Lead poisoning: clinical, biochemical, and haematological aspects of a recent outbreak

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Abstract

The clinical, biochemical, and haematological aspects of a recent outbreak of lead poisoning, in which exposure was related to the oxyacetylene cutting of red lead painted ironwork, were investigated. Initial suspicion was raised when a blood film showed punctate basophilia which remains a simple and useful method of picking up lead toxicity. Estimations of blood lead concentration and conventional laboratory data confirmed the diagnosis. Although there was prominent punctate basophilia, spectrophotometric analysis showed only negligible accumulation of pyrimidine-5'-nucleotides despite severe suppression of pyrimidine-5'-nucleotidase activity. The pattern of the red cell glycolytic intermediates, investigated for the first time, suggested that lead may also affect glycolysis at the hexokinase step. Once the diagnosis was made intravenous chelation treatment was begun with a rapid improvement in symptoms.

Long term follow up is required to assess any sequelae of intoxication. These cases emphasise the classic features of lead poisoning, and despite the currently available diagnostic tests, lead intoxication may still go unrecognised unless a thorough occupational history is taken.

The toxic effects of inorganic lead have been known since ancient times and were alluded to in the writings of both Hippocrates and Pliny.¹ The classic clinical descriptions were made by physicians in the nineteenth century with none better than the treatise by Tanquerel des Planches published in 1839.² Concern regarding the widespread occurrence of occupational lead poisoning during the second half of the nineteenth century brought about legislation designed to curb the problem. Lead poisoning first became a notifiable disease in 1899, and during that first year 1058 cases were notified. Exposure to lead at work is now strictly controlled by the Health and Safety Executive in their *Code of Practice*,³ but sporadic non-monitored cases of lead poisoning continue to occur, although only two cases were notified in 1984/5.⁴

The characteristic haematological effects of lead poisoning, such as anaemia and basophilic stippling, were fairly well established by the beginning of the twentieth century. Furthermore, excess porphyrines in the urine had been

noted as far back as 1895.⁵ Over the ensuing years numerous studies on the in vitro and in vivo effects of lead have shown inhibition at several sites of haem biosynthesis (fig 1)⁶⁻⁹ and have permitted specific delineation of the pathological events in lead poisoning. Despite this, cases may not be recognised unless there is a high degree of suspicion.

We report the clinical and laboratory features, some hitherto unreported, found in a recent outbreak of lead poisoning affecting four men who were carrying out demolition work using oxyacetylene torches to cut through metal structures covered in lead based paints. The outbreak was only recognised when the index case was admitted to hospital as an emergency.

CASE HISTORIES

Case 1

A 52 year old man presented as a surgical emergency with a two week history of colicky abdominal pain, and vomiting. He complained of anorexia, constipation, and weight loss of one stone. Examination showed that he had lower abdominal tenderness. His haemoglobin concentration was 9.8 g/dl with a mean cell volume of 82 fl, but a film was not initially requested. In view of the anaemia upper gastrointestinal pathology was looked for but no abnormality was found. After this initial delay a peripheral blood film was carried out which showed pronounced basophilic stippling (fig 2) with a reticulocyte count of 11.2%. On reviewing the patient a gum lead line was then

Figure 1 Simplified haem synthesis pathway. ALA synthetase, ALA dehydratase, and ferrochelatase are the three enzymes inhibited to the greatest degree by lead. Other intermediary enzymes such as uroporphyrinogen decarboxylase and coproporphyrinogen oxidase may also be affected.⁷

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Figure 2 Peripheral blood film from case 1 showing basophilic stippling. (Jenner-Giemsa.)

noted. Urea and electrolytes were normal. Liver function tests were abnormal with an alkaline phosphatase of 139 (normal range 30–85), aspartate transaminase 64 (10–50), and gamma glutamyl transferase 391 (5–55). An estimation of blood lead showed a concentration of $9.8 \mu\text{mol/l}$ (normal $<1.5 \mu\text{mol/l}$), confirming our initial suspicion.

Treatment was started with intravenous sodium calciumedetate BP (Ledclair-Sinclair Pharmaceuticals) at a dose of 80 mg/kg body weight/24 hours for five days. Symptoms subsided within 24 hours and his blood lead concentration fell to $2.9 \mu\text{mol/l}$ with a peak urinary lead of $9700 \mu\text{g/l}$. Within six weeks his blood lead had risen to $3.9 \mu\text{mol/l}$ so he was given a five day course of oral chelation with Dimaval (2,3 dimercaptopropane-1-sulphonate; DMPS) a new water soluble British anti-Lewisite (BAL) derivation¹⁰ (kindly supplied by the New Cross Hospital Poisons Unit, London). Blood lead diminished but the patient developed severe erythema multiforme with severe depigmentation, a hitherto unrecognised complication. This slowly improved on withdrawal of the drug and his blood lead concentration dropped to $2.5 \mu\text{mol/l}$.

Case 2

A 55 year old man, a colleague of the index case, was referred by the Employment Medical Advisory Service. His haemoglobin concentration was 10.8 g/dl , mean corpuscular volume 84 fl, and a blood film showed pronounced basophilic stippling with a reticulocyte count of 5.2%. Interestingly, despite close questioning, he was asymptomatic. The blood lead concentration, however, was high at $8.1 \mu\text{mol/l}$. He was therefore treated with intravenous chelation. His peak urinary lead was $6300 \mu\text{g/l}$ and his blood lead concentration reduced to $2.9 \mu\text{mol/l}$.

Case 3

A 48 year old man working on the same site was referred by the Employment Medical Advisory Service. He complained of anorexia, lethargy, impotence, acid taste in the mouth, colicky abdominal pain and constipation. He had been drinking two to three pints of milk a day with

some relief of his symptoms. Aperients prescribed by his general practitioner had not relieved his constipation. His haemoglobin was 12.6 g/dl , mean corpuscular volume 81 fl, reticulocyte count 2.6% and a blood smear showed basophilic stippling. His blood lead concentration was raised at $5.0 \mu\text{mol/l}$. His symptoms rapidly improved following intravenous chelation and he remains well with a blood lead of $2.8 \mu\text{mol/l}$.

Case 4

A 26 year old labourer had been working with the three men described above for the previous five weeks. He was asymptomatic. His haemoglobin concentration was 15.2 g/dl , mean corpuscular volume 93 fl, reticulocyte count 2.0%, with an occasional stippled red cell. Blood lead concentration was $3.1 \mu\text{mol/l}$. He did not reattend after his first visit so further investigations were not possible.

Methods

Blood lead concentrations were estimated on venous blood anticoagulated with K_2EDTA , in a vacutainer. The blood was diluted and the lead measured using graphite furnace atomic absorption spectrophotometry (GFAAS) on a Perkin Elmer 5000, with an HGA 400 furnace. Additional dilution was necessary for samples from cases 1 and 2 which were much higher than usual industrial samples—that is, below $4.0 \mu\text{mol/l}$.

Urine lead excretion was measured at the New Cross Supraregional Assay Service (SAS) trace element laboratory using GFAAS. Urinary aminolaevulinic acid and porphobilinogen were measured by standard methods.¹¹ Uroporphyrin I + III and total coproporphyrin I + III were measured by specific high performance liquid chromatographic techniques.¹² Erythrocyte zinc protoporphyrin (ZPP) was measured by standard techniques.¹³ Erythrocyte aminolaevulinic acid (ALA) dehydratase with and without dithiothreitol was measured using high performance liquid chromatography to determine porphobilinogen formation.¹⁴

The physiological effects on renal glomerular function were assessed by measuring creatinine clearance, urinary amino acids, phosphate and glucose. Arsenic, antimony and cobalt were also measured. Pyrimidine-5'-nucleotidase (Pyr-5'-N) activity was measured by a modified¹⁵ non-isotopic method.¹⁶ Perchloric acid (PCA) extracts of blood were used for spectral analysis¹⁷ to search for evidence of accumulation of pyrimidine nucleotides in the red cells. This is a characteristic finding in all homozygous patients with hereditary deficiency of Pyr-5'-N and is detected as a shift of the normal absorption maximum of adenine nucleotides at 257 nm towards longer wavelengths (265–270 nm).

PCA extracts were also used, after neutralisation, for the measurement of all red cell glycolytic intermediates by modified¹⁸ fluorimetric methods.¹⁹ Bone marrow was assessed using standard methods.²⁰

Biochemical variables at presentation

	Case 1	Case 2	Case 3	Case 4	Normal range
Blood lead	9.8	8.1	5.0	3.1	< 1.5 µmol/l
Erythrocyte zinc protoporphyrin	1300	730	1360	480	< 500 nmol/l
Erythrocyte aminolaevulinic dehydratase activity (without added dithiothreitol)	1.2	1.2	1.2	NA	22.1–36.5 µmol/l porphobilogen/l cells/min
With added dithiothreitol	12.1	12.1	16.5	NA	22.1–36.5 µmol/l
Erythrocyte pyrimidine 5' nucleotidase activity	0.034	0.020	0.028	NA	0.12–0.224 EU/gHb
Urine lead	200	410	1100*	NA	< 80 µg/l
Urine 5' aminolaevulinic acid	560	NA	NA	NA	< 46 µmol/l/24 hrs
Porphobilinogen	18	NA	NA	NA	< 9 µmol/l/24 hrs
Total uroporphyrin I + III	87	NA	NA	NA	< 40 nmol/24 hrs
Total coproporphyrin I + III	4750	NA	NA	NA	< 280 nmol/24 hrs

*Day 2 of chelation treatment.
NA—Not available.

Results

The results of confirmatory investigations are shown in the table. Lead concentrations in both urine and blood were raised as was the ZPP. ALA dehydratase and Pyr-5'-N were noticeably reduced, although there was some increase in the ALA dehydratase activity after the addition of dithiothreitol. Spectral analysis showed only a minimal increase in pyrimidine nucleotides in case 2, with the absorption maximum shifting minimally from 257 nm to 260 nm. Urinary porphyrin intermediates when measured were also raised. Iron, magnesium, zinc, copper and other heavy metal studies (arsenic, antimony, and cobalt) registered normal concentration as did B12, folate, thyroid stimulating hormone, urate, urinary amino acids and haemoglobin electrophoresis. Creatinine clearance was reduced at 45 ml/minute in case 1 but was normal in the other cases. Glycolytic intermediates showed a moderate increase in fructose-1,6-diphosphate (FDP) and dihydroxyacetone phosphate (DHAP). All the other intermediates were within the normal range. A liver biopsy specimen taken from case 1 showed mild hepatitis consistent with lead toxicity. A bone marrow aspirate in case 1 showed dyserythropoiesis with prominent karyorhexis and nuclear cytoplasmic asynchrony (fig 3), but ringed sideroblasts were absent on Perls's stain.

Discussion

In developed countries lead poisoning no longer occupies the prominent position which it once did, although in other parts of the world both industrial and environmental exposure are common problems. In the United Kingdom

the Health and Safety Executive monitor the number of registered lead workers, with recent figures showing 2535 in the demolition and scrap industries.²¹ Despite this, inadequate employer surveillance may nullify their efforts and lead to outbreaks of poisoning such as this.

Lead poisoning is often not recognised because of the non-specific nature of the symptoms.²² Moreover, the severity of the symptoms is not always proportional to the blood lead concentration, as illustrated by case 2 who remained asymptomatic despite a high lead concentration. Recent work has indicated that the synthesis of an intraerythrocytic low molecular weight lead binding protein may be induced by exposure to lead, thus providing a protective mechanism.²³ This response may be reduced in susceptible persons. Therefore a thorough occupational history may clinch the diagnosis which can then be confirmed by assessing the blood lead concentration.

In Britain the performance of blood lead analysis has improved dramatically since the seventies and the test is widely available through the SAS and other laboratories. This has been due to: (i) the advent of GFAAS; (2) the establishment of quality assessment schemes (SAS Trace Element Reference Laboratory, University of Surrey and the United Kingdom National External Quality Assessment Scheme); and (iii) the characterisation of stable reference materials, which have enabled the SAS laboratories to achieve coefficients of variation less than 6%.²⁴

The definitive test for assessing the body burden of lead is the urinary excretion of lead following a challenge with calcium EDTA. Blood lead concentrations have shown the best correlation with this, provided that the lead exposure is current.²⁵ Erythrocyte protoporphyrin or ZPP depends on the lead present as the erythrocyte was formed, so the blood concentrations reflect the exposure over a longer period than blood lead. Although raised in our cases, it is not reliable for diagnosing or monitoring mild degrees of exposure, but its simplicity had led to wide application in screening programmes. Similarly, urinary lead, ALA, and coproporphyrins are either too insensitive or too variable for this purpose with sample volume and renal function being further additional limitations.²⁶ Direct measurement of ALA dehydratase activity has been used, but the estimation is very sensitive to even small excesses of lead and furthermore,

Figure 3 Bone marrow aspirate from case 1 showing nuclear cytoplasmic asynchrony, cytoplasmic vacuolation, and multinuclearity. (Jenner-Giemsa.)

alcohol affects the enzyme activity.²⁷ For these reasons it is little used as a marker of lead exposure. Lead also inhibits ferrochelatase (haem synthetase) which catalyses the introduction of ferrous iron into the porphyrin ring to form haem. This test, however, is limited in its availability and reproducibility. In general, analysis of blood lead remains the single most useful index of recent exposure with the other estimations providing supportive evidence.²⁸

Anaemia, reticulocytosis, and basophilic stippling have been recognised in lead poisoning since the start of the century. Lead binds avidly to the red cells⁹ with up to 50 times as much being found in the bone marrow.⁸ The abnormalities are confined to the erythroid series, with ineffective erythroid hyperplasia being prominent. Ring sideroblasts are commonly reported⁸ but these were absent in our patient (case 1) despite dyserythropoietic changes (fig 3). The formation of ringed sideroblasts has been attributed to a decreased activity of the haem synthetic enzymes, particularly ALA synthetase, leading to intramitochondrial iron accumulation.²⁹ Their absence in case 1 may have been related to the short duration of exposure, although the exact mechanism remains unclear. Globin synthesis is also depressed but the importance of this in vivo is unclear.³⁰ Shortened red cell survival⁸ has been ascribed to the profoundly depressed activities of erythrocyte Pyr-5'-N and increased concentrations of pyrimidines in red cells of patients with lead poisoning,^{31,32} akin to that described in the hereditary deficiency of Pyr-5'-N.¹⁸ The accumulated pyrimidine nucleotides inhibit RNA breakdown, resulting in aggregates of undegraded and partially degraded ribosomes, which cause basophilic stippling.⁷ The Pyr-5'-N activity in cases 1-3 was very low (13-22% of the normal mean), but the results of the spectral analysis were quite unusual. Absorption spectra were normal in cases 1 and 3 with a minor shift of the absorption maximum from 257 nm to 260 nm in case 2, suggesting only minimal accumulation of pyrimidine nucleotides. The absence of appreciable amounts of pyrimidine nucleotides in the erythrocytes of these cases in the presence of pronounced basophilic stippling suggests that lead may interfere with the normal degradation of ribosomal RNA by ribonucleases in the maturing reticulocytes.

Glycolytic intermediates, which represent functional abnormalities in vivo, have not previously been measured in lead exposure. The concentrations of glycolytic intermediates in cases 1-3 were normal except for FDP and dihydroxyacetone phosphate (DHAP) which were moderately increased. Reticulocytosis, as found in cases 1-3, usually leads to increases in all the intermediates at the early part of the glycolytic pathway—that is, glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), FDP and DHAP. The failure of the concentration of G6P and F6P to rise above normal may be an indication that lead, at the concentrations found in these patients, affects glycolysis at the hexokinase step. Low level lead exposure,

albeit producing only about a 40% reduction in Pyr-5'-N activity, has been reported to have no effect on the maximum activities of the glycolytic enzymes, as measured in vitro.³² The effect of acute lead intoxication on glycolytic intermediates needs further assessment.

The treatment of lead poisoning is initiated by prompt removal from exposure. Chelation treatment, which can be given both orally and intravenously, should be considered when the symptoms are severe or when the lead concentration is dangerously high ($>5.0 \mu\text{mol/l}$). In severe poisoning sodium calcium EDTA is most effective but a rebound phenomenon may occur when the agent is stopped. New water soluble derivatives of BAL called dimercaptosuccinic acid (DMSA) and 2,3 dimercaptopropene-1-sulphonate (DMPS) have been shown to be effective. Skin rashes (case 1) are recognised with DMPS,¹⁵ although erythema multiforme has not previously been reported.

The acute clinical, biochemical, and haematological features of lead toxicity are well documented, yet the long term sequelae remain uncertain and despite large cohort studies no reproducible abnormality has been reported in adults.^{33,34} With demolition and reconstruction work again becoming prevalent in many inner cities de novo lead poisoning should be considered if there are clinical pointers. This initial suspicion can easily be confirmed with reproducible biochemical and haematological investigations.

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