Determination of plasma lead levels in normal subjects and in lead-exposed workers

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ABSTRACT Lead levels in whole blood and in plasma were measured in 64 non-exposed and in 29 exposed subjects with signs and symptoms of varying severity. Lead was determined by atomic absorption spectrophotometry after chelation with ammonium pyrrolidine dithiocarbamate and extraction with methyl isobutyl ketone. The method has a sensitivity of 0·4 µg/100 ml (0·02 µmol/l) for whole blood and of 0·2 µg/100 ml (0·01 µmol/l) for plasma and is reliably accurate and precise. Plasma lead increases progressively and significantly with the increase of whole blood lead, while its relative percentage in the plasma remains practically constant at all concentrations in whole blood. In exposed subjects a highly significant correlation was found between lead in plasma and lead in urine (r = 0·549) but the correlation coefficient was higher for whole blood lead versus urinary lead (r = 0·938). Aminolevulinic acid excretion in urine appeared to be significantly related to plasma lead concentration (r = 0·563) but to a greater extent to whole blood levels (r = 0·801). There was no significant correlation between lead in plasma and the logarithm of aminolevulinic acid dehydratase. The hypothesis is advanced that plasma lead, the more biologically active fraction of the metal, could be related to different individual sensitivities which would condition the development of toxic effects in various organs at different levels of lead.

Although normally considered to be a single compartment (Rabinowitz et al., 1976) blood contains lead (Pb) in two forms, a non-diffusible form bound to erythrocytes and a diffusible form in plasma. Because of the uneven distribution of the metal between red blood cells and plasma, two physiological pools may be considered to exist. The diffusible form, because of its greater bioavailability, is likely to influence lead concentration of the other compartments directly and, as a consequence, to produce critical effects in the various organs, with the possible exception of erythrogenetic effects.

There is little information so far relating to plasma lead levels; this may be attributable either to the technical difficulties involved in their measurement or to the lack of evidence of correlation with whole blood concentrations (Ambrosi and Chiantera, 1962; Rosen et al., 1974) which are normally considered to be a good indication of the degree of lead exposure.

Varying concentrations have been reported for serum lead: in adults, Ambrosi and Chiantera (1962) found levels ranging from 4·0 to 8·6 µg/100 ml (0·19-0·41 µmol/l) in non-exposed subjects and from 5·0 to 13·0 µg/100 ml (0·24-0·63 µmol/l) in workers showing toxic effects, by using a dithizone method. Butt et al. (1964) by means of an emission spectrochemical technique, estimated lead values of 3·9, 2·7, and 2·8 µg/100 ml (0·19, 0·13 and 0·13 µmol/l) respectively in three non-exposed groups. Lloyd Davies and Rainsford (1967) reported figures of 1·2 µg (0·05-0·1 µmol/l) of lead in serum of non-exposed subjects and of 3·4 µg/100 ml (0·14-0·19 µmol/l) at whole blood values of 100 µg/100 ml (4·83 µmol/l). In children, Robinson et al. (1958) found plasma concentrations varying from 0 to 9·0 µg/100 ml (0·43 µmol/l) by a dithizone method; Rosen et al. (1974) examining the blood of normal and lead-poisoned children by a flameless atomic absorption spectrophotometric technique (AAS) found lead levels ranging from 1·0 to 7·0 µg/100 ml (0·05-0·34 µmol/l), the mean concentration proving remarkably constant regardless of whole blood levels, haematocrit values and symptoms, and without

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finding any differences in lead levels between serum and plasma of the same blood samples.

To evaluate lead concentrations in plasma of normal subjects and of exposed workers, to assess the usefulness of this measurement in industrial exposure and the possible relationship with other biological measurements, we have assayed plasma lead levels in a group of non-exposed subjects and in others with a history of definite undue exposure, and who displayed signs and symptoms of varying severity.

Material and methods

Lead concentrations in plasma and in whole blood were assayed in 64 hospitalised subjects with no previous exposure to lead. At the same time lead levels were determined in 29 subjects with a history of occupational exposure, some of whom presented severe symptoms of lead intoxication. The 24-hour urinary concentration of the metal was checked in 63 of the non-exposed and in 22 of the exposed group.

To relate lead concentrations to the biological indices of intoxication in the exposed subjects, aminolevulinic acid (ALA) urinary excretion was measured in 14 subjects and ALA-dehydratase activity of blood red cells (ALAD) in 12.

Collection of samples

By venepuncture 3-4 ml of whole blood were drawn into a plastic syringe. A 1 ml sample was placed in a plastic tube containing K₃EDTA and stored at 4°C for subsequent determination of whole blood lead. The remainder of the sample, placed in another tube also containing K₃EDTA, was immediately centrifuged at 800 rev/min for 15 min; the plasma obtained was stored at −20°C for subsequent lead determination.

AAS analytical conditions

All determinations were performed using a Perkin Elmer atomic absorption spectrophotometer with a lead hollow cathode lamp, equipped with a HGA-74 graphite furnace and a deuterium background corrector. Nitrogen was used as purge gas for the graphite furnace. As the extraction procedure removes the chelated lead from the plasma matrix, the use of a deuterium corrector is not strictly necessary. Eppendorf micropipettes with disposable plastic tips were used for sample injection.

The method adopted for the determination of whole blood lead was the standard chelation extraction procedure reported by Volosin et al. (1975) with some minor modifications.

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Procedure for plasma lead

One millilitre of plasma, water blank or one of the lead standard solutions which had been prepared by adding known amounts of lead to plasma containing a low concentration of the metal, was pipetted into a glass-stoppered tube. To this were added 0·5 ml H₂O and 1 ml of a 1% aqueous ammonium pyrrolidine dithiocarbamate solution. After these had been mixed for 15 s, 0·5 ml methyl isobutyl ketone were added and mixed for 15 s. The tubes and contents were centrifuged for 5 min at 2000 rev/min. Then 0·3 ml of the solvent layers obtained from the blanks, standards and samples were transferred to a microtube, and 50 µl of each were injected into the graphite furnace.

The correction for endogenous lead is obtained by subtracting the amount of lead found in the sample used for preparation of the standards from the values obtained.

The method has a sensitivity of 0·2 µg/100 ml (0·01 µmol/l) of plasma. Its accuracy and precision are shown in Table 1.

<table>
<thead>
<tr>
<th>Number of determinations</th>
<th>Lead added to plasma (µg/100 ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0·5</td>
<td>85±2</td>
</tr>
<tr>
<td>20</td>
<td>1·0</td>
<td>89±0</td>
</tr>
<tr>
<td>20</td>
<td>2·0</td>
<td>90±1</td>
</tr>
<tr>
<td>20</td>
<td>3·0</td>
<td>91±0</td>
</tr>
</tbody>
</table>

Within-run reproducibility

<table>
<thead>
<tr>
<th>Lead levels in plasma (µg/100 ml)</th>
<th>CV* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0·3</td>
<td>5±0</td>
</tr>
<tr>
<td>0·6</td>
<td>3±6</td>
</tr>
<tr>
<td>0·9</td>
<td>1±9</td>
</tr>
<tr>
<td>1·2</td>
<td>2±6</td>
</tr>
<tr>
<td>1·5</td>
<td>1±7</td>
</tr>
</tbody>
</table>

*Coefficient of variation.

Results

The mean values and ranges of lead concentration in plasma from 4 groups of subjects divided according to whole blood levels are given in Table 2. The mean plasma concentrations in each group appear to increase progressively with the increase in whole blood levels; differences between groups, regardless of some slight overlapping of individual values, are highly statistically significant, except for the comparison between the first two groups.

The significant correlation between plasma and whole blood values is shown in Fig. 1 (r = 0·648) and
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Table 2  Plasma lead determinations compared with whole blood lead concentrations

<table>
<thead>
<tr>
<th>Range of whole blood lead (µmol/l)</th>
<th>Number of subjects</th>
<th>Plasma lead (µmol/l ± SD)</th>
<th>Range of plasma lead values (µg/100 ml ± SD)</th>
<th>Plasma lead Lead in whole blood (µg/100 ml) (Range)</th>
<th>Plasma lead Plasma lead (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5-9</td>
<td>30</td>
<td>0.54 ± 0.16</td>
<td>0.03 ± 0.008</td>
<td>0.3-0.9</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td>0-1-9</td>
<td>34</td>
<td>0.61 ± 0.24</td>
<td>0.03 ± 0.01</td>
<td>0.2-1.3</td>
<td>2.3 ± 0.9</td>
</tr>
<tr>
<td>2-4-0</td>
<td>17</td>
<td>1.69 ± 0.96</td>
<td>0.08 ± 0.05</td>
<td>0.7-4.0</td>
<td>2.8 ± 1.6</td>
</tr>
<tr>
<td>&gt;4-0</td>
<td>12</td>
<td>4.42 ± 3.75</td>
<td>0.21 ± 0.18</td>
<td>0.2-12.0</td>
<td>3.3 ± 2.8</td>
</tr>
</tbody>
</table>

Fig. 1  Correlation between lead levels in whole blood and in plasma of exposed and non-exposed subjects.

the correlation improves further (r = 0.721) upon correction for haematocrit values.

If lead plasma concentrations are expressed as a percentage of whole blood lead, values appear practically constant in all groups (means ranging from 2.3 to 3.3%) with no statistically significant differences between values from exposed and non-exposed subjects.

As shown in Fig. 2 the correlation between plasma lead and whole blood lead of the exposed group versus urinary levels proved statistically highly significant, this being more pronounced for blood (r = 0.938) than for plasma levels (r = 0.549). In the non-exposed group, the correlation was highly significant for blood lead versus urinary lead (r = 0.429) while no significance was shown for plasma lead (r = 0.18).

ALA urinary excretion appeared to be significantly related to lead plasma concentrations (r = 0.563) and to an even greater extent to whole blood levels (r = 0.801) (Fig. 3). The logarithm of ALAD activity was found to be significantly related to whole blood lead (r = 0.745) but not to plasma values (r = 0.297).

Discussion

Plasma lead values found by our method in non-
exposed and in low-exposure subjects are remarkably lower not only than those found using chemical (Ambrosi and Chiantera, 1962) and spectrochemical (Butt et al., 1964) techniques but also than those obtained by AAS (Rosen et al., 1974), with the exception of the data referred to by Lloyd Davies and Rainsford (1967) who do not mention the method used, however.

In our opinion these differences may be explained by the greater sensitivity of our method with respect to the chemical and spectrochemical techniques and to the AAS methods so far adopted, and by a greater degree of precision and accuracy in assaying low levels. If we consider that Rosen et al. (1974) inject 1 μl of plasma directly, on the hypothesis of a lead concentration of 0.5 μg/100 ml (0.02 μmol/l) of plasma, the amount of lead to be determined in the injected sample will be 0.005 ng. Using our method and taking into account our recovery coefficient at this level, which is of the order of 85%, the amount of lead injected is 0.425 ng. In addition, the extraction by an organic solvent eliminates the background interference (Volosin et al., 1975) and greatly improves the sensitivity of the method.

This would explain why the mean results referred to by Rosen et al. (1974) are so strikingly uniform, with figures approaching ours only in high exposure groups; this is not attributable to the relatively unlimited protective capacity of the red cells, suggested by these authors.

The highly significant correlation which we found between plasma and whole blood lead levels shows that the increase in red-cell-bound lead is accompanied by a linear increase in plasma levels, while the lead plasma percentage remains practically constant at all concentrations of lead in whole blood. We would emphasise that the correction of lead values for haematocrit levels in whole blood further improves such a correlation.

These data suggest that blood red cells behave as a storage compartment for lead, but not with unlimited capacity, and that lead reaching the blood stream distributes itself in a constant percentage between plasma and erythrocytes: this applies also when high lead concentrations are present.

The difference between individual results may be ascribed to the differences in ability of red cells of different individuals to adsorb lead (Mueller, 1956).
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Fig. 3 Lead levels in whole blood and in plasma of exposed subjects plotted against ALA urinary excretion.

The comparison between the lead levels of the two compartments and urinary concentrations is evidence that the correlation is more significant statistically for whole blood and urinary lead, than for plasma and urinary lead. This seems surprising, for lead filtered by the kidney and subsequently excreted is known for certain to derive from the plasma compartment. A likely explanation for this is that the plasma pool contains limited amounts of the metal and is continuously supplied with lead released by the erythrocytes because of the dynamic equilibrium. It is therefore to be expected that urinary concentrations would reflect the lead content of the pool of greater capacity.

On the other hand, the better correlation between whole blood lead and biochemical signs of disturbance of haemoglobin synthesis may be easily explained. Whole blood lead is almost entirely bound to erythrocyte membranes and the metal is therefore able to exert toxic effects on the cells directly by inhibiting ALAD and thus inducing increased urinary ALA excretion.

Conclusions

Plasma is a pool which contains the more biologically active fraction of lead, the percentage content of which is constant in respect to that of whole blood.

Lead absolute plasma concentration increases linearly with the increase in blood lead level and because it is the most rapidly exchangeable aliquot is likely to be in dynamic equilibrium with the interstitial fluid and to diffuse into the extravascular space, thus coming into direct contact with various tissue cells which will be damaged according to their sensitivity.

If the assumption of Mueller (1956) is correct, and there are differences between individuals in the ability of their red cells to adsorb lead, the scatter of some of our figures could be explained by individual sensitivity to lead. This hypothesis is merely speculative and further studies are needed to assess its validity.

Be that as it may, the availability of a reliable method for plasma lead determination may provide a new tool for evaluation of the kinetics of lead transport in the human body and shed some light on many aspects of lead metabolism that are still poorly understood.

References


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