Erythrocyte zinc protoporphyrin determination for screening for lead exposure

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Summary
Free erythrocyte protoporphyrin (FEP), erythrocyte zinc protoporphyrin (EZP) and blood lead concentrations were measured in 150 male subjects, 110 of whom were employed in industries where lead exposure was possible. Eighty-seven per cent of these 110 workers had blood lead levels above the maximum safe level of 40 μg/dl, which suggests that the factory control of lead toxicity is inadequate. The AVIV dedicated front-face fluorometer (AVIV Instruments, New Jersey, USA), which measures EZP, was evaluated as a simple, rapid method of screening for lead accumulation. EZP concentrations agreed well with conventional estimations of FEP (r = 0.987, P < 0.0001), and both correlated well with the blood lead concentration (r = 0.558, P < 0.0001 for EZP; r = 0.633, P < 0.0001 for FEP). The specificity of the EZP level as a screening test was found to be high (98%) and the sensitivity moderately good (83%). In view of the ease and speed of operation of this portable fluorometer, it should prove useful as an aid to the detection of early lead intoxication.

Subjects and materials
Venous blood samples from 150 male subjects were collected in Vacutainers, with lithium heparin as anticoagulant. Of these subjects, 110 were working in industries in which lead is used, but they were not specifically selected as those most directly exposed to the metal. Of this group 15 were white and 95 were black; 96 were lead engineers and 14 were lead burners. The blood samples were taken during the course of a regular survey carried out by the National Centre for Occupational Health. The 40 control subjects were all black men working in industries in which they were not exposed to lead.

Blood lead concentration was measured by atomic absorption spectrophotometry on a perchloric acid extract of whole blood. The estimation of free erythrocyte protoporphyrin (FEP) (protoporphyrin IX) was carried out according to the method of Chisholm and Brown in which 0.02 ml of mixed whole blood is evenly spread over the bottom of a test-tube and extracted with 1 ml of a 1:1 mixture of ethyl acetate and acetic acid. After centrifugation the supernate is decanted and a second extraction is carried out with 1 ml of ethyl acetate. The protoporphyrin content of the combined extracts separates into the aqueous phase on addition of 2 ml of 3N HCl, and its fluorescence is measured in a sensitive filter fluorometer. The concentration of FEP is then read off a standard curve as μg/dl whole blood.

EZP was measured in an AVIV dedicated front-face fluorometer. A single drop of blood is placed on a glass coverslip and inserted into the instrument, which gives a digital reading (μg protoporphyrin/g haemoglobin (Hb)) within 6 seconds. The instrument calibrates itself automatically before each reading. Hb concentrations were measured by the cyanmethaemoglobin method and haematoctrits were determined after centrifugation for 6 minutes in a Hawksley microhaematocrit centrifuge.

To compare the two measurements of erythrocyte protoporphyrin, the FEP level, originally measured in μg/dl whole blood, was converted to give μg/g Hb. Since EZP and FEP have molecular weights of 625 and 563 daltons respectively, the free figure was corrected by multiplying by 625/563 (i.e. 1.110) in order to get the equivalent EZP value.

Results
The correlation between EZP concentration as measured on the dedicated fluorometer and the FEP level estimated by...
The correlation coefficient was 0,987 for the whole group and 0,924 for the lead-exposed subjects. These figures were highly significant (P < 0,0001). Both measures of erythrocyte protoporphyrin showed a good correlation with blood lead concentrations measured by atomic absorption spectrophotometry (Figs 2 and 3). The correlation coefficients for the whole group were 0,558 for EZP and 0,633 for FEP. The corresponding values for the lead-exposed subjects were 0,519 and 0,541 respectively, and for the control group 0,822 and 0,832 respectively. In all cases the P value was less than 0,0001.

Fig. 1. The correlation between blood concentrations of FEP and EZP in 150 subjects (r = 0,987, P < 0,0001).

Fig. 2. The correlation between blood concentrations of FEP and lead in 150 subjects (r = 0,633, P < 0,0001).

It is noteworthy that only 13% of the subjects tested had blood lead concentrations below the maximum safety level of 40 µg/dl suggested by WHO. Two of the subjects used as controls had blood lead levels of 62 and 44 µg/dl. It was only possible to trace exposure in the one with the highest concentration; he had been exposed to lead within the previous month. The subject with the second highest lead concentration also had a very high urinary lead concentration (110 µg/l), an elevated EZP concentration (4,8 µg/g Hb) and low erythrocyte pyrimidine-5'-nucleotidase activity. These findings suggest that he too had recently been exposed to lead. Apart from these 2 subjects, the blood lead concentrations were low in the remainder of the control group: the mean value was 13,8 ± 10,1 µg/dl, compared with 65,5 ± 23,0 µg/dl in the lead-exposed group.

There was no significant difference (P > 0,1) between the Hb concentrations in the exposed (16,4 ± 1,5 g/dl) and the control (16,6 ± 1,0 g/dl) groups. This finding suggested that the raised lead concentrations in the exposed group had not had a significant effect on haematopoietic activity.

Discussion

Of the 110 lead-industry workers examined in the present study, 87% had blood lead concentrations above the recommended maximum safety level. While no general conclusions on the prevalence of lead intoxication in industry can be made on the basis of the findings of the present limited study, it is worth noting that subjects were not chosen on the basis of known excessive exposure. This suggests that excessive exposure to lead is indeed common in South Africa and that current monitoring procedures are inadequate. In this context, one of the problems in the past has been the fact that direct measurement of blood levels of lead or of the metabolic sequelae of lead intoxication (such as raised FEP concentrations in red cells and o-aminolaevulinic acid in the urine) is difficult to carry out. It has therefore not been possible to monitor enough workers frequently enough to ensure that the working environment is satisfactory. The introduction of a rapid and simple new screening method is therefore a potentially important development, and the aim of our study was to attempt to validate this new approach by testing a portable front-face fluorometer. We were able to show that values for blood EZP concentrations obtained on this instrument correlated extremely well with results obtained using the more conventional and tedious extraction procedure for FEP determination. EZP concentrations also correlated well with blood lead concentrations. The advantage of the instrument is that it requires no skill to operate and gives a digital readout within seconds. It is unnecessary to obtain venous blood, since a finger-prick sample is sufficient for the test.

As a predictive tool for lead intoxication, EZP appears sufficiently specific and moderately sensitive. The mean value for EZP in the 38 subjects with normal lead concentrations...
was 3.8 ± 0.58 μg/g Hb. If an EZP concentration two standard deviations above this mean is taken to indicate excessive lead accumulation (i.e., a blood lead concentration greater than 40 μg/dl), the test would be accurate in 83% of cases. Only 3 of the 150 subjects tested yielded false-positive results, but screening for lead loading by using EZP determination would miss 16.6% of workers with lead concentrations marginally higher than 40 μg/dl. (The mean lead concentration of subjects with false-negative results was 56.76 ± 12.07 μg/dl, compared with 74.93 ± 20.69 μg/dl for those correctly detected by the EZP method.) Although a higher sensitivity would obviously be preferable, we feel that as a screening method the rapid measurement of EZP has numerous advantages over the conventional methods since the apparatus is portable, requires no trained personnel and allows for frequent screening at low cost. While its major value promises to be in the industrial field, it may also find use as a more general monitor in South Africa, where lead is still added to petrol. Preliminary data indicate that blood lead concentrations may not only be raised in motor mechanics but also in marathon runners.

REFERENCES

Erythrocyte pyrimidine-5'-nucleotidase activity as a sensitive indicator of lead exposure

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Summary
Since the red cell enzyme pyrimidine-5'-nucleotidase (PN) is inhibited by lead, we examined the feasibility of using the activity of this enzyme as a measure of lead exposure. Erythrocyte PN activity was measured in 110 blood samples obtained from subjects working in industries which utilize lead and 40 control subjects. The measurements were then compared with a number of traditional indices of lead poisoning. These included blood and urine lead concentrations and free erythrocyte protoporphyrin, erythrocyte zinc protoporphyrin, urinary coproporphyrin and urinary δ-aminolaevulinic acid levels. There was a highly significant negative correlation between erythrocyte PN activity and blood lead concentration (-0.83; P < 0.0001), which was greater than that for any of the other measurements. It was therefore concluded that erythrocyte PN activity is an excellent indicator of lead exposure.


It has long been recognized that excessive exposure to lead is deleterious to health. The toxic results are seen in nearly every organ, the major being on the haematological and neurological systems. In South Africa, blood levels of lead in employees working in industries which utilize this metal are often above the maximum safety level of 40 μg/dl recommended by the World Health Organization, which suggests that present methods of factory monitoring are unsatisfactory. The presence of lead in petrol is another matter for local concern. In this connection, preliminary data suggesting that blood lead levels may be higher than normal in motor mechanics and in marathon runners are of interest, as are reports that lead concentrations in deciduous teeth are higher in children living in urban areas than in those living in rural areas. Valentine et al. were the first to demonstrate that red blood cells contain a pyrimidine-specific nucleotidase, hereditary