

EVIDENCE FOR INTERACTIONS AMONG LEAD, ZINC AND IRON IN CHILDREN

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ABSTRACT

Experimental observations about interaction among various metals point out a strong possibility that nutritional factors may well account for some of the variability in biological responses to lead in man. The evidence strongly suggests that nutritional status relative to the essential trace metals, iron and zinc, can modify the early stages of interference in heme synthesis due to lead.

A growing body of experimental evidence indicates that interactions among essential and non-essential trace elements have significant toxicological and nutritional implications. In rats, induced dietary deficiencies of calcium, iron, copper, zinc and selenium are reported to enhance absorption and retention of lead⁵. Zinc is said to be protective against some of the neurotoxic effects of lead in horses¹⁰. Delta-aminolevulinic acid dehydratase (ALAD) is a zinc-dependent enzyme and lead-induced inhibition of this enzyme can be reversed *in vivo* by zinc^{6,7}. Lead is a competitive inhibitor of the utilization of iron for heme formation⁹. These experimental observations point to a strong possibility that nutritional factors may well account for some of the variability in biological responses to lead in man. Studies in children on this question are the subject of this report.

METHODS

The children studied were drawn from a special clinic at The John F. Kennedy Institute to which children are referred for evaluation of their lead absorption status. Forty-five children were selected for study on the basis of various combinations of screening test results such as: low blood lead-low FEP, low blood lead-high FEP, high blood lead-high FEP and high blood lead-low FEP. The average age of the 45 children was 36 months with a range of 16 to 66

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months; their average blood lead concentration was $50 \mu\text{g}/100 \text{ ml}$ whole blood with 43 of the 45 values falling between 30 and $65 \mu\text{g Pb}/100 \text{ ml}$ whole blood. After informed parental consent was obtained, each child was admitted to the Pediatric Clinical Research Unit of The Johns Hopkins Medical School for study under controlled dietary and environmental conditions. Blood lead and FEP were measured daily. A four day control period was used to allow a somewhat more steady state to be reached before calcium EDTA was given. On the fifth day, calcium EDTA with added procaine was given intramuscularly in a dose of $1000 \text{ mg}/\text{m}^2$ of body surface area/day. This was divided into two doses given at 12 hour intervals. Urine was quantitatively collected for 24 hours during three of the four control days and the 24 hours of calcium disodium EDTA administration for determination of creatinine, ALA-U, lead, zinc, iron, cadmium, manganese and cobalt. Calcium EDTA causes mobilization and diuresis, not only of lead, but also of zinc, iron, cadmium and manganese. Thus, the calcium EDTA mobilization test may serve as a "chemical biopsy" of the mobile stores of these trace metals in the tissues. Hereafter, I shall use the terms "chelatable iron", "chelatable zinc" and "chelatable lead" to indicate the results found following this test. FEP³ and ALA-U⁴ were determined as previously described. Lead in blood was determined by anodic stripping voltammetry on $100 \mu\text{l}$ aliquots of venous blood. Metals in urine were determined by flame atomic absorption spectrophotometry after wet digestion with nitric acid, neutralization with formate and tris buffers, chelation with APDC and extraction into cyclohexane-methyl-isobutyl-ketone solvent. Bovine liver (U.S. National Bureau of Standards CRM # 1577) was used for quality control. Polynomial regression, multiple regression and intersecting lines techniques were used for statistical analyses of the data⁸.

RESULTS

During the four-day control period, serial data showed that blood lead concentration remained steady in some, but that it decreased by up to $15 \mu\text{g}/100 \text{ ml}$ whole blood in others. Generally, FEP and urinary delta-aminolevulinic acid (ALA-U) remained unchanged.

Figure 1 shows a plot of FEP against blood lead for this particular group. According to the design of the study, no statistically significant relationship was anticipated between these two variables and none was found. In fact, no statistically significant relationship was found between FEP and any of the other parameters measured. Although preliminary data pointed to relationship between FEP and the chelatable iron to lead plus manganese ratio, further increase in the sample size suggested a relationship of only borderline significance. Experimental data suggest that lead and manganese may have additive inhibitory effects on heme synthesis⁹.

Figure 2 shows a plot of ALA-U on the ordinate against blood lead as the independent variable on the abscissa. Because ALA-U is related to body surface area in children, it is expressed as $\text{mg}/\text{m}^2/24 \text{ hr}$. The normal value as determined in a separate group of children with a mean blood lead concentration of $20 \mu\text{g}$

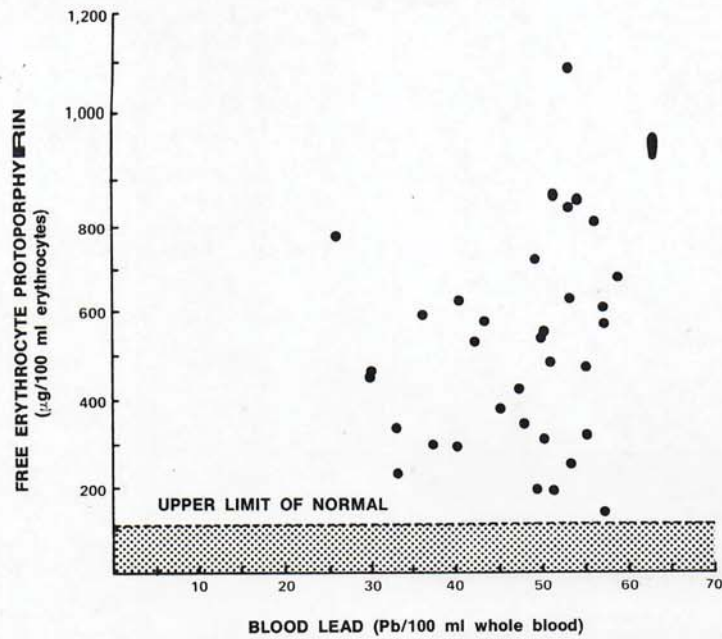


FIG. 1 - Paired blood lead (PbB) and free erythrocyte protoporphyrin (FEP) data on 45 children. This figure shows PbB and FEP values just prior to injection of calcium disodium EDTA. During four day control period in hospital prior to chelation, PbB decreased by as much as 15 µg Pb/100 ml whole blood in some children, but did not change in others: FEP remained steady in all.

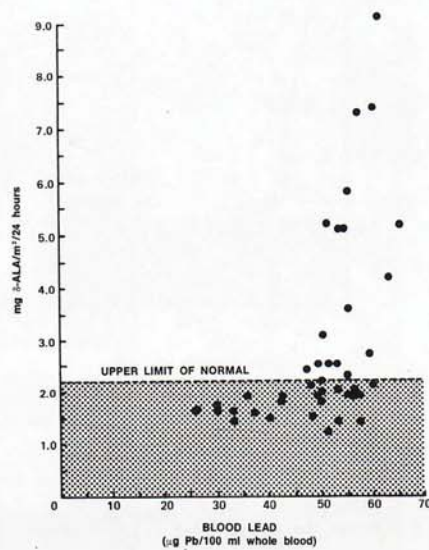


FIG. 2 - Relationship between urinary delta-aminolevulinic acid (ALA-U) and blood lead (PbB) concentration. ALA-U was determined in the 24-hours prior to chelation and PbB just prior to first injection of calcium disodium EDTA.

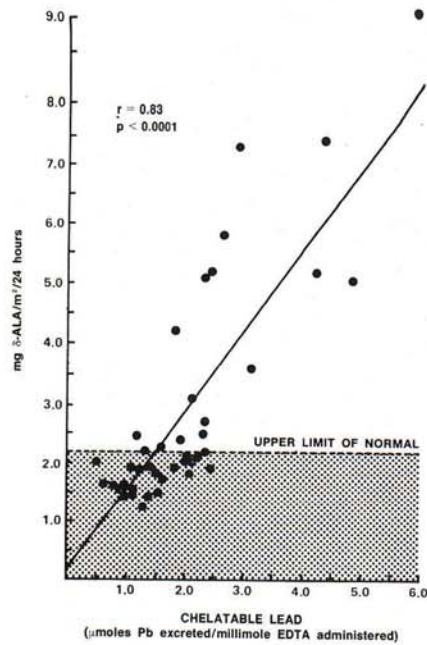


FIG. 3 - Relationship between ALA-U and chelatable lead. ALA-U was measured during 24 hours prior to administration of calcium disodium EDTA. Shaded areas show normal range for ALA-U (1.11 ± 0.37 mg ALA-U/ $m^2/24$ hr) in children with PbB < 35 μ g (mean PbB = 20 μ g Pb/100 ml whole blood). A highly significant linear relationship was found ($p < 0.0001$, $r = 0.8311$).

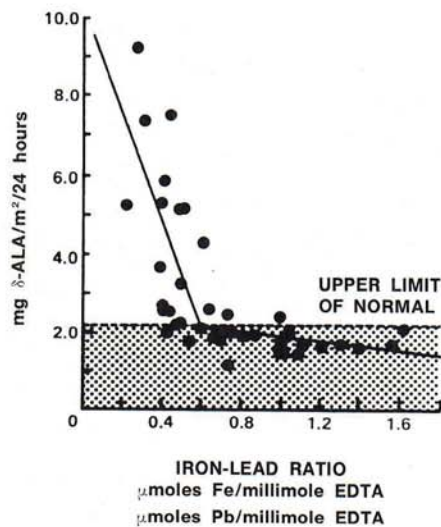
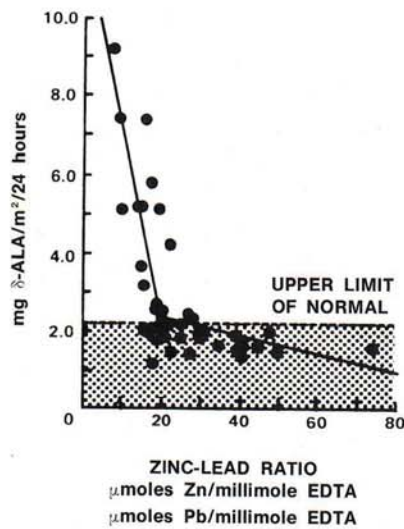


FIG. 4 - Relationship between ALA-U and chelatable zinc-lead and iron-lead ratios. Highly significant linear, quadratic and cubic statistical relationships were found. However, the data were best fitted by an intersecting lines technique, as shown in these figures. Note that the lines intersect at the upper limit of the normal range for ALA-U.

Pb/100 ml is 1.11 ± 0.37 mg/m²/24 hr². The shaded area in this and subsequent figures indicates the normal range for ALA-U. Although there is a highly significant linear relationship ($p < 0.001$) between these variables, the correlation coefficient ($r = 0.485$) is only moderate. Polynomial regression analysis indicated no significant curvilinear relationship nor any well defined threshold for ALA-U in relation to blood lead concentration in this group.

However, among these children selected on the basis of blood lead and FEP data, the most impressive relationship found is that between ALA-U and chelatable lead (Fig. 3). The relationship is a highly significant linear one ($p < 0.0001$) with a correlation coefficient (r) of 0.831. Similar studies show no relationship between ALA-U and chelatable iron or chelatable zinc. However, when ALA-U is plotted against the chelatable zinc-lead and chelatable iron-lead ratios, interesting relationships are found (Fig. 4).

Polynomial regression analysis indicated highly significant curvilinear relationships; however, the best fit with the smallest variance was found with an intersecting lines approach. Statistically, well defined points of intersection were found. The coordinates of the points of intersection are as follows: The lines intersect at a chelatable zinc-lead ratio of 18.45 and an ALA-U value of 2.29 mg/m²/24 hr. The lines also intersect at a chelatable iron-lead ratio of 0.593 and an ALA-U value of 2.14 mg/m²/24 hr. These ALA-U values closely approximate the 99% confidence limit for the upper limit of normal ALA-U, which is 2.22 mg/m²/24 hr. Thus, the data strongly suggests that there is a well defined threshold for increasing ALA-U and that increase in ALA-U above the normal range is inversely proportional to decreases in the mobile stores of zinc and iron in combination with increasing concentrations of mobile lead in the tissues.

Multiple regression analysis of the data indicates that decrease in labile tissue iron stores contributes significantly to the ALA-U effect, although the major component in the ALA-U effect is increase in labile tissue lead. The multiple regression equation for the iron-lead interaction involving ALA-U is as follows: $ALA-U$ (mg/m²/24 hr) = $0.3917 + 1.5658$ (chelatable lead) - 0.4246 (chelatable iron)² ± 0.975 ($r = 0.853$). In relation to blood lead concentration, the data suggest that compensatory mechanisms begin to be overburdened as PbB rises through the 45-60 μ g/100 ml range.

DISCUSSION

Biologically, accumulation and excretion of ALA is the consequence of the relative activities of delta-aminolevulinic acid synthetase, which controls the formation of ALA, and delta-aminolevulinic acid dehydratase, which catalyzes the conversion of ALA to porphobilinogen, the next step in the biosynthesis of heme. The chelatable zinc-lead data are readily explained by a zinc-lead interaction involving delta-aminolevulinic acid dehydratase. The iron-lead data probably reflect increased activity of delta-aminolevulinic acid synthetase¹ secondary to reduced formation of heme which, in turn, can result from reduced availability of iron for heme formation. In conclusion, the evidence strongly

suggests that nutritional status relative to the essential trace metals, iron and zinc, can modify the early stages of interference in heme synthesis due to lead.

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These studies were carried out under protocols approved by the Committee on Clinical Investigation, Johns Hopkins Medical Institutions. Informed written consent was obtained prior to study.

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