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INTERNATIONAL SYMPOSIUM

Environmental health aspects of lead

Die gesundheitlichen Aspekte der Umweltverschmutzung durch Blei

Les problèmes sanitaires posés par le plomb présent dans l'environnement

AIR LEAD / BLOOD LEAD IN G-6-PD DEFICIENT BLACK SCHOOL CHILDREN

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Synopsis-Abstract

Forty-four black children at 2 elementary schools within 0.7 miles of a battery plant had significantly higher ($p < .001$) blood leads (34.2 ± 9.7 ug%, mean and S.D.) than 122 students (26.2 ± 7.1) living at 0.7 to 4 miles. Comparable differences were found in red cell leads 5 months later (54.8 ± 18.6 vs 35.5 ± 12.2) although composite air lead monitored at a site 0.7 downwind from the plant was not increased above urban levels. Independent of the effect of location, glucose-6-phosphate dehydrogenase (G-6-PD) deficient children had higher ($p < .005$) blood leads, after standardization to a hematocrit of 38% (32.9 ± 9.7), than the non-deficient (25.7 ± 8.8) and higher ($p < .001$) red cell leads (47.3 ± 14.7 vs 35.6 ± 15.8). An increased red cell lead with hypochromia was independent of geographic exposure or enzyme deficiency.

This is a report of the effect of urban sources of air lead on the concentration of lead in the red cells and whole blood of normal and glucose-6-phosphate dehydrogenase (G-6-PD) deficient black school children. It is presented as evidence for a genetic factor that may contribute to the higher blood leads of black children in endemic poisoning as found by the USPHS and as a more complete documentation of the role of urban atmospheric exposure on blood lead.(1)

The epidemiologic correlation of urban atmospheric exposure to lead with increasing blood lead levels is still under investigation. The position paper of the Environmental Protection Agency considers children, because of their higher metabolic rate, to be more at risk for respiratory absorption but, as both Chisholm (2) and Barltrop (3) point out, adequate data has not been obtained to support these speculations.

In Omaha, a city of 340,000 population situated in the mid Great Plains, the wind patterns and infrequency of marked temperature inversion reduce the possibilities of air pollution. Despite this, a single comparative sampling in May, 1968 by the National Air Sampling Network showed central Omaha to have the highest dustfall lead of 22 midwestern cities, although dustfall lead does not distinguish particles below 1 mg, the only size contributing to respiratory absorption. Since automobile emission should relate to the relatively low population, the possibility was considered of a significant contribution to air lead by lead emission sources in the central city area.

Our hypothesis of genetic vulnerability to urban levels of lead is primarily directed to the possibility that the G-6-PD deficient red blood cells (rbc) may have a different binding capacity for lead independent of any effects of anemia. Normally, 95% of blood lead is bound to the red cell (4-7), although there seem to be individual variations in partition that are as low as 50% (7). Partition is not affected by either acute or chronic toxicity except at high experimental levels of lead (4).

Since anemia results in a low total blood lead concentration, a standard correction of blood lead for hematocrit such as used by the British Ministry of Labour may eliminate this major variable in epidemiologic studies (8). Utilization of the red cell lead (Pb-rbc), as proposed by numerous investigators (9) as a more specific index of biotoxicity than blood lead (Pb-B), supplies, in addition, information of rbc binding capacity.

We also made an initial inquiry into the possible interaction of a hemolytic effect of lead, at urban concentrations, with G-6-PD deficiency, a hypothesis supported by the association of clinical plumbism with a hemolytic anemia (10), shortened red cell survival time (11), positive

Coombs test (12), spherocytosis, decreased cell volume (13), decreased osmotic fragility (10), accelerated loss of K from the cell interior with altered Na/K flux and decreased Na/K adenosine triphosphatase (ATPase) (14). Ganzoni and Rhomberg (15) and Shafer and Tague (16) have both reported acute hemolytic crises in G-6-PD deficient subjects, enzyme type unknown, with only moderate increases in the blood lead. In vitro studies by Vergnano et al., (17) and Vasiliu (18) show a decrease in reduced glutathione suggesting to us interaction with the hexose monophosphate shunt. Rausa has demonstrated progressive depression of G-6-PD activity in rats at Pb-B of 10-40 mg% as well as a synergistic depression of enzyme activity in human rbc by lead and carbon monoxide, although not by either toxin alone (19).

METHODS

From May through November, 1970, monitoring of air lead in Omaha was conducted with high volume (Hi Vol) sample collectors, using millipore filters, shown in Figure 1, at five sites: 1 industrial (i), 1 commercial (c), 1 mixed (m), and 2 residential (r). All samples were taken at 15 feet elevation, all data reported as the average of 24 samples collected three times weekly. Lead assays were performed by the Air Quality Analytical Laboratory of the EPA at Cincinnati.

In April, 1970, 3,900 school children (of over 10,000 volunteers) from both suburban and inner city Omaha were screened for hematocrit and G-6-PD activity employing the fluorescent spot technique of Beutler (20). In May, 1970, 102 G-6-PD deficient and 64 non-deficient blacks, ages 5-19, attending and living in the districts of 9 schools (Figure 1) in proximity to the sites c and m were retested for Pb-B (9) and G-6-PD activity (21). Hematocrits were determined on 79 of these students. The schools are numbered 1-9 according to their distance from emission source A, a battery plant immediately adjacent to School 1; there are, however, multiple emission sources such as brass foundries, auto paint shops, and printers in the area, especially in proximity to Schools 7 and 8. School 5, grades 7-9, and School 9, grades 7-12, have students residing throughout the area. Students at the 7 elementary schools all live within 0.3 miles of the school. In November, 108 of the May group were retested for Pb-rbc with rbc indices and haptoglobins. The differences in group size for each parameter relate to the logistics of mass venepuncture.

Both Pb-B and PB-rbc were done after 2-3 days refrigerated storage of heparinized blood collected in lead free vacuum tubes and employed the Farrelly and Pybus extraction prior to atomic absorption spectrometry at 2171 Å (9). The standard deviation of 126 duplicate samples was 4.2 mg/100 ml whole blood.

RESULTS

Air Lead:

The monthly composite average of air lead at the sampling sites is diagrammed in Figure 2. The acute increase in mean air lead to 5.0 mg/M^3 at the industrial site c in July deviates from the pattern of seasonal variation noted at the other sites.

Blood Lead - Geographic Distribution:

In the 166 black students tested in May, mean age 10.5, 52% male, the Pb-B (mean and standard deviation, mg/100 ml, as used throughout this report for all values) was 28.4 ± 9.0. No significant increase was found in either older students or males.

As outlined in Table 1, the Pb-B of the 44 students at elementary schools 1 and 2, all living within 0.7 miles of battery plant A, was 34.1 ± 9.7, significantly higher ($p < .001$) than that of the 122 students at Schools 3-9, 26.3 ± 7.1. A comparable geographic pattern was found after standardization to a hematocrit (hct) of 38% by the formula Pb-B x $\frac{38\%}{\text{hct}\%}$.

Pb-rbc of the 108 students tested in November, mean age 10.8, 61% male, was 41.9 ± 16.2. The geographic distribution was again similar and significantly increased at Schools 1 and 2.

Venous hct at Schools 1 and 2, 36.6 ± 3.1% (N=26), was not significantly lower than that at Schools 3-9: 37.7 ± 4.6% (N=67). Hct ranged from 34.8 ± 2.8% (N=12) in school 2, the only significant ($p < .05$) variation, to 38.7 ± 3.3% (N=37) at the junior-senior high school 9.

Students at Schools 1 and 2 had a relative hypochromia, characterized by a lower hemoglobin (12.4 ± 0.9) and mean corpuscular hemoglobin (MCH) (28.6 ± 2.0γγ) than the total students at Schools 3-9, (13.1 ± 1.4 gm%, $p < .005$; 30.0 ± 2.6γγ, $p < .01$) but no different from those at the five other elementary schools. The correlation coefficients (Pearson's r) for the entire group were -0.19 for Pb-B and hematocrit and -0.03 for Pb-B and hemoglobin, neither one being significant.

Blood Lead - G-6-PD:

On quantitative assay in May, 73 (84% male, mean age 10.5) of the 166 students had deficient G-6-PD activity (0-4 uMoles NADPH/min/10^{11} rbc), 29 had intermediate values (4.1 - 14.9 uM) and 65 (49% male, mean age 11.4) were non-deficient (>15 uM). As listed in Table 1 and analyzed in the rows, t for enzyme, Pb-B of the 73 deficient did not differ significantly from that of the 65 non-deficient. The Pb-B x $\frac{38\%}{\text{hct}\%}$, however, of the deficient, 32.9 ± 9.7 (N=32), was significantly higher ($p < .005$) than that of the non-deficient, 25.7 ± 8.8 (N=29); this difference was also significant at Schools 3-9 ($p < .025$) although not statistically significant

for the very small number of students at Schools 1 and 2. Two way analysis of variance, employing the Doolittle correction for samples of unequal size (23), showed the variation related to enzyme deficiency to be independent of that related to location.

Pb-rbc (Table 1) was also significantly ($p < .001$) increased in the deficient, 47.3 ± 14.7 (N=48) compared with the non-deficient, 35.6 ± 15.8 (N=38). Two way analysis of variance again showed this difference to be independent of the variation correlated with location.

The hematologic parameters, hct, hgb, rbc, MCV, MCH, reticolocytes, and haptoglobins, showed no significant difference between deficient and non-deficient. No correlation of either hematologic or lead values was found with a history of recent infection, medications or smoking as reported on the consent form.

Blood Lead - Hypochromic Anemia:

As shown in Table 2, the 39 children with relative hypochromia, a MCH below the mean value of 29.6γγ, had no increase in Pb-rbc, but analysis of data for these 79 children who had both rbc indices and Pb-B shows a significant increase in Pb-B in G-6-PD deficient children with or without concurrent hypochromia. The Pb-rbc of the hypochromic group, 47.3 ± 16.7, was significantly ($p < .01$) higher than the 38.2 ± 15.2 of those with MCH > 29.6. Two way analysis of variance (23) showed this to be independent of the increased Pb-rbc of the G-6-PD deficiency.

COMMENT

Air lead in central Omaha at sites c and i, May-November, 1970, was not only comparable to that of similar sites in Chicago, Manhattan, and Houston for the same months as reported by the EPA (Oct. 7, 1971) but the mean maximum peak in July at site i exceeded the maximum monthly mean of any of these cities. Air lead was not markedly increased at site c directly downwind from emission source A, but it is well known that the localized microclimate is more responsible than prevailing winds for pollution by atmospheric lead.

The May sampling of Pb-B and the November assay of Pb-rbc in this study preclude any seasonal correlations between atmospheric lead and blood lead, although the reproducible geographic distribution suggests the role of environmental factors. All children were beyond the usual age of pica, had a common water supply, similar food sources and comparable socioeconomic status. Since all children, except at Schools 5 and 9, lived within 0.3 miles of the school, localized atmospheric contamination might well be reflected in the differences in blood lead.

The mean blood lead, 28.4, of these 166 urban black school children is higher than the maximum value of 20.6 for adult women in the U.S. Seven City Study (EPA, June 4, 1971). Failure to find the expected increase in

older students, males, and cigarette smokers also suggests exogenous factors contributing to blood leads in the range considered indicative of an increased body burden (2, 3). The Pb-rbc of these urban black children, 41.9 ± 16.2, was significantly higher ($p < .001$) than the 24.3 + 7.5 of freshman medical and nursing students, predominantly from rural Nebraska. For comparison, Robinson et al., found the median Pb-B and Pb-rbc to be, respectively, 27 and 65 mg% in urban children 6 months to 4 years (25).

The biologic validity of Pb-rbc as an index of hematopoietic toxicity is supported by our data showing its correlation with rbc ALAD (log ALAD = 2.3713 - .01 Pb-rbc, $r = 0.888$; $N = 90$) (24) to be similar to that reported by Hernberg et al. (26, among others) for whole blood.

The possibility of an altered rbc permeability or binding capacity for lead in G-6-PD deficiency is strongly suggested by the increase in red cell lead in the deficient subjects, whereas the whole lead, before standardization for hct, did not differ significantly.

One explanation of this increase in Pb-rbc could be a decrease in the trapping of serum in the preparation of packed rbc in G-6-PD deficiency, but this is not supported by any difference in hct or MCV. A concurrent iron deficiency anemia was also associated with an increase in red cell lead, but no change in blood lead; this correlation was independent of enzyme deficiency and geographical location.

There is an extensive body of evidence suggesting that lead combines with the rbc membrane, although Barltrop has shown that in hemolysates of red cells, lead is bound with hemoglobin and possibly another intracellular protein (3); this binding is independent of free sulphydryl groups since it is not inhibited by blocking the - SH groups of hemoglobin with iodoacetic acid (27).

The strong evidence supporting the role of the rbc membrane in the surface binding and transport of lead by the intact erythrocyte and the susceptibility of the membrane to lead cannot, however, be discounted, (13,14) and is relevant to the data here reported suggesting that G-6-PD deficiency is associated with increased rbc binding of lead. It is not known whether this decreases the serum lead available for equilibrium with the tissues or enhances the body burden of lead as noted experimentally by Goyer in iron deficiency (28). All our subjects were black and statistically the enzyme deficiency is most likely the A^- type, but the effects of specific variants of G-6-PD as well as iron deficiency anemia, hemoglobinopathies and other hemolytic anemias deserve consideration for their role in the prediliction of U. S. preschool black children to high blood lead.

If validated, the apparent increase in blood lead in G-6-PD deficient blacks is of potential significance to the 12% of black males and 1.4% of black females with this genetic variant, most of whom live in inner city areas of high ambient lead.

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TABLE I

BLOOD AND RBC LEAD AS RELATED TO LOCATION AND G-6-PD ACTIVITY

Blood Lead (May) µg%	Schools 1 & 2	Schools 3 - 9	Total	t for Location (1-2 vs 3-9)
G-6-PD 0- 4	34.2 ± 9.5 (20)	27.2 ± 7.1 (53)	29.1 ± 8.4 (73)	p < 0.005
4.1-15	34.2 ± 8.8 (11)	25.3 ± 4.1 (18)	28.6 ± 7.6 (29)	p < 0.005
>15	32.8 ± 10.8 (13)	25.8 ± 7.9 (51)	27.3 ± 9.0 (64)	p < 0.05
Total	34.1 ± 9.7 (44)	26.3 ± 7.1 (122)	28.4 ± 9.0 (166)	p < 0.001
t for enzyme (0-4 vs >15)	n.s.	n.s.	n.s.	

Blood Lead µg% x $\frac{38\%}{Hmt\%}$	Schools 1 & 2	Schools 3 - 9	Total	t for Location (1-2 vs 3-9)
G-6-PD 0- 4	38.2 ± 12.8 (9)	30.9 ± 7.1 (23)	32.9 ± 9.7 (32)	p < 0.1
4.1-15	38.3 ± 10.4 (7)	27.2 ± 8.3 (11)	31.5 ± 10.7 (18)	p < 0.05
>15	27.0 ± 11.2 (5)	25.4 ± 8.2 (24)	25.7 ± 8.8 (29)	N.S.
Total	35.6 ± 12.6 (21)	27.9 ± 8.2 (58)	29.9 ± 10.2 (79)	p < 0.025
t for enzyme	N.S.	p < 0.025	p < 0.005	

(Mean & S.D.)

Rbc Lead µg%	Schools 1 & 2	Schools 3 - 9	Total	t for Location (1-2 vs 3-9)
G-6-PD 0- 4	57.0 ± 15.5 (16)	42.4 ± 11.5 (32)	47.3 ± 14.7 (48)	p < 0.001
4.1-15	52.0 ± 16.4 (17)	36.1 ± 13.13 (15)	33.1 ± 11.6 (32)	p < 0.05
>15	48.8 ± 25.5 (6)	33.1 ± 11.6 (32)	35.6 ± 15.8 (38)	p < 0.025
Total	54.1 ± 18.5 (29)	37.4 ± 12.6 (79)	41.9 ± 16.2 (108)	p < 0.001
t for enzyme	N.S.	p < 0.005	p < 0.001	

(Mean & S.D.)

LEGENDS

Figure 1: Location of 5 air sampling stations: i, industrial; c, commercial; m, mixed (commercial - residential); and r, residential, and of 9 schools. Emission sources A and C are battery plants; B is a lead smelter-refinery. The prevailing winds and percent of time observed are indicated by the large arrows

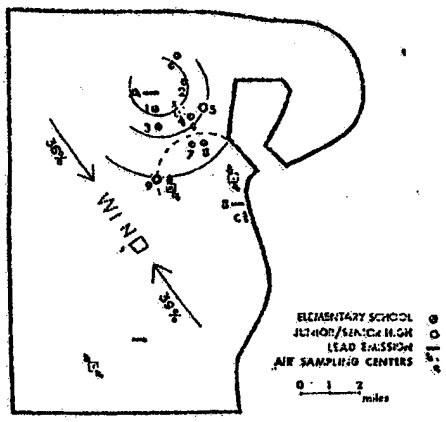
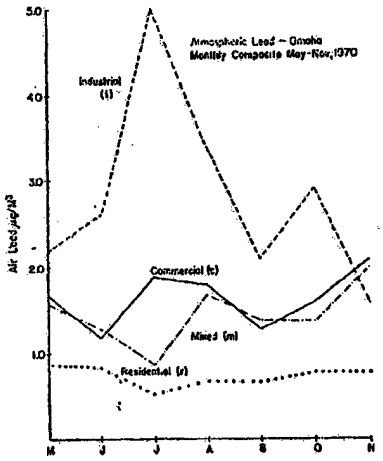


Figure 2: Mean monthly composite atmospheric lead at industrial (i), commercial (c), mixed (m), and 2 residential (r) sites. Mean is that of representative 24-hour samples collected three times weekly. The autumnal peak at all but the industrial site parallels the usual Omaha pattern for particulates.



M.

Table 2

Blood Lead µg% (Mean + S.D.) As Related to G-6-PD Deficiency and Mean Corpuscular Hemoglobin (MCH)

	G-6-PD µM				t test for enzyme
MCH, γγ	0-4	4.1 - 14.9	≥15	Total	(0-4 vs ≥15)
< 29.6	32.9 ± 9.6 (18)	31.7 ± 7.3 (7)	26.9 ± 9.4 (14)	30.5 ± 9.6 (39)	n.s.
≥ 29.6	32.2 ± 8.1 (15)	26.8 ± 7.5 (10)	24.5 ± 7.5 (15)	27.9 ± 8.4 (40)	p < .02
Total	32.6 ± 9.0 (33)	28.8 ± 7.8 (17)	25.7 ± 8.6 (29)	29.2 ± 9.1 (79)	p < .005
t test for hypchromia (<29.6 vs ≥ 29.6)	n.s.	n.s.	n.s.	n.s.	