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COMMUNICATIONS

Diagnostic value of free erythrocyte porphyrins and blood lead as a screening test for lead exposure

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It is generally accepted that lead interferes with haem synthesis at three sites i.e. to inhibit δ-aminolevulinic acid (δ-ALA) dehydratase, coproporphyrinogen oxidase and the ferrochelatase catalysed transport of iron to protoporphyrin. Labbe (1977) however finds that lead does not inhibit ferrochelatase but interferes with intracellular iron transport, to produce an iron-deficiency response. Irrespective of the mechanisms involved, it is clear that one result of lead intoxication is an accumulation of protoporphyrins in red blood cells. In the present text it is shown that measurement of this protoporphyrin combined with the determination of blood lead provides a useful diagnostic test for the evaluation of lead intoxications.

Samples. Analyses were made on unclotted samples originating from patients, suspected of lead or heavy metal intoxication between April 1, 1977 and September 30, 1978. If 24 h urine samples were also returned, urinary lead and δ-ALA were also measured.

Free erythrocyte porphyrins (FEP). The method of Piomelli (1973) was used with slight modifications of the sample and reagents volumes, without changing their ratios.

Results were expressed in μg FEP/100 ml of red blood cells because some sources returned red blood cells instead of whole blood. Measurements were made on an Aminco Bowman SPF 4-8202 spectrophotofluorometer equipped with a photomultiplier RCA 1P21, and an Aminco Bowman X-Y recorder. The excitation wavelength was set at 405 nm. The emission peak between 550 and 700 nm was recorded and its height used as a measure of protoporphyrin concentration. Both excitation and emission slits were opened at 1 mm. Standard solutions were prepared from protoporphyrin IX dimethyl ester (Sigma Chemical Co. St. Louis, Mo.) according to Piomelli (1973).

Blood and urinary lead. Blood lead was measured by the method of Hessel (1968). The same procedure was used for urine analysis except that the pH of each sample was adjusted before treatment with a chelating agent (Sprague & Slavin 1964).

δ-*Aminolevulinic acid.* This was measured according to the method of Mauzerall & Granick (1956).

A histogram where lead in μg/100 ml of blood in seven intervals of 10 μg/100 ml is plotted against the average free erythrocyte porphyrins (FEP) concentration, expressed in μg/100 ml of red blood cells is shown in Fig. 1. Its overall aspects seems to corroborate the widely accepted idea that FEP increases exponentially with blood lead concentration (Piomelli 1973); the relationship can be expressed in a function of the form

$$y = a\,e^{bx}$$

where y = FEP in μg/100 ml red cells, x = lead in μg/100 ml blood, a and b > 0.
This equation is linearized into

$$\log y = \log a + \frac{bx}{2{\cdot}303}$$

and upon linear regression by the method of the least squares, taking the centre of each interval as the mean lead concentration, yields an equation:

$$\log(\text{FEP}) = 1{\cdot}51 + 0{\cdot}019\,(\text{Pb})$$

with a correlation coefficient r = 0·97. This correlation is reasonably good although by expressing the results in the form of a histogram, much information is lost. If all the experimental data are taken into account, an exponential curve fit is obtained. Its equation can be written as

$$\log(\text{FEP}) = 1{\cdot}39 + 0{\cdot}017\,(\text{Pb})$$

with a correlation coefficient of r = 0·71. These data fit surprisingly well with those from other authors (Lamola et al 1975; Piomelli 1973).

If of 388 patients those with a blood lead ⩽40 μg/100 ml (upper limit) are considered and the average of the corresponding FEP-values is calculated, a value of 56 (s.d. 33) μg/100 ml of red blood cells is found (n = 264). According to Houk et al (1975) the FEP test is positive when the value is equal to or greater than three standard deviations above normal. For our investigation this means ⩾155 μg/100 ml of packed red cells. This figure too corresponds well with the value of 160, suggested by these authors. If 155 μg/100 ml of red cells is considered as the ceiling value for FEP, 102 (26%) patients of 388 showed a positive result. Of these 85 had a blood lead above 40 μg/100 ml. Similarly if 40 μg/100 ml of blood is taken as the maximum normal value, 113 (29%) had a FEP value beyond 155 μg/100 ml of red cells. These data are compiled in Fig. 2.

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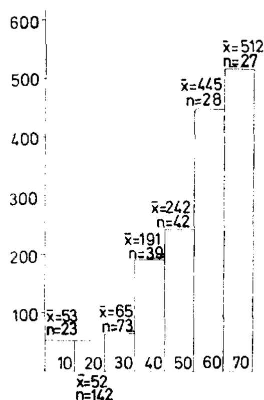

FIG. 1. Histogram of the distribution of experimental Pb and FEP values. Lead is compiled in intervals of ten μg/100 ml blood with n patients in each interval (abscissa). The mean value (X) of the corresponding FEP values (μg FEP/100 ml red cells) is on the ordinate.

In cases where urine was tested 25 had a FEP value above 155 μg/100 ml among which urinary lead varied from 10 times below to 15 times above the reference value (< 150 μg/24 h), and δ-ALA was below the highest acceptable value (3 mg litre^{-1}) in 10 and above in 15 of the cases.

In 23 patients with an increased blood lead concentration (>40 μg/100 ml) it was possible to measure simultaneously urinary δ-ALA and lead. In 15 patients urinary lead increased (>150 μg/24 h) and in 13 cases δ-ALA was above 3 mg litre^{-1}. Despite the limited number of observations, this shows a poor correlation between FEP and urinary lead or δ-ALA.

By mathematical transformation the likelihood of encountering a pair of FEP and Pb values could be calculated. The X, Y diagram of Fig. 3 shows curves

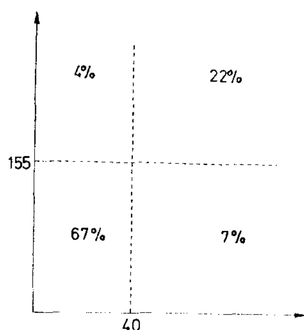

FIG. 2. Distribution of 388 pairs of Pb and FEP values on both sides of the ceiling normal values. Ordinate: μg FEP/100 ml red cells. Abscissa: μg Pb/100 ml blood.

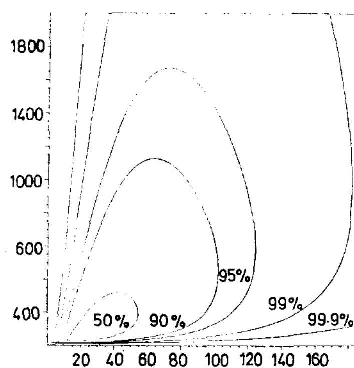

FIG. 3. Equal probability density curves for a given pair of Pb (μg Pb/100 ml blood) and FEP abscissa (μg FEP/100 ml red cells) ordinate.

of equal probability densities. The figures mentioned on it indicate the probability that a given pair of values will be situated within the area of each of the curves.

As a conclusion, there is a good correlation between blood lead and free erythrocyte porphyrins. As a consequence of this correlation it may be stated that a positive FEP-test with a normal blood lead should be checked by differential diagnosis for other possible physiological abnormalities, such as impaired iron metabolism, anaemias or overproduction of porphyrins. These results from a random population, selected on the basis of a suspicion of lead or heavy metal poisoning, are comparable with those obtained from epidemiological or clinical studies. The FEP test can be performed on a small sample volume (25 μl), and is less susceptible to contamination or alteration, than is the case for blood lead.

We did not have the facilities to relate positive laboratory tests (blood lead and/or FEP) to clinical symptoms.

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