The effect of metal ions on the activity of δ-aminolevulinic acid dehydratase

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ABSTRACT The effects of lead, iron, copper, and zinc ions on δ-aminolevulinic acid dehydratase from red blood cell haemolysates in humans, both in the absence and presence of plasma proteins, have been investigated. δ-aminolevulinic acid dehydratase (ALAD) was not found to be a specific indicator of blood lead concentrations since it was also inhibited by copper and activated by zinc. Plasma protein protected the enzyme from both inhibition and activation. ALAD activity was found to be an indicator of the total metal ion concentration in the blood and was therefore considered to be of doubtful value in screening large populations for increased lead absorption.

The inhibitory effect of lead on δ-aminolevulinic acid dehydratase (E.C. 4.2.1.24, ALAD) in patients with lead poisoning was first demonstrated by Lichtman and Feldman (1963). Numerous workers (Bonsignore et al., 1965, 1969; Bonsignore, 1966; Nakao et al., 1968) later confirmed these findings. The degree of inhibition has been correlated with blood lead levels (Hernberg et al., 1970; Millar et al., 1972) and these workers showed that even so-called normal levels of lead affected this enzyme. Some workers, however, have found the inhibitory effect of lead on ALAD to be too sensitive to be a useful indication of over exposure to lead (Haeger-Aronsen et al., 1971).

There have been conflicting reports regarding the inhibition of ALAD by other inorganic ions (Gibson et al., 1955; Calissano et al., 1965; Abdulla and Haeger-Aronsen, 1971; Lauwers et al., 1973). It seemed appropriate to investigate the effect, not only of lead but also of zinc, copper, and iron which are normally present in whole blood in humans, and the possible interaction of these ions on ALAD. Such an investigation would be of value in understanding the interrelationship that may exist between these ions in blood.

Materials and methods

SAMPLE PREPARATION
Blood was taken by venepuncture into calcium heparin. The red blood cells and plasma were separated by centrifugation of 10 ml at 1500 g for 10 minutes, and the red cells were washed twice in pre-cooled 0.9% (x/v) saline. Packaged cells from 20 blood samples were pooled, 1 ml aliquot from each pool was mixed with 1 ml of 0.9% saline, and the packed cell volume (PCV) estimated. The red cells were subsequently treated as described below. The plasma from each sample was also pooled.

PREPARATION OF METAL ION SOLUTIONS
Standard solutions of lead, copper, and zinc were obtained from BDH, Poole, Dorset at a concentration of 1 mg/ml. The iron was a standard solution obtained from Merck, Germany at a concentration of 1000 g/l. All metal ion solutions were diluted with deionised water or pooled plasma.

TREATMENT OF POOLED CELLS WITHOUT PLASMA
1 ml of pooled packed cells was added to metal ion solutions of lead, copper, zinc, and iron. The ranges of concentrations tested were lead 1.2—19.3, copper 8.0—78.0, zinc 76—900, and iron 1785—17800 μmol/l. Additions were made in increments of 2.4, 8.0, 8.5, and 1785 μmol/l of lead, copper, zinc, and iron respectively. The lowest four concentrations of lead, copper, and zinc were used to investigate the combined effects of lead and copper, copper and zinc, zinc and lead.

TREATMENT OF POOLED CELLS WITH PLASMA
The pooled red blood cells were haemolysed in an ultrasonic disintegrator for 40 minutes using a
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frequency in the range of 20-30 kHz. 1 ml was added to 1 ml of the metal ion solutions, the concentrations of which were similar to those described for pooled cells without plasma. Plasma was used as diluent. The haemolysate was frozen at a temperature of −4°C overnight and thawed immediately before ALAD determination.

No difference in ALAD activity was found whether the cells were haemolysed by rapid freezing and thawing twice or haemolysed in an ultrasonic disintegrator.

DETERMINATION OF LEAD, ZINC, COPPER, AND IRON
In all experiments the initial concentration of each metal ion present in the pooled red cells was estimated on an aliquot from the pool that had been mixed with an equal amount of deionised water or pooled plasma. Each ion was estimated using an A3000 atomic absorption spectrophotometer (Southern Analytical, Frimley Road, Camberley, Surrey). Blood lead was estimated by the Delves’ boat technique (Delves, 1970). Zinc and copper were estimated as recommended by Shandon Southern Instruments, Camberley, Surrey. The iron was estimated colorimetrically using the technique of Ramsay (1954).

EXPERIMENTS
In order to investigate the combined effects of the metals on ALAD, a series of solutions was prepared, each containing two metal ions. For each concentration of one metal there were increasing concentrations of another. The metal pairs were lead and copper, lead and zinc, zinc and copper.

Further experiments were performed to examine the possible effect of the sequence in which the ion was added. Solutions containing one metal ion were added to the blood 18 hours before the other metal ion was added. Thirty minutes later the enzyme activity was estimated.

The metal concentrations in the graphs and tables listed in the results are the sum of the concentration in the blood and the solutions added.

DETERMINATION OF ALAD
The method used was that of Nakao et al. (1968) automated by us using a Technician auto-analyser continuous flow system. The porphobilinogen liberated from δ-aminolevulinic acid was estimated using Ehrlich’s reagent and compared to a set of porphobilinogen standards ranging from 69 μmol/l to 276 μmol/l. The porphobilinogen was obtained from Sigma Chemicals Co., Kingston-Upon-Thames, Surrey. The results were expressed in μmol per porphobilinogen per min/l and corrected to a PCV of 40%.

RESULTS
LEAD
Lead, at a concentration of 150 μmol/l inhibited ALAD by 85% and at 50 μmol/l by 38%. The inhibition was not linear but a straight line was obtained when the activity was plotted against the logarithm of copper. The presence of plasma protein, however, had an inhibition of 70% and 20% at these two lead concentrations.

COPPER
Similarly, a non-linear inhibition was obtained for copper and a straight line was obtained when the activity was plotted against the logarithm of lead. A 90% inhibition occurred at a copper concentration of 940 μmol/l and in the presence of plasma protein the inhibition was 38%.

ZINC
Zinc activated ALAD, the highest activity occurring at a zinc concentration of approximately 652 μmol/l at which point saturation of the enzyme had presumably occurred. Above this level the activation decreased and at a concentration of 955 μmol/l inhibition occurred. When plasma protein was included in the experiments the activation was decreased and no inhibition was detected throughout the range of zinc concentrations investigated (61—1030 μmol/l).

IRON
ALAD activity was not affected by iron concentrations between 0 and 17 857 μmol/l.

LEAD AND COPPER
When copper and lead were used together at the concentrations tested, the inhibition of ALAD was greater than either of these alone, and again plasma protein reduced the inhibitory effect.

LEAD AND ZINC
Increasing concentrations of zinc added simultaneously with lead caused greater inhibition than that caused by lead alone. For example, an increase of 150 μmol/l (60—210 μmol/l) of zinc caused an 83% decrease in activity of ALAD at 2·6 μmol/l of lead and 24% decrease at 1·6 μmol/l of lead.

When plasma protein was added with these two metals there was activation of the enzyme, although less activation than when zinc was used alone. Thus, an increase of 152 μmol/l of zinc (61—212 μmol/l), in the presence of plasma protein caused a 14% increase in activity at 2 μmol/l and a 5% increase in activity at 4·4 μmol/l of lead. The results are illustrated in Fig. 1.
When zinc was added 18 hours before the lead or when lead was added 18 hours before the zinc, in the absence of plasma protein and at zinc concentrations below 212 μmol/l, no activation or inhibition occurred. At zinc concentrations above this level, when zinc was added 18 hours before the lead at a concentration of 1·6 μmol/l, there was 55% greater inhibition than when lead was added 18 hours before the zinc (Fig. 2).

Numerous workers have described the inhibition of
ALAD by lead in vivo and in vitro (Weissberg et al., 1971; Beattie et al., 1972; Millar et al., 1972). Haas et al. (1972) using lead, and Lauwers et al. (1973) using lead and cadmium, reported ALAD to be a specific indicator of lead absorption. The inhibition of ALAD by lead and other metal ions conforms the work of Abdulla and Haeger-Aronsen (1971). The inhibitory effect of zinc with other metal ions has been investigated by Abdulla and Haeger-Aronsen (1973), who found that at high zinc concentration ALAD was partially protected from the inhibitory effect of other metal ions. A four-hour incubation period was used by these workers, whereas in the present work an 18-hour period was employed.

The results of the present investigation show that with increasing concentrations of lead, the inhibition of the enzyme ALAD was enhanced in the presence of zinc. When the two ions lead and zinc were added to the crude red blood cell homogenate, some ions would be bound and others in the free state. The red cell has an affinity for lead which probably binds the cholesterol, phospholipids, and lipoproteins contained in the red cell membrane (Watanabe and Yana, 1953). After haemolysis these compounds would, we suggest, be liberated into the surrounding medium and that with increasing concentrations of zinc, lead would be displaced from the binding sites of these compounds, and therefore would become available for ALAD inhibition, by binding to the sulphhydryl group. It has been shown (Waldren and Stofen, 1974) that lead has a greater affinity for binding with the sulphhydryl group in the enzyme than zinc. Thus, the enhanced inhibition by lead in the presence of zinc is not due to the direct action of zinc but to the displacement of lead from binding sites (a mass action effect) and its greater availability for reaction with ALAD.

It therefore follows that ALAD activity in haemolysates would not be a specific indicator of blood lead but rather the result of a dynamic equilibrium between the two ions, lead and zinc. As further evidence for this hypothesis, the addition of zinc before the lead did not materially change the results. When lead was added before the zinc the zinc was found to replace the lead at zinc concentrations above those found in vivo. At these high concentrations an equilibrium would exist.

The action of copper and zinc parallels that for lead and zinc and it is probable that the same phenomenon applies. The addition of copper and lead together to ALAD causes a greater inhibition than that by either alone and is presumably an additive effect.

Plasma protein provides additional binding sites for lead, copper, and zinc and it has been shown (Passow et al., 1961) that amino-acids in the plasma protein have a greater affinity for lead and copper than for zinc. Thus, more lead and copper would be absorbed than zinc and less would be available for reaction with ALAD, so that, in experiments involving plasma protein, activation of the enzyme occurred when lead and zinc or copper and zinc were added simultaneously to ALAD. The heavy metal binding by protein found during this investigation was not specific but some conclusions can be made concerning the order of affinities. The addition of increasing amounts of the metal to a solution containing a mixture of molecules with different types of ligands resulted in excessive binding in order of decreasing affinity. Not only the affinity of the protein molecule to the copper or lead but also the relative concentrations of the metals influenced the protective mechanism of plasma protein. It has been found (Martell and Calvin, 1952) that chelation may produce patterns that invalidate the relative affinities. Amino-acids are capable of forming chelates. On the other hand, chelates of high specificity play an important role in the activation of the enzyme by zinc.

The protective mechanism of plasma protein during this investigation would suggest that haemolysed blood has a built-in mechanism that provides some degree of protection against the effects of lead, copper, and zinc. The binding of lead by plasma protein may have little relevance in the in vivo state but in subjects with lead poisoning where the fragility of the red blood corpuscle is decreased and haemolysis occurs, the plasma protein may be of importance in binding metal ions. Iron was not found to activate or inhibit the enzyme at the concentrations tested although Calissano et al. (1965) using purified enzyme found strong activation. During the current investigation crude homogenates of red blood corpuscles were used which would of necessity contain high concentrations of iron and therefore the enzyme would be likely to be fully saturated.

Passow et al. (1961) found that the rate of substrate utilisation was proportional to the concentration of the enzyme-substrate complex and that this complex was formed by mass law governed reactions. This effect was likely to occur when increasing concentrations of lead were found to displace a zinc molecule from ALAD. It is also possible that the metal binding ligands of ALAD which have no functional significance may play an indirect role. These non-functional ligands may combine with the metals to reduce the amount available for reaction with functional ligands thereby affording protective action. The active centre of ALAD may only be functional when the activator zinc is present and zinc combines reversibly with the enzyme. If zinc is then displaced by lead there would be no activating power.
and the enzyme would be inhibited.

In the in vivo state many metal binding ligands have been found to be essential to the maintenance of the membrane as a diffusion barrier (Passow et al., 1961). In turn, the membrane plays an important role in active transport phenomena. The localisation of the lead effect on membrane structure using red blood cells has been found by Passow et al. (1961), and therefore the effect of lead on whole red blood cells in vivo may not reflect the effect of haemolysed red blood cells in the in vitro work although the results do not suggest that different mechanisms occur. Zinc, lead, and copper in serum have been found to be in two forms. Ionised plasma zinc constitutes 60% and the bound fraction constitutes 40% of the total serum zinc. It would therefore be likely that only the ionised fraction of each metal ion would affect ALAD. It is also likely that with the removal of the free zinc, more zinc would be removed from the protein molecule to maintain an equilibrium.

In some of the experiments metal ion concentrations were greater than those found in vivo. It is also clear from the results that concentrations of metal ions found in blood from subjects who are exposed to these metals may well interact with ALAD causing an activating or inhibitory effect. Since all published techniques for ALAD activity use haemolysates we suggest that care should be taken in the interpretation of the results where the subjects have been exposed to metal ions other than lead.

References


Jill Thompson, D. D. Jones, and W. H. Beasley

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J Thompson, D D Jones and W H Beasley

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