Erythrocyte 6-aminolaevulinic acid dehydratase activity and blood protoporphyrin concentrations as indices of lead exposure and altered haem biosynthesis

P. A. MEREDITH, M. R. MOORE AND A. GOLDBERG
Department of Materia Medica, University of Glasgow, Stobhill Hospital, Glasgow, Scotland, U.K.

(Received 18 April 1978; accepted 14 August 1978)

Summary

1. The activity of erythrocyte 6-aminolaevulinic acid (ALA) dehydratase and blood protoporphyrin concentrations have been measured in patients with various anaemias, a group of subjects with known lead exposure and a group of control subjects. Leucocyte ALA synthase was measured in subjects from the last two groups.

2. Erythrocyte ALA dehydratase activity was significantly depressed in the group of lead-exposed subjects and showed a highly significant negative exponential relationship with blood lead concentration.

3. Blood protoporphyrin concentrations were significantly elevated in the group of lead-exposed subjects and patients with iron-deficiency anaemia and showed a significant positive exponential relationship with blood lead concentration.

4. Comparison of the least-squares regression analysis of these relationships and incidence of false positive and false negative results indicates that erythrocyte ALA dehydratase activity is a more accurate measure of environmental and moderate industrial lead exposure than blood protoporphyrin concentrations.

5. The correlations of erythrocyte ALA dehydratase and leucocyte ALA synthase activity, and of blood protoporphyrin concentrations and leucocyte ALA synthase activity, suggest that blood protoporphyrin more accurately reflects haem synthesis than does erythrocyte ALA dehydratase activity.

Key words: 6-aminolaevulinate dehydratase, 6-aminolaevulinate synthase, haem biosynthesis, lead, protoporphyrin.

Abbreviation: ALA, 6-aminolaevulinic acid.

Introduction

In recent years there has been considerable interest in both clinical and subclinical lead exposure. It has, however, become apparent from the inter-laboratory variations in determination of blood lead (Queen Elizabeth Hospital, Birmingham, U.K. Quality Control Scheme) that blood lead analysis is not necessarily the most reliable or consistent index of lead exposure. This has resulted in the quest for an alternative diagnostically valid screening test for lead exposure.

The anaemia of lead poisoning was first noted nearly 150 years ago and since that time lead has been shown to have many dramatic effects on haem biosynthesis (Goldberg, 1972). It is therefore not surprising that many tests have revolved round these effects of lead on this pathway. The activity of erythrocyte 6-aminolaevulinic acid (ALA) dehydratase (EC 4.2.1.24) and blood protoporphyrin concentrations have aroused the greatest recent interest although other measurements, such as urinary ALA and coproporphyrin, have been
given some consideration. The use of erythrocyte ALA dehydratase as a bioanalytical measure of lead exposure has been aided in Europe by the development of a standardized assay (Berlin & Schaller, 1974) for use as indicated in the council directives of the Commission of the European Communities (1977). The use of blood protoporphyrin has received much attention, especially in the United States of America, as a biological index of lead exposure. This has involved the use of classical extraction procedures (Alessio, Bertazzi, Monelli & Foa, 1976), the development of micromethods (Kammholz, Thacher, Blodgett & Good, 1972; Sassa, Granick, Granick, Kappas & Levere, 1973; Piomelli, 1973; Chisolm & Brown, 1975; Lamola, Joselow & Yamane, 1975; Orfanos, Murphey & Guthrie, 1977) and also the development of a portable spectrofluorimeter, with front face optics, for the determination of protoporphyrin in an untreated drop of blood (Blumberg, Eisinger, Lamola & Zuckerman, 1977). Such tests have resulted in the publication of recommendations for the use of protoporphyrin as a primary screening test in lead intoxication (Centre for Disease Control, 1975).

The effects of lead on haem biosynthesis may easily be followed by assay of the enzymes of the pathway in peripheral blood (Campbell, Brodie, Thompson, Meredith, Moore & Goldberg, 1977). On the basis of these methods the present study is an evaluation and critical analysis of haem biosynthesis and the effects of lead on the kinetics of haem biosynthesis during both environmental and industrial lead exposure.

Subjects and methods

Studies were carried out on 209 adult subjects, 115 (54 male and 61 female) with no known exposure to lead, 49 (all male) with known industrial lead exposure, 20 (10 male and 10 female) with iron-deficiency anaemia, 17 (eight male and nine female) with secondary anaemia and eight subjects (four male and four female) with megaloblastic anaemia. Iron-deficiency anaemia was diagnosed on the basis of hypochromic erythrocyte indices, lowered serum iron and raised iron-binding capacity. Megaloblastic anaemia was diagnosed on macrocytic erythrocyte indices, on bone-marrow aspirate and depressed serum B12 and/or folate concentrations. The normochromic, normocytic anaemias were secondary to such conditions as carcinoma, renal disease, chronic infection or collagen disease.

Marrow examination in this group revealed normoblastic erythropoiesis with adequate stainable iron.

Erythrocyte ALA dehydratase activity was determined in all subjects by the method of Berlin & Schaller (1974) and results were expressed as nmol of ALA utilized min⁻¹ ml⁻¹ of erythrocytes. Blood protoporphyrin was determined in 50 µl of blood with an ESA ZnP model 400 Hematofluorometer (Environmental Sciences Associates, Burlington, Mass., U.S.A.). After correction for packed cell volume (normalized to the value of 42) results were expressed as µmol of erythrocyte protoporphyrin/1 of whole blood.

Leucocyte ALA synthase activity (EC 2.3.1.37) was measured in 19 lead-exposed subjects and 37 subjects with no known lead exposure by the method of Brodie, Thompson, Moore, Beattie & Goldberg (1977). Results were expressed as nmol of ALA produced h⁻¹ g⁻¹ of protein.

Blood lead concentrations were measured by flameless atomic absorption spectrophotometry (Perkin–Elmer 306 with HGA 72) with deuterium background correction. Consistency of results was maintained by participation in the Queen Elizabeth Hospital, Birmingham, U.K. Quality Control scheme.

Results were analysed on the Northumbrian Universities multiple access computer (NUMAC) by using non-linear regression analysis programmes based on numerical analysis group (NAG) routines. All results were expressed as means ± SD.

Results

The elevation of blood lead concentrations in the lead-exposed group (Table 1) was associated with a highly significant (P < 0.001) decrease in erythrocyte ALA dehydratase activity and a highly significant (P < 0.001) rise in blood protoporphyrin concentrations. There was a highly significant (P < 0.001) rise in blood protoporphyrin in iron-deficiency anaemia when compared with the control group. In the group of subjects with iron-deficiency anaemia there was a small but significant (P < 0.05) increase in blood lead concentration than the iron-deficiency anaemia group. For this reason a matched control group was selected (from the original 115 subjects) on the basis of sex, age and blood lead concentration. This matched control group was not
**Lead exposure and haem biosynthesis**

**TABLE 1. Blood lead concentrations, erythrocyte ALA dehydratase activity and blood protoporphyrin concentrations in subjects with no known lead exposure, known lead exposure, iron-deficiency anaemia and megaloblastic anaemia.**

Significance: *P < 0.001 and †P < 0.05 with respect to control as assessed by Student's t-test; ‡P < 0.001 with respect to matched control group, as assessed by paired t-analysis. The 20 matched control subjects (selected from the original 115) were age-, sex- and blood lead-matched to subjects with iron-deficiency anaemia (see the text).

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Blood lead (μmol/l)</th>
<th>Erythrocyte ALA dehydratase activity (nmol of ALA utilized min⁻¹ ml⁻¹ of erythrocytes)</th>
<th>Blood protoporphyrin (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>115</td>
<td>1.40 ± 0.83</td>
<td>28.1 ± 11.3</td>
<td>0.87 ± 0.50</td>
</tr>
<tr>
<td>Lead-exposed subjects</td>
<td>49</td>
<td>3.11 ± 1.17*</td>
<td>11.5 ± 7.6*</td>
<td>2.04 ± 1.53*</td>
</tr>
<tr>
<td>Iron-deficiency anaemia</td>
<td>20</td>
<td>1.09 ± 0.58</td>
<td>34.2 ± 9.4†</td>
<td>2.42 ± 2.22‡</td>
</tr>
<tr>
<td>Matched control group</td>
<td>20</td>
<td>1.10 ± 0.08</td>
<td>33.0 ± 8.6</td>
<td>0.72 ± 0.32</td>
</tr>
<tr>
<td>Secondary anaemia</td>
<td>17</td>
<td>1.48 ± 0.52</td>
<td>26.1 ± 9.6</td>
<td>0.87 ± 0.38</td>
</tr>
<tr>
<td>Megaloblastic anaemia</td>
<td>8</td>
<td>1.23 ± 0.57</td>
<td>30.6 ± 13.0</td>
<td>0.63 ± 0.39</td>
</tr>
</tbody>
</table>

**TABLE 2. Parameter values obtained by computer fit of the data to equation (1) (A, B, C) and equation (2) (D, E, G).**

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th>All subjects excluding iron-deficiency anaemia</th>
<th>All subjects excluding secondary and megaloblastic anaemias</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>64.0 ± 3.8</td>
<td>64.9 ± 4.4</td>
<td>65.4 ± 3.9</td>
</tr>
<tr>
<td>B</td>
<td>0.62 ± 0.06</td>
<td>0.64 ± 0.06</td>
<td>0.64 ± 0.05</td>
</tr>
<tr>
<td>C</td>
<td>1.40 ± 1.44</td>
<td>1.25 ± 1.43</td>
<td>1.25 ± 1.32</td>
</tr>
<tr>
<td>Sum of squares/pt</td>
<td>0.147</td>
<td>0.153</td>
<td>0.157</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>-0.891</td>
<td>-0.898</td>
<td>-0.899</td>
</tr>
<tr>
<td>D</td>
<td>0.13 ± 0.02</td>
<td>0.14 ± 0.01</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>F</td>
<td>0.68 ± 0.05</td>
<td>0.72 ± 0.03</td>
<td>0.67 ± 0.05</td>
</tr>
<tr>
<td>G</td>
<td>0.62 ± 0.08</td>
<td>0.43 ± 0.05</td>
<td>0.64 ± 0.08</td>
</tr>
<tr>
<td>Sum of squares/pt</td>
<td>0.201</td>
<td>0.165</td>
<td>0.200</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.674</td>
<td>0.877</td>
<td>0.669</td>
</tr>
</tbody>
</table>

significantly different from the iron-deficiency anaemia group in respect to ALA dehydratase, but the rise in blood protoporphyrin in iron-deficiency anaemia as assessed by paired Student's t-analysis was still highly significant (P < 0.001).

Regression analysis of the relationship between erythrocyte ALA dehydratase activity and blood lead was carried out on the basis of equation (1) (values for A, B and C obtained by computer fit of the data to this equation are shown in Table 2).

ALA dehydratase = A exp. [−B (blood lead)] + C

(1)

The regression line based on the parameters A, B and C obtained for all subjects is shown in Fig. 1.

The correlation of blood protoporphyrin and blood lead concentrations was performed on the basis of equation (2) and the values for D, F and G obtained by computer fit of the data to this equation are shown in Table 2.

Blood protoporphyrin

= D exp. [+F (blood lead)] + G

(2)

The theoretical parameter values of this equation based on those obtained from the regression analysis of equations (1) and (2) and the actual parameter values obtained by computer analysis of the data from all subjects excluding those with iron-deficiency anaemia are shown in Table 3. The
I. FIG. 1. Relationship between erythrocyte ALA dehydratase activity and blood lead concentrations in control subjects (■), subjects with known lead exposure (□) and subjects with iron-deficiency anaemia (○). The regression line, for all subjects, $y = 64.0 \exp(-0.62) + 1.40$ ($r = -0.89$), is shown.

The relationship between leucocyte ALA synthase activity and erythrocyte ALA dehydratase activity was evaluated on the basis of the equation obtained in earlier studies (Meredith, Moore, Campbell, Thompson & Goldberg, 1978) (equation 4).

Erythrocyte ALA dehydratase

$$J \times \left[ \frac{\text{leucocyte ALA synthase} - K}{L} \right]^{-M} \quad (4)$$

The parameter values obtained by computer fit of the data to equation (4) were $J = 59.3 \pm 7.2$, $K = 100.1 \pm 11.2$ and $L = 45.6 \pm 0.29$. (Sum of squares/pt = 0.269; correlation coefficient ($r$) = $-0.810$.) The regression line based on these parameters is shown in Fig. 4.

A regression line based on these parameters is shown in Fig. 3.

An evaluation of predictive validity of both screening tests was carried out on the basis of an assessment of the percentage of false positive and false negative results. Two blood lead concentrations were selected: the first, at 1.75 μmol/l, was selected as being the now generally accepted desirable upper limit for subjects with no known lead exposure, and the second, 3.0 μmol/l, was selected as being representative of a blood lead value that could be encountered in industrial lead exposure. For each of these two blood lead values, two values of erythrocyte ALA dehydratase activity and two values of blood protoporphyrin concentrations were considered. Bearing in mind the earlier regression analysis, these values were selected arbitrarily as being those most likely to result in high predictive validity. Predictive validity was assessed in terms of the percentage of false negatives, that is a failure to detect subjects with raised blood lead, and percentage of false positives, that is a false prediction of raised blood lead. The results of this analysis, based on the data obtained from all the subjects in the present study, excluding
FIG. 2. Relationship between blood protoporphyrin and blood lead concentrations in control subjects (■), subjects with known lead exposure (□) and subjects with iron-deficiency anaemia (○). The regression line, for all subjects excluding those with iron-deficiency anaemia, $y = 0.14 \exp (0.72 + 0.43 (r = 0.877)$, is shown.

**TABLE 3. Theoretical parameter values for equation (3) and actual values obtained by computer analysis of data from all subjects excluding those with iron-deficiency anaemia**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Theoretical values</th>
<th>Parameter values obtained by computer fit to eqn (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D$</td>
<td>0.14</td>
<td>$0.13 \pm 0.02$</td>
</tr>
<tr>
<td>$A$</td>
<td>64.9</td>
<td>$60.1 \pm 6.2$</td>
</tr>
<tr>
<td>$H$</td>
<td>1.13</td>
<td>$1.20 \pm 0.15$</td>
</tr>
<tr>
<td>$G$</td>
<td>0.43</td>
<td>$0.36 \pm 0.08$</td>
</tr>
<tr>
<td>Sum of squares/pt</td>
<td>—</td>
<td>0.175</td>
</tr>
<tr>
<td>Correlation coefficient ($r$)</td>
<td>—</td>
<td>$-0.848$</td>
</tr>
</tbody>
</table>

those with iron-deficiency anaemia, are shown in Table 4. This Table shows that, at both blood lead concentrations, erythrocyte ALA dehydratase activity has a higher predictive validity than blood protoporphyrin concentrations, both in terms of false positives and false negatives.

**Discussion**

The validity of any screening test for lead exposure should depend not only upon its sensitivity but also upon its specificity.

It is well recognized (Baloh, 1974; Lamola et al., 1975; Posner, 1977) that the use of blood protoporphyrin as a screening test for lead exposure has certain limitations with respect to its specificity, especially in view of the raised blood protoporphyrin concentrations associated with iron-deficiency anaemia and iron deficiency without anaemia. The findings of the present study confirm this in two ways. First, blood protoporphyrin concentrations were significantly elevated in iron-
deficiency anaemia. Secondly, there was an improved fit in the relationship between blood protoporphyrin concentrations and blood lead concentrations after exclusion of the subjects with iron-deficiency anaemia as assessed by an increased regression correlation coefficient and decreased sum of squares.

Other metals besides lead can alter the activity of ALA dehydratase (Meredith, Moore & Goldberg, 1977). Of these, zinc has been shown to have the most profound effect in activating the enzyme. However, although significant, this effect is very small at physiological concentrations (Meredith & Moore, 1978). Carbon monoxide has been shown to depress the activity of erythrocyte ALA dehydratase significantly but again at physiological concentrations the effect is very small (Moore & Meredith, 1978). Chronic ethanol consumption has been shown to decrease erythrocyte ALA dehydratase activity (Moore, Beattie, Thompson & Goldberg, 1971). The activity, however, increases as the blood alcohol concentration declines. There is contradictory evidence concerning the effects of iron-deficiency anaemia on erythrocyte ALA dehydratase activity. The results of the present and earlier studies (Lichtman & Feldman, 1963; Battistini, Morrow, Ginsburg, Thompson, Moore & Goldberg, 1971; Mazza, Pescarmona, Bianco, Ricco & Gallo, 1972) suggest that iron-deficiency anaemia has no effect on erythrocyte ALA dehydratase. This is contrary to the recent findings (Campbell, Meredith, Moore & Goldberg, 1978; Chalevelakis, Lyberatos, Manopoulous, Pyrovolakis & Gardikas, 1977) that erythrocyte ALA dehydr-
Lead exposure and haem biosynthesis

Fig. 4. Relationship between leucocyte ALA synthase activity and erythrocyte ALA dehydratase activity, in control subjects (■) and subjects with known lead exposure (□). The regression line for all subjects, \( y = 59.3 \times [(x - 100.1)/45.6]^{-0.18} \) \((r = -0.810)\), is shown.

Table 4. Predictive validity of erythrocyte ALA dehydratase activity and blood protoporphyrin concentration at two blood lead concentrations

Predictive validity was assessed in terms of the percentage of false negatives, that is a failure to detect subjects with raised blood lead values, and percentage of false positives, that is a false prediction of raised blood lead values. The values of erythrocyte ALA dehydratase activity and blood protoporphyrin concentration were selected arbitrarily as being those most likely to result in high predictive validity. This analysis was carried out on the data from all subjects, excluding those with iron-deficiency anaemia.

<table>
<thead>
<tr>
<th>Blood lead (μmol/l)</th>
<th>Erythrocyte ALA dehydratase activity (nmol of ALA utilized min(^{-1}) ml(^{-1}) of erythrocytes)</th>
<th>Blood protoporphyrin (μmol/l of whole blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>~1.75</td>
<td>≤20</td>
<td>3.7</td>
</tr>
<tr>
<td>~1.75</td>
<td>&lt;20</td>
<td>7.4</td>
</tr>
<tr>
<td>~1.75</td>
<td>≤10</td>
<td>1.6</td>
</tr>
<tr>
<td>~1.75</td>
<td>&lt;10</td>
<td>4.8</td>
</tr>
<tr>
<td>&gt;=0.75</td>
<td>≥1.00</td>
<td>3.7</td>
</tr>
<tr>
<td>&gt;=0.75</td>
<td>≥12.5</td>
<td>11.6</td>
</tr>
<tr>
<td>&gt;=0.75</td>
<td>≥2.0</td>
<td>2.6</td>
</tr>
<tr>
<td>&gt;=0.75</td>
<td>≥1.5</td>
<td>9.0</td>
</tr>
</tbody>
</table>

Rubino, Teso & Rasetti (1960) and Heilmeyer (1963) have suggested that the activity of the enzyme is depressed by this anaemia. Battistini et al. (1971) and Campbell et al. (1978) showed increased ALA dehydratase activity in megaloblastic anaemias. The present study shows a small but non-significant rise in activity. Despite the dubiety concerning the effects of anaemia on erythrocyte ALA dehydratase activity, it is clear
that it is a more specific index of lead exposure than blood protoporphyrin concentrations.

The relative sensitivity of these tests as indices of lead exposure has already received some consideration. By virtue of the characteristics of the relationship between blood protoporphyrin and blood lead (Fig. 2) it is apparent that blood protoporphyrin concentration is a less-sensitive index of low blood lead than of high blood lead. The activity of erythrocyte ALA dehydratase, however, is more sensitive at low blood lead concentrations, with lower sensitivity at higher blood lead values (Fig. 1). Previous comparisons of the relative value of erythrocyte ALA dehydratase activity and blood protoporphyrin concentrations as monitors of lead exposure have proved somewhat inconclusive. Roels (1975) found that erythrocyte ALA dehydratase activity was more sensitive than blood protoporphyrin concentrations to changes in blood lead. Both Sassa et al. (1973) and Tomokuni & Ogata (1976) provided the rather contradictory finding that although erythrocyte ALA dehydratase was more useful for the detection of acute lead exposure and blood protoporphyrin for the detection of chronic lead exposure, both were equally sensitive for the detection of subclinical blood lead concentrations. Alessio et al. (1976) and Zielhuis (1974) have, however, cast some doubt on the validity of erythrocyte ALA dehydratase activity at blood lead values below 2 µmol/l. The present study suggests that the coefficients of correlations of erythrocyte ALA dehydratase activity and blood protoporphyrin concentrations (excluding iron-deficiency anaemia) with blood lead are similar. However, as Zielhuis (1974) has already suggested, a high coefficient of correlation does not necessarily imply a high predictive validity. On the basis of the present analysis it is apparent that the predictive validity of erythrocyte ALA dehydratase activity is higher than that of blood protoporphyrin concentrations at both the selected blood lead concentrations (Table 4). Furthermore, it is important to bear in mind that these figures were based on data excluding subjects with iron-deficiency anaemia. In the screening of a population for lead exposure, the number of false positive results with the use of blood protoporphyrin concentrations will be further increased.

In the evaluation of any screening test consideration should be given to the relative expense, time involved and to the ease of assay. It is probable that the determination of erythrocyte ALA dehydratase activity and the measurement of blood protoporphyrin concentrations by classical extraction procedures are little different on the basis of these criteria. However, the determination of blood protoporphyrin concentrations with the ‘hematofluorometer’ does have the major advantage of rapidity and simplicity combined with relatively cheap operating costs. Thus it is likely that the use of this instrument will be expedient in the monitoring of large numbers of subjects industrially exposed to lead.

The inter-relationships of blood lead concentrations, leucocyte ALA synthase activity, erythrocyte ALA dehydratase and blood protoporphyrin concentrations can be of considerable metabolic importance in determining the depression of haem production in lead exposure, provided that one assumes that these parameters parallel those in the bone marrow and liver, the main sites of haem biosynthesis. This is a reasonable assumption, provided that the subjects are in a steady-state condition. It is clear from the present studies that the relationship between erythrocyte ALA dehydratase and blood protoporphyrin and the relationship between erythrocyte ALA dehydratase and leucocyte ALA synthase are of a similar form. Thus, when erythrocyte ALA dehydratase activity falls below 20 nmol of ALA utilized min⁻¹ ml⁻¹ of erythrocytes, there is a concomitant accelerating rise in both blood protoporphyrin concentrations and leucocyte ALA synthase activity. We have previously demonstrated that ALA synthase activity is inversely related to haemoglobin and haemoprotein concentrations in lead exposure (Campbell et al., 1977; Goldberg, Meredith, Miller, Moore & Thompson, 1978). It is therefore clear that a rise in blood protoporphyrin concentrations more accurately reflects a depression in haem production than does a depression in erythrocyte ALA dehydratase. This consideration is in concordance with our previous suggestions (Meredith et al., 1978) that there are ‘critical’ tissue lead concentrations, represented by a blood lead value of approximately 2 µmol/l, an erythrocyte protoporphyrin value of 0.75 µmol/l and an erythrocyte ALA dehydratase activity of 20 nmol of ALA utilized min⁻¹ ml⁻¹ of erythrocytes. Above these concentrations, and below this activity, the inhibition of haem synthesis is sufficiently great to reduce free haem and thus to induce ALA synthase activity.

**Acknowledgments**

We thank Miss M. A. Hughes for skilled technical assistance. P. M. was supported by the Medical Research Council (U.K.).
References


