Red Cell Lead, Whole Blood Lead, and Red Cell Enzymes*

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Simultaneous assay of blood lead (Pb-B) and red cell lead (Pb-Rbc) in 123 samples from 104 urban and suburban students, ages 10–18, shows the ratio of concentration (Pb-Rbc)/(Pb-B) to increase as the hematocrit decreases. On direct assay in 40 samples, plasma lead (Pb-P) was fixed in a narrow range. In 28 students with Pb-Rbc >40 μg/100 ml, the mean red cell 2,3-diphosphoglycerate (2,3-DPG) was 6.05 ± 0.28 (±S.E.), significantly higher (P < .025) than the 5.25 ± 0.18 of 51 students with Pb-Rbc < 40 μg/100 ml, although hemoglobin values were comparable (13.83 ± 0.31 versus 13.55 ± 0.20). Analysis of the individual population groups showed this correlation of Pb-Rbc with 2,3-DPG to be primarily related to the intercorrelation of each parameter with hemoglobin.

Rbc membrane Na/K ATPase, as per cent of total membrane ATPase, had a median value of 60% in 48 subjects. Na/K ATPase below 60% was found in 10 (77%) of the 13 students with Pb-Rbc ≥ 40 μg/100 ml, but in only 14 of the 35 with Pb-Rbc < 40 μg/100 ml (χ² = 5.1, df = 1, P < .05).

Correlation of significant enzyme changes with Pb-Rbc, but not with Pb-B in the normal urban range of Pb-B < 35 μg/100 ml suggests Pb-Rbc, increased in anemia, to be a critical factor in the hematotoxicity of lead.

Introduction

This is a report of the effect of modest increases of blood lead, as found in urban children, on two red cell enzymes 2,3-diphosphoglycerate (2,3-DPG) and Na/K ATPase. It is predicated on the hypothesis that red cell enzyme activity is more likely to be correlated with the intracellular concentration of lead (red cell lead) than with the extracellular concentration (plasma lead or whole blood lead).

It is well accepted that most of the lead in blood is found in the red cell fraction. The concentration of lead in plasma as measured directly by Rosen (1) is in the range of 2–7 μg/100 ml even at high levels of blood lead.

It follows that if plasma lead has a ceiling value, possibly related to binding capacity of a low molecular weight protein or polypeptide of the serum (2), then the concentration of lead in the red cell will increase with anemia, as well as with increased blood lead.

If, for example, 90% of blood lead is associated with the red cells, it can be calculated that, at a blood lead of 20 μg/100 ml, the red cell lead concentration is 40 μg/100 ml at a hematocrit of 45%, but 51 μg/100 ml at a hematocrit of 35%; this theoretical increase in red cell lead in anemia is supported by our own studies.

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Red Cell Lead, Plasma Lead, and Hematocrit

Venous blood samples from 40 high school students, ages 14–18, were immediately iced and centrifuged within 2 hr. Four lead assays, all employing atomic absorption spectroscopy, were done: (1) plasma lead (Pb-P), as assayed by the Delves sampling cup method (3), which is less sensitive than the methodology of Rosen (1), ranged from 4.5 to 7.2 μg/100 ml; (2) blood lead (Pb-B), as assayed by the Farrelly and Pybus (4) extraction of macro samples, ranged from 5.6 to 27.4 μg/100 ml; (3) blood lead assayed by the Delves assay (3) had a correlation coefficient of 0.62 (significant at the 99th percentile) with the macro assay; (4) red cell lead (Pb-Rbc), done by the same method as the macro blood lead, ranged from 14.5 to 41.8 μg/100 ml. The reliability of these assays is supported by the correlation coefficient of 0.868 for the red cell lead of 40 students (mean and S.E.: 23.61 ± 0.99) with the red cell lead (26.6 ± 1.69) as calculated from blood lead, plasma lead and hematocrit.

The ratio of red cell lead (Pb-Rbc) concentration to that of whole blood (Pb-B) versus hemoglobin as found in 123 blood samples from 104 students, ages 10–18, is plotted in Figure 1. In this narrow range of values for hemoglobin and blood lead, the correlation coefficient is 0.2. Ratios below 1.0 presumably represent error variance. Although the reliability of assay of Pb-Rbc is improved by the fact that its concentration is 3.5 times that of Pb-B, the previously noted correlation of calculated and actual Pb-Rbc establishes the methodology to be reliable.

The wide range of variance suggests that factors other than hemoglobin may alter the plasma/red cell partition of lead (5,6). The biologic significance of red cell lead is, however, supported by its correlation with red cell enzymes (7).

2,3-DPG

Red cell 2,3-diphosphoglycerate (2,3-DPG), as is well known, binds deoxyhemoglobin, resulting in a liberation of oxygen at a lower oxygen tension. It is increased in anemia, hypoxia, reticulocytosis, hyperphosphatemia, and related phenomena (8). It also increases in the presence of oxidants and with a decrease in the ratio of ATP to ADP (9). Mercury, in vitro, blocks glutathione (GSH) and shifts the oxygen dissociation to the left, presumably decreasing 2,3-DPG activity (10), but the effect of lead does not appear to have been studied.

2,3 DPG—was assayed by the enzymatic method of Maeda (8) and reduced glutathione (GSH) by the method of Beutler (11) in 73 students, ages 10–18, from three populations. Groups A comprised 25 black children, ages 10–14; 12 boys, 13 girls, attending an elementary school adjacent to a battery plant. Group B consisted of 35 high school students, ages 14–18; 27 white, 8 blacks; 26 boys, 9 girls; 13 attending an urban high school and 22 a suburban high school. Group C comprised 23 black males, ages 12–16, attending urban schools other than that of group A, all with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency (presumably homozygous for the A-enzyme) with activity less than 4.0 μmole NADP/min/1012 Rbc by the method of Bishop (12).

As shown in Table 1, the mean 2,3-DPG levels of the 30 students with Pb-Rbc at or
Table 1. Red cell lead and 2,3-DPG.*

| Pb-Rbc, µg/100 ml | Pb-Rbc, µg/100 ml | t test |
|---------------------|---------------------|--------|
| <40 µg/100 ml       | >40 µg/100 ml       |        |
| (n=53)              | (n=30)              |        |
| Hgb, gm/100 ml      | 5.26±0.18           | 6.05±0.28 | P<0.025 |
| (mean±S.E.)         |                     |         |
| 2,3-DPG mmole/l. Rbc | 13.55±0.20          | 13.83±0.31 | N.s. |
| (mean±S.E.)         |                     |         |

* Pb-B range: 4.9–33.4 µg/100 ml; Pb-Rbc range: 9.0–65.0 µg/100 ml.

above 40 µg/100 ml was 6.05±0.28 mmole/l. (mean ± S.E.). This was significantly higher (P < 0.025 by t test) than the 5.26 ± 0.18 of the 53 students with Pb-Rbc below 40 µg/100 ml.

The mean ± S.E. of the values for Pb-Rbc, hemoglobin, 2,3-DPG, and GSH for the three groups are given in Table 2. A correlation matrix for the intercorrelations of these values is given for each group in Table 3. Red cell lead was inversely correlated with hemoglobin in the high school group without enzyme deficiency. The correlation coefficient for 2,3-DPG with Pb-Rbc ranged from 0.27 to 0.42, although it was significant only in the G-6-PD-deficient subjects. There was a significant correlation in all three groups of Pb-Rbc with hemoglobin plus 2,3-DPG. This is a provocative association but is considered a factor of the previously noted intercorrelation of 2,3-DPG and hemoglobin.

As expected, there was a negative correlation of GSH with hemoglobin in the G-6-PD-deficient subjects, but no significant correlation of GSH with Pb-Rbc in any of the groups.

Table 2. Red cell lead, 2,3-diphosphoglycerate, and glutathione in three populations.*

| Group | Pb-Rbc, µg/100 ml | Hgb, gm/100 ml | 2,3-DPG mmole/l. Rbc | GSH µmole/g Hgb |
|-------|-------------------|----------------|----------------------|-----------------|
| A. Ages 10–12 | 41.6±2.5          | 13.1±0.1        | 5.40±0.23            | 5.05±0.19       |
| N=25               |                   |                 |                      |                 |
| B. Ages 14–18 | 37.4±1.6          | 14.8±0.3        | 5.61±0.29            | 6.17±0.20       |
| N=35               |                   |                 |                      |                 |
| C. Ages 12–16 | 29.5±2.8          | 13.0±0.2        | 5.28±0.28            | 4.41±0.14       |
| G-6-PD-deficient |                   |                 |                      |                 |

* All values given as mean±S.E.

b Group A: black, attend school adjacent to battery plant; B: 27 whites, 8 blacks; 26 males, 9 females; attending urban (12) and suburban (22) high schools; group C: black males, urban, G-6-PD less than 4.0 µM NADP/min/10⁶ Rbc.

Table 3. Correlation coefficients for red cell lead with hemoglobin, 2,3-DPG, and reduced glutathione (GSH) for three population groups.

| Pb-Rbc, µg/100 ml | Hgb, gm/100 ml | 2,3-DPG mmole/l. Rbc | GSH µmole/g Hgb | r1.25 |
|-------------------|----------------|----------------------|-----------------|-------|
| Ages 10–12        | -0.32          | 0.27                 | -0.05           | 0.44  |
| Ages 14–18        | -0.41*         | 0.31                 | -0.11           | 0.51  |
| G-6-PD-deficient, ages 12–16 | -0.01          | 0.42                 | -0.10           | 0.41  |
| Hgb Ages 10–12    | -0.33          | 0.17                 | -0.17           |       |
| Ages 14–18        | -0.19          | -0.25                | -0.61           |       |
| G-6-PD-deficient, ages 12–16 | -0.02          | 0.13                 | -0.61           |       |
| 2,3-DPG Ages 10–12 | 0.13           |                      | -0.61           |       |
| Ages 14–18        | 0.20           |                      | -0.61           |       |
| G-6-PD-deficient, ages 12–16 | 0.09           |                      | -0.61           |       |

* P<0.05.

b P<0.01.

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Na/K ATPase

A decrease in Na/K ATPase of the red cell membrane was reported in lead workers with blood leads of 63–130 µg/100 ml by Hasan, Vihko, and Hernberg (13) and by Secchi (14) in another 10 workers with blood leads of 42–100 µg/100 ml. In both reports, this decrease was a threshold effect without a linear correlation of blood lead and enzyme activity. Increase in the potassium loss from the red cell, considered an index of decreased Na/K ATPase activity, has been associated with increased blood lead in vitro (15,16) and in vivo (17).

Assay of red cell membrane ATPase by the method of Post (18) was carried out on 48 samples of blood from 36 urban and suburban males, ages 10–18, taken from the population groups A and B of the 2,3-DPG study. There was no significant correlation of either total ATPase activity (0.94–2.63 µg Pi/mg protein/hr) or Na/K ATPase (ouabain-inhibited: 0.54–1.71 µg Pi/mg protein/hr) with blood lead or red cell lead. Neither value was significantly affected by age or race.

As shown in Table 4, Na/K ATPase as percent of total membrane ATPase had a median value of 60% in the 48 samples (Pb-B; 9.6–31.8; Pb-Rbc, 12.5–60.0).

Table 4. Red cell lead and red cell membrane Na/K ATPase for urban and suburban students, ages 10–18.*

| Na/K ATPase×100 | Pb-Rbc | Pb-Rbc | Total ATPase |
|-----------------|--------|--------|--------------|
|                 | <40 µg/| >40 µg/|              |
|                  | 100 ml | 100 ml | subjects     |
| <60%            | 14     | 10     | 24           |
| ≥60%            | 21     | 3      | 24           |
| Total subjects  | 35     | 13     | 48           |

* Pb-B, range, 9.6–31.8 µg/100 ml; Pb-Rbc range, 12.5–65.0 µ/100 ml; df=1; χ²=5.1, P<0.05.

Na/K ATPase was less than 60% of total membrane ATPase in 10 of the 13 samples with a red cell lead above 40 µg/100 ml, but in only 14 of the 35 samples with red cell lead below 40 µg/100 ml (χ² = 5.1, P < 0.05).

This distribution of decreased Na/K ATPase suggests an enzymatic sensitivity even at normal urban levels of blood lead. In experimental lead poisoning in rats, Lessler and Cardona (19) noted an immediate decrease in red cell membrane Na/K ATPase even though anemia did not develop until the ninth week of the high lead intake. The mechanism of the decrease in activity is not yet defined although physicochemical changes in the membrane, interaction with other cations, as well as impaired glycolytic activity of the cell may cause decreased activity of Na/K ATPase and an associated increase in loss of intracellular K+ (16). Secchi and Alessio (20) found that the in vitro inhibition of the enzyme by lead could be prevented by the addition of cysteine to the incubation medium suggesting that the inhibition related to −SH binding by lead or that lead otherwise interfered with the ability of the cell to maintain the reduced thiol. Decreased GSH has been clinically correlated with lead poisoning by Vasiliiu et al. (21) and Batolska et al. (22), among others, but was not documented in this study.

Summary

Correlation of a decrease in Na/K ATPase with red cell lead but not with blood lead at normal urban values of blood lead, 5–35 µg/100 ml, suggests that red cell lead is a more sensitive index of red cell enzyme toxicity than blood lead, a low hematocrit contributes to a relatively higher red cell lead and may enhance hematotoxicity, and significant biologic effects relate to lead at normal urban blood levels of lead.

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