*Brit. J. industr. Med.*, 1967, 24, 203.

# Early Signs of Lead-exposure

## A comparative study of laboratory tests

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Previous work has shown that urinary lead, coproporphyrin (CP), and δ-aminolaevulinic acid (ALA) are closely related after exposure to lead. We have confirmed the relationship in 50 automobile workers with different exposures, and have also estimated porphobilinogen (PBG) levels. There was some evidence that a raised ALA level gave an earlier sign of lead absorption than a raised CP level. ALA-dehydratase activity (converting ALA to PBG) was greatly reduced by exposure, on the average to 20% of normal. This reduction in activity was strongly correlated with the increases in urinary lead, CP, and ALA and is the most sensitive test of exposure to lead.

A weak correlation was found between the number of coarsely stippled cells, or the basophilic quotient, and the urinary levels of lead, CP, and ALA.

In addition, the serum aspartate aminotransferase (SGOT) and serum alanine aminotransferase (SGPT) levels were estimated and were not affected by exposure to lead. The serum protein patterns and haemoglobin levels were also unaffected.

In the last decade several investigators have shown that workers exposed to inorganic lead have considerably raised δ-aminolaevulinic acid (ALA) levels in the urine (Holmqvist, 1960; Haeger-Aronsen, 1960; de Kretser and Waldron, 1963; Gattner and Schrantz, 1964). Most investigators found a linear relationship between the urinary concentrations of coproporphyrin (CP) and ALA both of which have also been correlated with lead concentrations in urine or in whole blood.

Raised ALA levels in urine are closely correlated with raised levels in serum (Chiesura and Brugnone, 1963; Saita and Moreo, 1961). ALA in serum appears to be the better test of lead absorption as it gives earlier signs of lead exposure, but its determination is difficult, because of its low concentrations.

Tanabe (1959) found raised urinary concentrations of porphobilinogen (PBG) in cases of lead poisoning. Haeger-Aronsen (1960) likewise reported an increased urinary output in lead-poisoned rabbits. These findings have been confirmed by other authors (Watson, Hawkinson, and Bossenmaier, 1953; Bashour, 1954) in lead-exposed workers. Griggs and Harris (1958) and de Kretser and Waldron (1963), however, have stated that the excretion of PBG is not changed by lead exposure.

Waldman and Borman (1959) found increased serum aspartate aminotransferase (SGOT) levels in lead workers. Waldron (1964) did not find any rise in SGOT, nor in serum alanine aminotransferase (SGPT) levels. It is important to determine the SGOT/SGPT quotient, because in hepatitis and under the influence of hepatotoxic agents this quotient (normal = 1) may be changed. The albumin:globulin (A/G) ratio also may point to disturbance of the liver function.

Because of the conflicting results reported above, and because few data on the interrelationships of the parameters mentioned have been published, we decided to investigate the correlation between the parameters in a group of workers exposed to inorganic lead.

### Subjects

The group examined consisted of 50 workers from an automobile factory, most of them working in the car-body polishing department. An alloy of lead and tin is spread on the car-body and has to be abraded and polished by electrically-driven grinding and polishing apparatus. In the areas of relatively high exposure the

Received for publication December 3, 1966.

workers wore ventilated hoods. Other workers were employed in the car construction department. Lead in air was not determined, but the group was obviously not homogenous in regard to intensity and duration of exposure. The workers were physically examined; 15 of them reported some non-specific symptoms of fatigue, stomachache, and loss of appetite, but without constipation. Cases of manifest lead intoxication were not reported.

Because the aim of the investigation was to examine the relationships between several parameters of increased lead absorption within individuals and not the relation between these parameters and the degree of lead exposure or the intensity of symptoms, we did not separate the workers into groups corresponding to their supposed degree of exposure or to the non-specific symptoms they reported.

In order to avoid the problems associated with obtaining 24-hour urine samples, we collected the urine at about 10.30 a.m. The workers had previously voided their bladders at about 8.00 a.m.

## Methods

The following laboratory analyses were carried out:

In blood: haemoglobin (Hb), ALA-dehydratase, SGOT, SGPT, serum protein pattern, basophilic count, and haematocrit value

In urine: ALA, CP, PBG, lead (in only 24 cases), creatinine, density, and volume.

Urinary lead was estimated by the dithizone method of King and Thompson (1961). We modified the method by extracting twice with dithizone, transferring the lead after the first dithizone extraction to the acidified solution. After bringing the solution to the appropriate *p*H, the second dithizone extraction was performed.

ALA and PBG in the urine were determined by the method of Mauzerall and Granick (1956). The compounds were separated using Dowex ion-exchange resins, followed by elution of the substances and colour development with Ehrlich reagent.

Coproporphyrin was determined by the method of Zondag and van Kampen (1956). This method involves quantitative extraction of CP from urine with ether, followed by purification of the ether layers and extraction of the CP into dilute HCl. The concentration was determined spectrophotometrically at 400 m$\mu$. Because of impurities interfering at 400 m$\mu$, the absorbence was corrected from the absorbence at two other wavelengths (430 and 380 m$\mu$) by an empirical formula.

The assay of ALA-dehydratase in the whole blood was made by direct colorimetric estimation of the amount of PBG produced from added ALA after incubation for one hour (Bonsignore, Calissano, and Cartasegna, 1965a). The results were corrected to a constant haematocrit value as the enzyme only occurs in the erythrocytes. The activities reported in the Results section are calculated from the formula

$$\frac{1{,}000 \times 12{\cdot}5\ (A_{60} - A_0)}{\text{per cent haematocrit}}$$

where $A_{60}$ and $A_0$ are the absorbencies at t = 60 and t = 0 min., and the factor 12·5 corrects for the dilution of blood.

For the basophil count the staining method of Lane (1949), as modified by Malcolm (personal communication), was used. The slides were examined in the dark field. Both the coarsely- and the finely-stippled cells were counted (Zielhuis, 1957). The coarse punctation corresponds to basophilic cells; both coarse and fine punctation correspond to reticulocytosis. Fifty microscopical fields, each containing about 200 erythrocytes, were counted. Because of the difficulty in obtaining blood smears with the same thickness (otherwise the total number of cells per field is variable) we assessed the number of coarsely-stippled cells on a numerical scale as follows:

1 = 0 − 0·5% (0 − 5 coarsely stippled cells per 10,000 erythrocytes)
2 = 0·5 − 1·0%
3 = 1·0 − 1·5%
4 = 1·5 − 2·0%
5 = >2·0%.

In addition, we determined the basophilic quotient $\left(\frac{\text{reticulocytes counted}}{\text{basophil count}}\right)$, the ratio of the total number of coarsely and finely stippled cells to the number of coarsely stippled cells (basophil count). In increasing lead absorption, this quotient decreases and approaches the value 2 (Baikie and Valtis, 1954). In this case the number of finely and coarsely stippled cells is about equal. This quotient is independent of the constancy of the number of erythrocytes per microscopical field.

The transaminases were assayed by the method of Reitman and Frankel (1957). Creatinine in urine was determined by the method of Jaffé (1886). Haemoglobin was determined as cyanmethaemoglobin using a spectrophotometer. Finally the serum protein fractions were separated by means of electrophoresis, using cellulose acetate as the supporting medium.

## Results

The results of the laboratory tests are shown in Table I, in order of increasing excretions of CP.

Table II presents the average values of urinary CP, ALA, lead and PBG, expressed in different ways. Division of the values by the creatinine level yielded creatinine-corrected data. Multiplying the values by the factor $\frac{0{\cdot}024}{(D - 1)}$, where D = density at room temperature, provided density-corrected data. According to Pinto, Elkins, and Ege (1941), we also corrected the lead values by the formula $Pb_c = Pb_d\sqrt{V/T}$, where $Pb_c$ is the corrected lead concentration in $\mu$g./l, $Pb_d$ is the uncorrected concentration in $\mu$g./l., V is the volume of the

TABLE I

CONCENTRATIONS OF CP, ALA, AND LEAD (Pb) IN URINE, HAEMOGLOBIN LEVELS, ALA-DEHYDRATASE ACTIVITIES, BASOPHIL QUOTIENTS, AND BASOPHIL COUNTS IN BLOOD

| *Case* | *CP* (*μg./g. creatinine*) | *ALA* (*mg./g. creatinine*) | *Pb* (*μg./g. creatinine*) | *Hb* (*g./100 ml.*) | *ALA-dehydratase* (*absorbence increase*[1] *1 ml. erythrocytes in 1 hr at 37°C.*) | *Basophil Quotient* $\left(\frac{\text{reticulocytes}}{\text{basophils}}\right)$ | *Basophil Count* (*numerical scale*) |
|---|---|---|---|---|---|---|---|
| 1 | 40 | 5·1 | — | 16·1 | 33 | 8·1 | 2 |
| 2 | 41 | 4·15 | 69 | 16·0 | 41 | 3·5 | 5 |
| 3 | 48 | 2·7 | — | 17·3 | 26 | >4·0 | 1 |
| 4 | 51 | 3·75 | — | 15·4 | 15 | 7·8 | 2 |
| 5 | 51 | 4·55 | — | 16·3 | 64 | 2·7 | 2 |
| 6 | 52 | 2·55 | — | 13·0 | 67 | >13·0 | 1 |
| 7 | 52 | 3·5 | 61 | 14·9 | 19 | 6·5 | 1 |
| 8 | 57 | 6·05 | 101 | 15·2 | 62 | >30·0 | 1 |
| 9 | 59 | 2·4 | 47 | 16·5 | 22 | 4·4 | 2 |
| 10 | 65 | 3·2 | — | 15·3 | 28 | 3·4 | 1 |
| 11 | 65 | 4·65 | — | 15·5 | 15 | 9·0 | 1 |
| 12 | 67 | 2·3 | — | 15·3 | 28 | 3·6 | 1 |
| 13 | 68 | 4·05 | — | 15·3 | 27 | 3·7 | 1 |
| 14 | 70 | 1·85 | 19 | 16·9 | 21 | 3·5 | 4 |
| 15 | 71 | 3·15 | — | 13·6 | 15 | 3·8 | 3 |
| 16 | 75 | 3·55 | 140 | 15·0 | 17 | 6·8 | 1 |
| 17 | 76 | 5·5 | 38 | 15·6 | 33 | >18·0 | 1 |
| 18 | 77 | 6·15 | — | 16·0 | 49 | >7·0 | 1 |
| 19 | 82 | 5·3 | 97 | 17·3 | 13 | 5·0 | 1 |
| 20 | 88 | 3·7 | 83 | 15·4 | 16 | 6·3 | 2 |
| 21 | 92 | 4·65 | — | 17·8 | 13 | 9·7 | 1 |
| 22 | 93 | 3·6 | 162 | 14·7 | 58 | 3·0 | 1 |
| 23 | 94 | 3·75 | 117 | 15·4 | 19 | 3·3 | 4 |
| 24 | 95 | 3·65 | — | 14·3 | 44 | 3·2 | 3 |
| 25 | 98 | 3·85 | — | 14·8 | 32 | 2·5 | 2 |
| 26 | 101 | 2·9 | — | 15·2 | 28 | 3·5 | 5 |
| 27 | 103 | 3·85 | 88 | 16·1 | 85 | 4·1 | 4 |
| 28 | 108 | 3·05 | — | 15·5 | 47 | >6·0 | 1 |
| 29 | 116 | 6·2 | — | 16·5 | 8 | 3·4 | 4 |
| 30 | 117 | 7·05 | 218 | 15·0 | 15 | 3·3 | 2 |
| 31 | 118 | 2·8 | 82 | 15·0 | 35 | 5·3 | 5 |
| 32 | 125 | 5·4 | 55 | 16·3 | 17 | 2·7 | 5 |
| 33 | 128 | 7·8 | 75 | 15·4 | 32 | 4·4 | 2 |
| 34 | 145 | 4·95 | 130 | 13·7 | 16 | 3·0 | 3 |
| 35 | 164 | 2·75 | — | 15·4 | 21 | 3·6 | 2 |
| 36 | 167 | 10·9 | — | 18·7 | 11 | 3·4 | 2 |
| 37 | 200 | 4·4 | — | 15·2 | 12 | 2·0 | 4 |
| 38 | 203 | 9·9 | 55 | 18·1 | 10 | 4·0 | 3 |
| 39 | 211 | 9·4 | — | 13·6 | 15 | 3·8 | 3 |
| 40 | 243 | 12·7 | 80 | 15·4 | 5 | 2·6 | 5 |
| 41 | 305 | 10·8 | — | 17·9 | 9 | 2·4 | 5 |
| 42 | 311 | 13·55 | — | 16·0 | 6 | 2·8 | 4 |
| 43 | 370 | 9·65 | — | 15·8 | 0 | >3·0 | 1 |
| 44 | 411 | 10·1 | — | 14·8 | 8 | 7·2 | 2 |
| 45 | 500 | 20·15 | 264 | 15·4 | 8 | 3·5 | 2 |
| 46 | 557 | 16·15 | 133 | 16·3 | 5 | 2·8 | 2 |
| 47 | 576 | 21·3 | 150 | 15·0 | 4 | 2·6 | 5 |
| 48 | 642 | 14·15 | — | 16·3 | 4 | 5·2 | 4 |
| 49 | 918 | 39·3 | 275 | 14·6 | 3 | 2·2 | 5 |
| 50 | 2,220 | 33·8 | 290 | 14·9 | 4 | 2·8 | 5 |

[1]See text (Methods) for definition.

The sign '>' indicates that the basophil count in these cases was zero, corresponding to an infinite quotient. The number shown is the reticulocyte count.

TABLE II

URINARY LEVELS OF CP, ALA, LEAD, AND PBG (AVERAGE VALUES)

| | *CP* (μg.) (*n* = 50) | *ALA* (mg.) (*n* = 50) | *Lead* (μg.) (*n* = 24) | *PBG* (mg.) (*n* = 50) |
|---|---|---|---|---|
| Conc. per litre | 316 | 11·64 | 172 | 1·30 |
| Conc. per g. creatinine | 216 | 7·61 | 118 | 0·86 |
| Conc. corrected to density = 1·024 | 339 | 11·04 | 166 | Not calc. |
| Conc. corrected with Pinto's formula | Not calc. | Not calc. | 149 | Not calc. |
| Quantity excreted in 2·5 hr | 32 | 1·06 | 15·8 | Not calc. |

Mean creatinine conc. = 1·32 g./l.
Mean urine density = 1·024.
Mean urine excretion in 2·5 hr = 113 ml.

TABLE III

VALUES FOR HB, ALA-DEHYDRATASE, BASOPHIL QUOTIENT, BASOPHIL COUNT, TRANSAMINASES, AND SERUM PROTEIN FRACTIONS

| | | | | | |
|---|---|---|---|---|---|
| Hb (g./100 ml.) | 15·63 (15·50, 15·59, 15·53, 15·68, 15·76) | | | | |
| ALA-dehydratase (Units under 'Method') | 24·5 (37·7, 23·4, 34·9, 17·4, 5·1). Mean normal value is 113 ± 13 | | | | |
| Basophil quotient $\left(\frac{\text{reticulocytes}}{\text{basophils}}\right)$ | 5·3 (8·4, 6·7, 4·2, 3·5, 3·7) | | | | |
| Basophil count | 2·6 (1·8, 1·6, 2·7, 3·4, 3·5) | | | | |
| S.G.O.T. (Karmen units) | 18·2 (20, 17, 18, 19, 17). Normal range: 8 to 40 | | | | |
| S.G.P.T. (Karmen units) | 18·1 (17, 17, 16, 23, 17). Normal range: 5 to 35. | | | | |
| Serum protein pattern (%) | albumin | $\alpha_1$-globulin | $\alpha_2$-globulin | $\beta$-globulin | $\gamma$-globulin |
| | 59·6 | 2·8 | 7·6 | 13·3 | 16·7 |
| Serum protein pattern (%) of a group of non-exposed persons (n = 26) | 59·6 | 3·0 | 7·3 | 13·1 | 17·1 |

The figures between the brackets are the average values in subjects 1 to 10, 11 to 20, 21 to 30, 31 to 40, and 41 to 50 (Table I), therefore in increasing order of CP concentration.

sample, and T is the time in minutes during which the sample was collected (150 min. in our experiments). This is stated to give the best estimate of the amount of lead excreted in 24 hours.

Table III gives the average values for Hb, ALA-dehydratase, basophil quotient, basophil count, SGOT, SGPT, and serum protein pattern in blood.

## Discussion and Conclusion

The average haemoglobin level in the exposed group was 15·63 g./100 ml., with 11 values below 15·0 and four below 14·0 g./100 ml. These levels were not significantly different from those in non-exposed Dutch workers. The low haemoglobin levels in cases 6, 15, 34, and 39, moreover, were not associated with increased CP or ALA excretion, *i.e.*, the anaemia was probably due to other causes.

The workers under observation were, therefore, not suffering from definite lead poisoning but only showed signs of increased lead absorption without anaemia. The exposure data and the absence of definite cases of poisoning in the past support this conclusion.

Except in three workers, the SGOT/SGPT quotient (average 1) did not come outside the range found in groups of non-exposed subjects. These

three values were not associated with increased levels in any of the other tests. In addition we did not find a significant difference in transaminase values between the 15 workers with the highest and the 15 workers with the lowest CP excretion. Thus transaminase levels are not increased in cases of slight to moderate lead absorption.

The same is true of the serum protein patterns. No statistically significant differences were found between the exposed group and a group of non-exposed subjects. The albumin: globulin ratio was also quite normal.

In contrast to Griggs and Harris (1958), we found an increased PBG excretion, although it was only moderate. The mean in the 15 workers with the highest CP levels was 2·00 mg./l., and in the 15 workers with the lowest CP levels it was 0·87 mg./l., which is significantly different (Wilcoxon, $P<0.01$). According to Haeger-Aronsen (1960), the upper limit of the normal PBG level in urine is 2 mg./l., with an average of 1 mg./litre. In our investigation we found in urine from eight workers more than 2 mg./l., and from 23 workers 1 to 2 mg./l., but from 19 less than 1 mg./litre. Although increased PBG excretion was evident in the group under study, it was only found in a fairly small part of the group. PBG excretion, therefore, does not appear to be a useful early indicator for increased lead absorption.

Although the basophil quotient did not reach the value 2 (*i.e.*, 50% of the reticulocytes), there was a tendency for it to decrease with increasing CP excretion (Table III). This decrease is negatively correlated ($r = -0.70$) with the increase of the basophil count (numerical scale). Table I indicates that the increase in basophil count generally occurred after the increase of CP excretion. The validity of the count as an early indicator is therefore less than that of other parameters.

Nearly all the subjects gave values of ALA-dehydratase activity far below normal, on the average only 20%. The normal levels determined in our laboratory on a group of 26 non-exposed subjects agree with those found by Bonsignore *et al.* (1965a). From Table I it is obvious that this is the most sensitive test examined, perhaps too sensitive to be of use in industrial medicine. It would be interesting to study the order in which various parameters deviate from the normal. This, however, requires a long-term study in animals, or in workers, not hitherto exposed, from the start of their employment in lead-processing industries.

We studied the correlation between the values of the various parameters in individual workers. High correlation coefficients were found between ALA and CP excretion ($r = 0.85$, $n = 50$), between CP and lead excretion ($r = 0.77$, $n = 24$), and between ALA and lead excretion ($r = 0.79$, $n = 24$). These coefficients are not significantly different. The relationships are shown in Figures 1, 2, and 3.

It should be noted that in Figs. 1 to 6 the x axis is vertical and the y axis is horizontal; and that as there is variance on both x and y, two lines can be drawn, the best estimate lying between the two.

The relations between the decrease of ALA-dehydratase and the increase of CP, ALA, and lead excretion are shown in Figures 4, 5, and 6. In order to obtain linear relationships we plotted the reciprocals of enzyme activity, 100/E, against the excretion levels. In Table IV the correlation coefficients (r) between 100/E and CP, ALA, and lead excretion, each expressed in different ways, are given. The difference between the correlation coefficients for the same parameters, but expressed in different ways, is not statistically significant.

In Table V the correlation coefficients between the basophil quotient and the various other parameters are given. These coefficients are signifi-

TABLE IV

CORRELATION COEFFICIENTS BETWEEN 100/[ALA-DEHYDRATASE ACTIVITY] AND CP, ALA AND LEAD

| | *CP* ($n = 50$) | *ALA* ($n = 50$) | *Lead* ($n = 24$) |
|---|---|---|---|
| Conc. per litre | 0·66 (0·47 to 0·79) | 0·64 (0·44 to 0·77) | 0·71 (0·43 to 0·86) |
| Conc. per g. creatinine | 0·71 (0·54 to 0·83) | 0·69 (0·51 to 0·81) | 0·48 (0·09 to 0·74) |
| Conc. corrected to density = 1·024 | 0·69 (0·51 to 0·81) | 0·67 (0·49 to 0·80) | 0·66 (0·35 to 0·84) |
| Conc. corrected with Pinto's formula | — | — | 0·66 (0·35 to 0·84) |
| Corr. on quantity excreted in 2·5 hrs | 0·55 (0·32 to 0·72) | 0·46 (0·21 to 0·65) | 0·54 (0·17 to 0·77) |

The 95% confidence limits are given in brackets. All coefficients are significant ($P<0.01$).

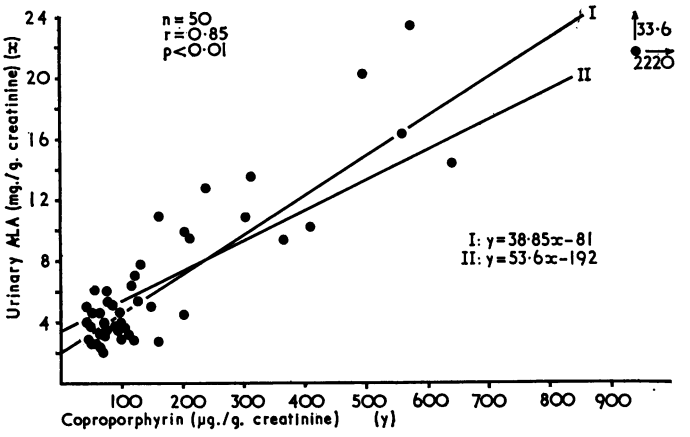


FIG. 1. Urinary levels of CP plotted against those of ALA.

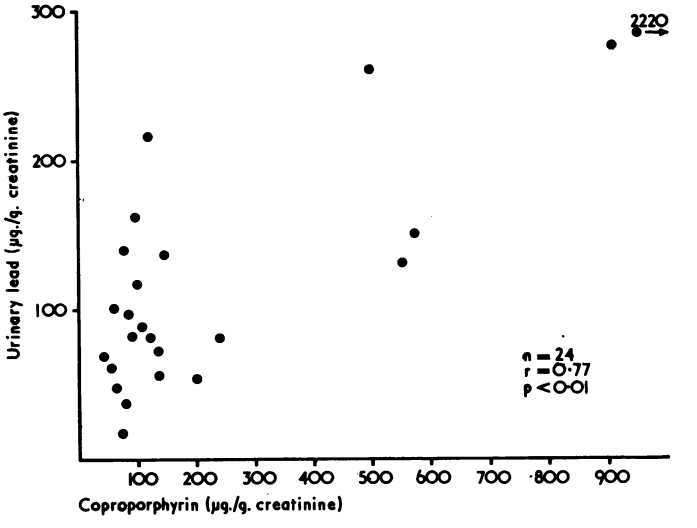


FIG. 2. Urinary levels of CP plotted against those of lead.

TABLE V

CORRELATION COEFFICIENTS BETWEEN THE BASOPHIL QUOTIENT AND CP, ALA, LEAD, AND 100/[ALA-DEHYDRATASE]

| | *CP* ($n = 50$) | *ALA* ($n = 50$) | *Lead* ($n = 24$) | $\frac{100}{(E)}$ ($n = 50$) |
|---|---|---|---|---|
| Conc. per litre | 0·36[1] (0·09 to 0·58) | 0·22 (−0·07 to 0·47) | 0·47[1] (0·08 to 0·74) | |
| Conc. per g. creatinine | 0·48[1] (0·23 to 0·67) | 0·35[1] (0·07 to 0·57) | 0·37 (−0·04 to 0·67) | |
| Conc. corr. to density = 1·024 | 0·53[1] (0·29 to 0·71) | 0·47[1] (0·22 to 0·66) | 0·57[1] (0·22 to 0·79) | 0·35[1] (0·07 to 0·57) |
| Conc. corr. with Pinto's formula | — | — | 0·42 (0·01 to 0·70) | |
| Corr. on quantity excreted in 2·5 hrs | 0·20 (−0·09 to 0·49) | 0·10 (−0·19 to 0·37) | 0·32 (−0·07 to 0·64) | |

[1] $P < 0·05$; 95% confidence limits are shown in brackets.

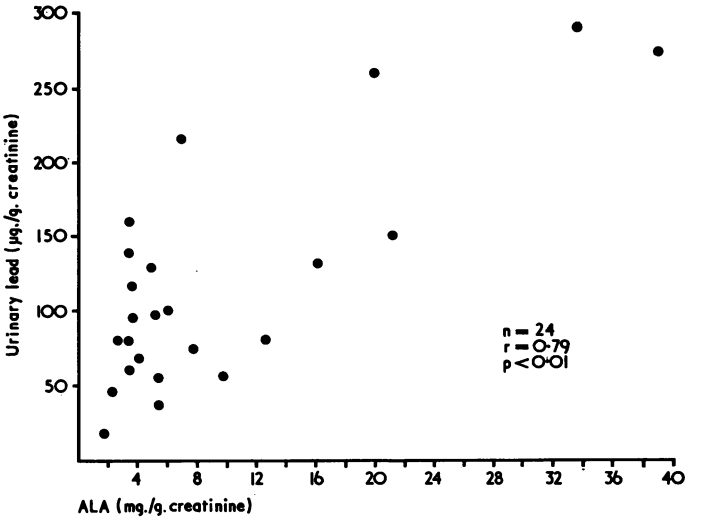


FIG. 3. Urinary levels of ALA plotted against those of lead.

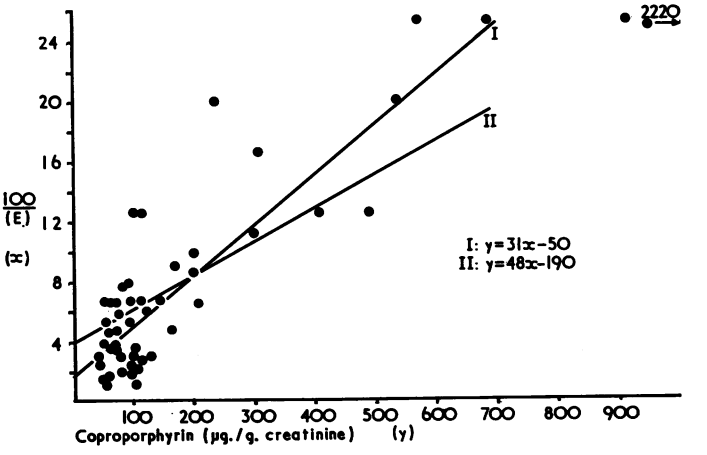


FIG. 4. Urinary levels of CP plotted against the reciprocal values of the activity of ALA-dehydratase.

TABLE VI

AVERAGE VALUES OF ALA AND CP AND CALCULATED CORRELATION COEFFICIENTS

| *Author* | *No.* | *Average CP (µg./l.)* | *Average ALA (mg./l.)* | *ALA/CP* | *Correlation Coefficient* |
|---|---|---|---|---|---|
| This paper .. .. .. .. .. | 50 | 216 (per g. creatinine) | 7·61 (per g. creatinine) | 36 | 0·85 |
| Gattner and Schrantz (1964) .. .. .. | 42 | 371 | 15·0 | 40 | 0·92 (P < 0·01) |
| de Kretser and Waldron (1963) .. .. | 100 | 236 | 7·5 | 32 | 0·74 (P < 0·01) |
| Djuric *et al.* (1966) .. .. .. .. | 28 | 131 | 6·1 | 46 | 0·43 (P < 0·05) |
| Cramér and Selander (1965) .. .. .. | 12 | 250 | 20·0 | 80 | 0·65 (P < 0·05) |

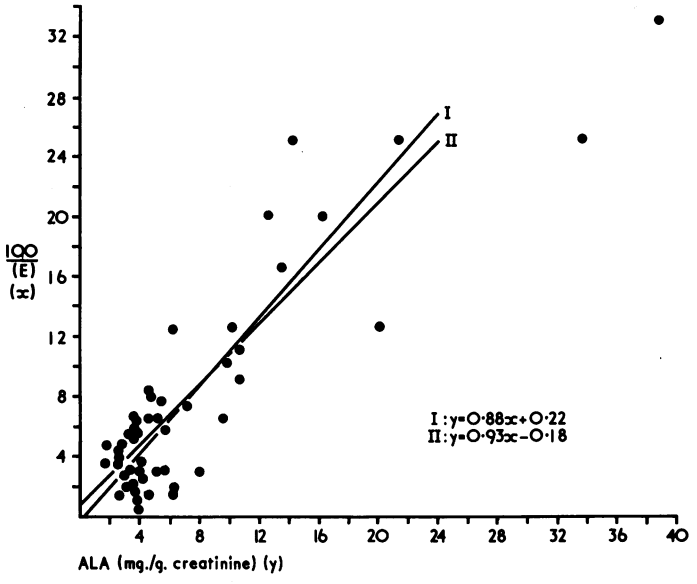


FIG. 5. Urinary levels of ALA plotted against the reciprocal values of the activity of ALA-dehydratase.

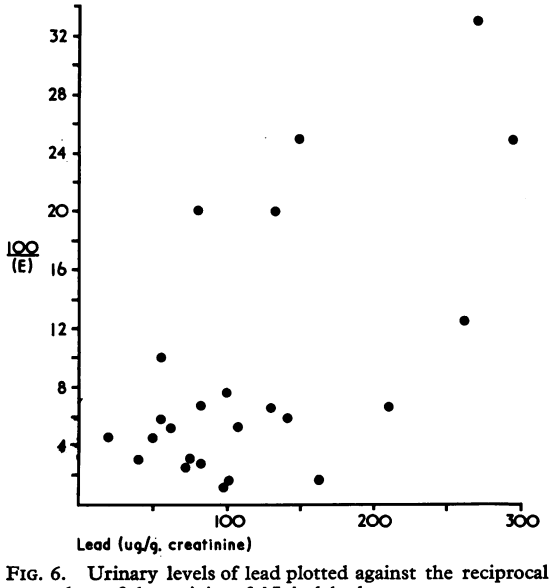


FIG. 6. Urinary levels of lead plotted against the reciprocal values of the activity of ALA-dehydratase.

cantly lower than those given in Table IV (g./100 g.). Punctate basophilia, therefore, is a less valid indicator for increased lead absorption than the other parameters investigated.

The relation between CP and ALA excretion shown in Table VI agrees with the results of most investigators. This shows high correlation, except for the data of Djuric, Novak, Milic, and Kalic-Filipovic (1966). de Kretser and Waldron (1963) concluded from their data that any significant or linear correlation between the excretion of the two substances may be ruled out but they did not calculate the correlation coefficient. From their graph a significant correlation ($r = 0{\cdot}74$, $P<0{\cdot}01$) could be calculated. As levels of both ALA and CP increase similarly, both provide tests of equal sensitivity for lead absorption, at least in the range studied. ALA excretion is more specific because coproporphyrinuria is a more common sign of increased absorption of other metals. High ALA excretion also occurs in porphyria acuta congenita; but in that case PBG excretion is also markedly raised. A slight increase in ALA excretion has also been reported in some rare anaemias.

The determination of ALA excretion is, however, still a highly specialized laboratory procedure whereas the simple method for the semi-quantitative estimation of CP excretion (Donath, 1956; Zielhuis, 1961) is much more practicable in small lead processing industries. There is an apparent need for a 'quick' method of determination of ALA excretion.

Does ALA excretion precede CP excretion? Djuric and his colleagues (1966) stated that ALA excretion is the first sign of lead absorption because ALA levels were increased in cases where CP levels were still within normal limits. In a group of 37 moderately exposed workers they found increased ALA levels in 27 subjects, but raised CP levels in only six. The same conclusions can be drawn from

the results of Balbo, Gualdi, and Marucci (1965) who found that, of the 50 subjects under investigation, the CP level was increased in 21 cases and the ALA level in 40 cases.

Haeger-Aronsen (1960) found 4 mg. ALA/g. creatinine and 120 μg. CP/g. creatinine to be the normal upper limits. Applying these limits to our data, we divided them into four classes of increasing CP levels (Table VII). All workers (n = 19) with CP levels higher than 120 μg./g. creatinine had ALA levels above 4 mg./g. creatinine, except one (No. 35, ALA 2·75 mg./g. creatinine; CP 164 μg./g. creatinine). In addition there were 12 subjects with increased ALA levels and normal CP levels (<120 μg./g. creatinine). These data strongly suggest that increased ALA excretion is an earlier sign of lead absorption than is increased CP excretion.

TABLE VII

NUMBER OF SUBJECTS ABOVE THE NORMAL UPPER LIMITS OF ALA AND CP EXCRETION

| *Coproporphyrin* | *Total No.* | *No. of Subjects with ALA ≥ 4 mg./g. Creatinine* |
|---|---|---|
| 40 to 70 μg./g. creatinine .. | 13 | 6 |
| 70 to 120 μg./g. creatinine .. | 18 | 6 |
| 120 to 200 μg./g. creatinine .. | 5 | 4 |
| > 200 μg./g. creatinine .. | 14 | 14 |
| Total .. .. .. .. | 50 | 30 |

This also follows from Figure 1. The regression lines do not run through the origin but intersect the ordinate at the positive side. Omitting the 19 subjects with normal CP excretion, we recalculated the data for the other 31 subjects. The resulting regression lines had almost the same slope as those of Figure 1. Comparing Figs. 4 and 5, we see that the regression lines between 100/E and ALA excretion run through the origins but not those between 100/E and CP excretion. All these results point to the conclusion that ALA excretion definitely precedes CP excretion. A more detailed evaluation of the sequence, however, can only be given by long-term studies in animals and men.

PBG excretion was increased, and the increase was rather strongly correlated with ALA excretion ($r = 0{\cdot}52$, $P < 0{\cdot}01$). It was only weakly correlated with CP excretion ($r = 0{\cdot}32$) and with 100/E ($r = 0{\cdot}28$). These values of r are barely significant ($P = 0{\cdot}05$). Thus, PBG excretion is more closely related to ALA than to CP excretion, apparently because PBG is directly produced from ALA. However, as PBG excretion is less influenced by lead exposure it is not useful as an early indicator.

The increase in ALA concentration can be accounted for by the inhibition of ALA-dehydratase. It is, however, surprising that increased PBG levels are found, as PBG is formed from ALA via the action of ALA-dehydratase. The well-known increase in protoporphyrin IX in blood is also surprising. It can only be supposed that although lead inhibits ALA-dehydratase the remaining activity is still enough in the presence of raised ALA levels to give an increase in PBG.

According to Bonsignore *et al.* (1965b, 1965c), the activity of ALA-dehydratase is strongly inhibited in experimental lead intoxication both *in vivo* and *in vitro*. The effect seems to be partly due to oxidation of sulphydryl groups, because the blockage is partly suppressed by substances containing essential thio groups such as cysteine and glutathione. Another manifestation of the overall disturbance of haem synthesis is the inhibition by inorganic leads of the incorporation of the ferric ion into the porphyrin molecule. This leads to an increase of protoporphyrin IX in the erythrocytes and of iron in the serum, ultimately resulting in a decreased haemoglobin level. It seems, therefore, that the anaemia due to the intake of relatively large amounts of lead is caused by inhibition of the 'incorporation enzyme' (Goldberg enzyme) rather than by inhibition of ALA-dehydratase.

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