had less than 13 g/100 ml (Table 5).

Thus, the prevalence of anemia was relatively low in this lead-exposed group; this finding is consistent with the distribution of blood lead levels, the distribution and dynamics of ZPP, and with the relatively lower (when compared to other exposed groups) prevalence of symptoms. Although most of the hemoglobin levels were above 14 g/100 ml and relatively few blood lead levels exceeded 60 μg/100 ml, the correlation between hemoglobin and blood lead levels was significant ($r = 0.24, p < 0.01$) (Fig. 2)

The correlation between blood lead and hemoglobin was not perceptibly influenced by the exclusion of the only case with blood lead in excess of 80 μg/100 ml and of the only female lead exposed worker ($r = 0.24$; $p < 0.01$). Hemoglobin levels showed a higher and more significant correlation (Fig. 3) with ZPP levels ($r = 0.41$; $p < 0.001$). This highly significant correlation between ZPP and hemoglobin levels was only minimally changed through the exclusion of the only case with blood lead above 80 μg/100 ml and of the one female worker ($r = 0.40$; $p < 0.002$).

Table 5. Hemoglobin in secondary lead smelter workers and nonexposed controls.

	Number examined	Hemoglobin, g/100 ml: <13 Number	<13 %	13–13.9 Number	13–13.9 %	≥14 Number	≥14 %
Lead smelter workers	109	1	0.9%	19	17.4	89	81.6
Controls	35	0	0	4	11.4	31	88.6

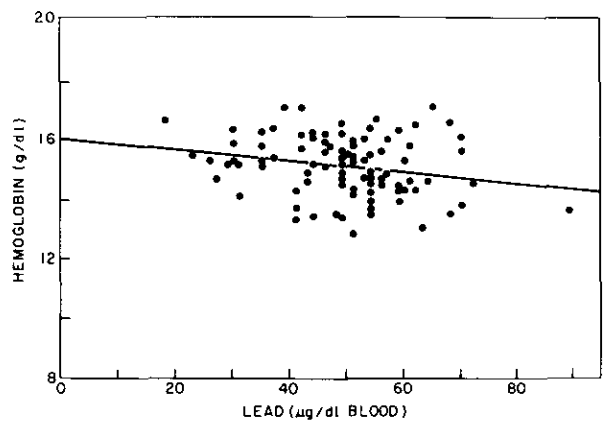

FIGURE 2. Hemoglobin levels of secondary lead smelter workers plotted against their blood lead levels and fitted with the least-square linear regression line; $r = 0.23$; $p < 0.05$.

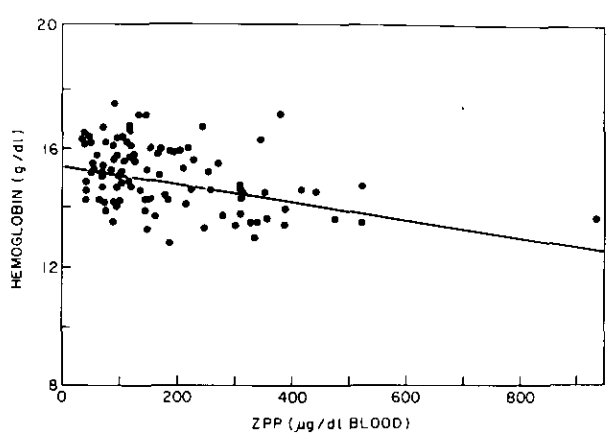

FIGURE 3. Hemoglobin levels of secondary lead smelter workers plotted against their zinc protoporphyrin levels and fitted with the least-square linear regression line; $r = 0.42$; $p < 0.001$.

Serum iron levels were found to be in the normal range for the great majority of the population studied. Females (one exposed, four nonexposed controls) were excluded from this analysis. The

mean serum iron level was 90.5 ± 31 μg/100 ml for the lead-exposed workers. In the control group, the mean serum iron level was 94.2 ± 28 μg/100 ml; the difference between mean serum iron in the lead-exposed and control group was not significant. The distribution of serum iron levels in the lead-exposed workers and controls is given in Table 6. No statistically significant difference was found.

In this lead-exposed population, no correlation between serum iron and blood lead (Fig. 4) or zinc protoporphyrin (Fig. 5) was found (r = 0.02 and r = 0.038).

The total iron binding capacity was also determined and found to range from 235 to 375 μg/100 ml in the control group, and 190 to 430 μg/100 ml in the lead-exposed workers, with all but 3 cases between 200 and 400 μg/100 ml. The mean TIBC for the lead smelter workers was 300.7 ± 44 μg/100 ml, very similar to that of the control group, 289 ± 36 μg/100 ml.

No correlation between total iron binding capacity and blood lead level was found in the lead-exposed population (r = 0.05, n.s.) or in the control group (r = 0.08, n.s.)

Table 6. Serum iron levels in secondary lead smelter workers and nonexposed controls.

Serum iron, μg/100 ml	Lead-exposed workers		Controls	
	Number	%	Number	%
<60	16	18.5	4	12.1
60–90	34	39.5	10	30.3
91–120	18	20.9	13	39.4
>120	18	20.9	6	18.2
Total	86		33	

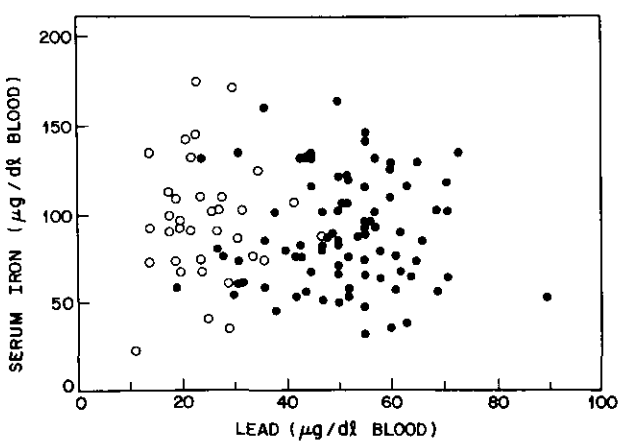

FIGURE 4. Serum iron levels vs. blood lead levels: (●) lead-exposed workers; (○) controls (persons with no exposure to lead). There is no significant correlation for the lead-exposed workers (r = 0.02; n.s.) or for the control group (r = 0.19; n.s.)

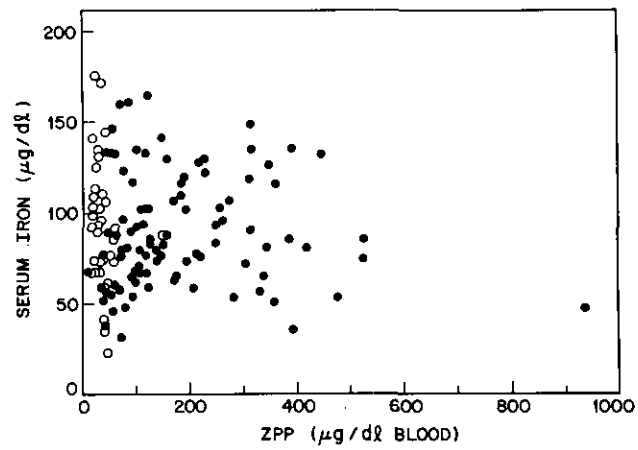

FIGURE 5. Serum iron levels vs. zinc protoporphyrin levels in (●) lead-exposed workers and (○) controls. There is no significant correlation for the lead-exposed group (r = 0.038; n.s.) or for the control group (r = 0.22; n.s.).

There was no significant correlation between total iron binding capacity and zinc protoporphyrin levels in the lead-exposed workers (r = 0.03) or in the control group (r = 0.06).

The mean value for the ratio serum iron/total iron binding capacity in the control population was 0.332 ± 0.122; in the lead-exposed workers the mean ratio was 0.301 ± 0.099. The difference was not statistically significant. The distribution of the Fe/TIBC ratio in the lead-exposed workers and control population (Table 7) also did not show any statistically significant difference.

No significant correlation was found between the ratio serum iron/total iron binding capacity and blood lead level in the control population (r = 0.021) or in the lead-exposed population (r = 0.002).

The ratio Fe/TIBC was not found to be significantly correlated to zinc protoporphyrin levels; the correlation factors were r = 0.023 (n.s.) for the lead-exposed population and r = 0.199 (n.s.) for the control population.

Since more than one third of the lead-exposed workers had had long (more than 10 years) exposure, an analysis of the relationship between duration of exposure, indices of lead absorption (blood

Table 7. Fe/TIBC in secondary lead smelter workers and nonexposed controls.

Fe/TIBC	Lead-exposed workers		Controls	
	Number	%	Number	%
<20%	10	11.6	4	12.1
20–40%	62	72.0	20	60.7
41–60%	13	15.1	9	27.3
>60%	1	1.2	0	—
Total	86		33	

lead and ZPP) and hemoglobin, serum iron and TIBC was also undertaken (male workers only). Mean values for blood lead, zinc protoporphyrin, hemoglobin, serum iron, and TIBC are given in Table 8.

The only significant difference between workers with longer and those with shorter duration of lead exposure was found in ZPP levels; the other differences did not reach the level of statistical significance. A significant negative correlation between hemoglobin and blood lead levels was found in workers with less than 10 years of exposure ($r = 0.029$; $p < 0.02$); there was no correlation in workers with longer exposure ($r = 0.15$; n.s.). Hemoglobin and zinc protoporphyrin however, showed significant correlations in workers with shorter exposure ($r = 0.33$; $p < 0.004$) as well as in those with longer lead exposure ($r = 0.49$; $p < 0.001$). Therefore the correlation between hemoglobin and ZPP does not seem to be dependent on duration of lead exposure, after a certain critical level of lead body burden is reached.

Table 8. Duration of lead exposure and levels (mean ± SD) of blood lead, ZPP, hemoglobin, serum iron, and TIBC.

	Lead-exposed workers		
	Exposure <10 yr	Exposure >10 yr	Controls
Blood lead, μg/100 ml	49.4 ± 11.0	53.4 ± 12.5	26.9 ± 9.2
ZPP, μg/100 ml	150.3 ± 97.3	240.4 ± 178.5	46.4 ± 30.4
Hemoglobin, μg/100 ml	15.1 ± 1.0	14.9 ± 1.2	15.3 ± 1.1
Serum iron, μg/100 ml	92.8 ± 28.1	86.3 ± 34.6	94.2 ± 28
TIBC, μg/100 ml	308.3 ± 45.2	288.0 ± 38.2	289 ± 36

Discussion

The effects of lead on the hematopoietic system have long been recognized, and lead-induced anemia has historically been an important component of the clinical picture of lead poisoning. This has included not only the occasional rather acute anemia, with an important hemolytic component, observed in cases of acute exacerbation of chronic lead poisoning, with lead colic or lead encephalopathy, but, more frequently, the chronic type of lead anemia, due to the toxic inhibition of heme synthesis.

Considerable progress in this area has recently been achieved, and has resulted in a more complete understanding of lead toxicity and also in the identification of free erythrocytic protoporphyrin (FEP), later shown to be, in fact, zinc protoporphyrin (ZPP), as the most sensitive indicator of biologic effects of lead (*6*).

In several cross-sectional clinical studies of lead-exposed male workers, we have found a significant prevalence of low hemoglobin levels (*1, 7*). Interestingly, significant correlations between hemoglobin levels and zinc protoporphyrin levels were also found in these occupationally-exposed populations.

Six and Göyer (*2*) have found that iron deficiency enhances both retention of lead and lead toxicity in rats. In children suspected to be suffering from lead poisoning, Delves, Bicknell, and Clayton found a negative correlation between blood lead and serum iron levels (*3*). In females, higher FEP levels were found than in males at similar blood lead levels (*8*). In a recent study on healthy, nonexposed young males, a negative correlation was observed between blood lead and serum iron (*4*). Nevertheless, the correlation did not reach statistical significance in a second, similar group.

Cool et al. (*5*), in a study on male volunteers ingesting lead acetate for 49 days to a maximum level of blood lead of 40 μg/100 ml, found no effect of lead on serum iron. No relationship between serum iron and FEP increase was identified in the study group of 11 male volunteers; there was no change in hemoglobin either, and no correlation between blood lead values and FEP levels. This was attributed to the rather short term of the exposure.

Van den Eijk, Wiltink, and Bos, in a study on 100 male and female patients, with hemoglobin levels ranging from 7.25 to 17.71 g/100 ml, found significant negative correlations both between hemoglobin and FEP and between serum iron and FEP (*9*). This study did not distinguish between females and males, blood lead levels were not measured and patients with marked anemia were included.

In the present study of an occupationally lead-exposed group of secondary smelter workers, we found a significant inverse correlation between hemoglobin levels and blood lead levels; the correlation between hemoglobin and zinc protoporphyrin levels reached a much higher level of significance. It is noteworthy that these correlations were found in a group in which the blood lead levels with one exception did not exceed 80 μg/100 ml. This indicates that the adverse effect of lead on heme synthesis may result in clinically detectable anemia at lower blood lead levels than hitherto accepted.

We also investigated the possibility that low serum iron contributed to low hemoglobin levels in this lead-exposed male population.

Serum iron levels were found to be in the normal range, and no significant difference was noted when comparisons were made with the values of the control, nonexposed group. No correlation between serum iron and zinc protoporphyrin was found. Therefore a contributing effect of iron deficiency on the hematologic findings in this group of lead-exposed workers can be excluded.

Conclusion

Hemoglobin values in male lead-exposed workers were found to be correlated with blood lead levels and highly correlated with ZPP levels, the hemoglobin decreasing with increasing blood lead or ZPP. Serum iron, total iron binding capacity, and the ratio Fe/TIBC were all found to be in the normal range, with mean values not significantly different from those of a non-exposed control group. There was no significant correlation between blood lead or ZPP and serum iron, TIBC, and Fe/TIBC.

Lead interferes not only with the biosynthesis of heme at several enzymatic steps but also with the utilization of iron. The mechanism for the latter effect is considered to be the inhibition of the ferrochelatase enzyme system, thereby impeding the insertion of iron into the heme molecule.

As a result of the interference with heme synthesis anemia may develop. Lead associated anemia is usually of the hypochromic type and may exhibit some morphologic features in common with iron deficiency anemia; occasionally these two conditions are not easily distinguished. However, bone marrow preparations from lead poisoned individuals tend to show a greatly increased number of sideroblasts, and the iron content in serum and bone marrow may be normal or even increased; these are characteristics that may help in differentiating between lead related and iron deficiency anemias.

The results of this study support the view that anemia (low hemoglobin levels) in lead-exposed male workers is related to the heme synthesis inhibiting effects of lead, as reflected by elevated zinc protoporphyrin levels, and is not due to iron deficiency.

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