

EFFECT OF LEAD ON HEME SYNTHESIS

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Recently, a fair amount of work has been done on the effect of lead on porphobilinogen dehydratase, which has been used as a sensitive indicator of lead poisoning. How far this is in itself harmful depends on the Michaelis constants of both the aminolaevulinic synthetase and of the dehydratase, and in addition on the relative activities of the two enzymes in a cell and also on the tissue concentration of glycine. Information on some of these points is still fragmentary, and a reliable judgement is at the present not very easy. Another step in the heme synthesis, which is sensitive to low concentrations of lead, is the incorporation of iron into protoporphyrin. Inhibition of this step may be important in accounting to a large extent for the anaemia found in individuals with lead poisoning. Reduction in the tissue concentration of heme or of heme-like compounds may also explain, through the mechanism of de-repression, the excretion of increased amounts of aminolaevulinic acid in the urine observed in cases of lead poisoning. A third step in heme synthesis, which might be sensitive to lead, is the oxidative decarboxylation of coproporphyrin to protoporphyrin, and this may explain why the former derivative is excreted in the urine.

Recent work of the Harvard Medical School has indicated that greatly reduced levels of ALA dehydratase may be found in most cases of severe liver damage due to alcoholism. In most of these cases the level of lead in the blood is within normal limits, and there is no history of exposure to toxic amounts of lead. We therefore have to assume that a reduction in the blood level of this enzyme is not necessarily an indication of lead poisoning.

Lead is widely distributed in the atmosphere, in ground water and the oceans, and trace amounts can be detected in most animal and plant tissues. There is thus every reason to believe that minute quantities of

lead have always been ingested or absorbed by Man and animals. Indeed, blood and tissues taken from individuals who are members of non-industrial, primitive, and isolated societies contain traces of this metal, which can be quantitatively measured by modern methods. Such minute amounts of lead have also been found in human remains which go back to early periods of Man's existence, a long time before lead was used in industry. We must therefore assume that these trace amounts of lead are either non-toxic, or that Man or other species have become adapted to these minute quantities of the metal. Our present concern therefore is not with the presence of lead in our tissues and body fluids as such, but with quantities which are significantly greater than those found in the tissues of pre-industrial Man.

This paper is concerned with the biological basis of lead toxicity. The clinical signs and symptoms consist of colic, the well-known lead line, an anaemia with some special features, peripheral neuritis and encephalopathy, and sometimes damage to the kidney. Biochemical features of lead poisoning are markedly increased excretion in the urine of *amino-laevulinic acid* (ALA); to a much lesser extent and more infrequently, a raised level of *porphobilinogen*; excretion in the urine of markedly increased amounts of *coproporphyrin*; and the presence of abnormally high quantities of *protoporphyrin* in the red cell. There has also been a tendency in recent years to ascribe less well-defined psychological symptoms, especially sub-normal mental development and hyperactivity, to the ingestion of relatively small amounts of lead over long periods.

It seems reasonable to ascribe the toxic effect of lead to its interaction with enzymes. Lead forms mercaptides with SH groups, as does Hg and Cd. It forms a stable complex with cysteine with a stability constant of 10^{12} . The Hg complex of cysteine has a much higher stability constant ($\log K$ of 45.4). Thus, Pb^{2+} has a lower affinity towards univalent SH groups than silver, mercury, or cadmium cations. This explains the fact that lead inhibits the majority of those enzymes which require a free SH group for their activity, only at concentrations of about 10^{-3} or $10^{-4}M$, concentrations which are of little practical biological significance. However, lead forms a very stable complex with certain dithiol compounds such as 2:3 dimercapto propanol. It seems likely that similar very stable complexes can be formed with proteins which have two thiol groups placed in a suitable spatial position to one another, so that Pb^{2+} can react with both SH groups and thus form a very stable linkage. It is also not impossible that certain isolated SH groups in proteins have, owing to their special environment, an unusually high affinity to Pb^{2+} , much greater than predicted from studies made with simple compounds such as cysteine or the reduced form of glutathione.

Several enzymes have been shown to be inhibited by Pb^{2+} concentrations of 10^{-5} or $10^{-6}M$. One such enzyme is the lipoamide dehydrogenase, which plays an essential part in the oxidation of pyruvic and α -oxoglutaric acids in mitochondria, and is of great importance in the metabolism of almost all cells. This enzyme, which is known to have two

thiol groups on its active site, is inhibited by about 50 percent. at a lead concentration of about $6 \cdot 10^{-6}$ (1).

Another group of enzymes which is inhibited by relatively low concentrations of Pb^{2+} is that of certain ATP-ases which are involved in the transport of Na^+ or K^+ . Inhibition of this group of enzymes might interfere with the generation and transmission of nervous impulses, and this may account at least in part, for the encephalopathy, peripheral neuropathy and intestinal colic found in lead intoxication.

A very considerable amount of work has been done on the effects of lead on the haemopoietic system. One might start in making the reasonable assumption that, in a multi-step pathway of biosynthesis, interference by a toxic agent will, in the absence of feed-back inhibition, lead to the accumulation and excretion of an intermediate, which is just prior to the enzymatic reaction or step which is inhibited. On this basis, one can argue that lead must interfere with the conversion of aminolaevulinic acid to porphobilinogen, with the conversion of coproporphyrin to protoporphyrin, and with the insertion of iron into protoporphyrin to give heme (Fig. 1). This general conclusion is supported, at least to a

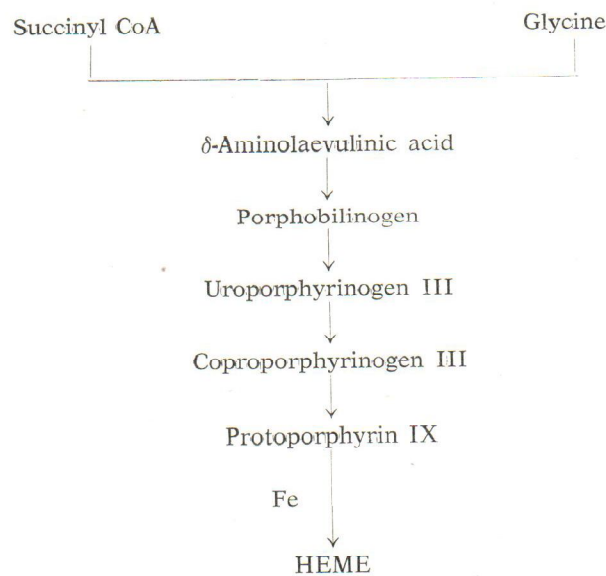


Fig. 1. General scheme of heme synthesis

large extent, by experimental results on isolated enzymes. It has already been shown about twenty years ago, in the first isolation of the aminolaevulinic acid dehydratase (2), that this enzyme depends for its

activity on SH groups, that these groups are oxidised by air very easily, and that active enzyme can only be obtained if throughout the preparation a reducing agent is present. In this first paper, it was shown also that very small amounts of lead inactivate the enzyme. It has also been suggested (3) that ALA synthetase is inhibited by lead; but the amounts required are relatively large, and the greatly increased concentrations of ALA in the urine do not support the belief that lead in actual clinical conditions interferes significantly with the formation of this particular metabolite. It was shown by *Kreimer-Birnbaum* and *Grinstein* (4) that lead inhibits the decarboxylation of uroporphyrinogen, and also interferes with the oxidative decarboxylation of coproporphyrinogen. This might explain the accumulation of coproporphyrin in cases of lead poisoning, as indicated by the presence of increased quantities of this porphyrin in the urine. Indeed, it has been suggested that measurement of urinary coproporphyrin may be a reliable guide in assessing lead intoxication. *Rimington* (5) was the first person to postulate that lead inhibits the incorporation of iron into protoporphyrin, and this effect on iron chelatase was demonstrated later by various authors (6—8). It is likely that one of the most important effects of lead is its antagonism to other metals, particularly iron, and this has been demonstrated, for instance, by *Ulmer* and *Vallee* (9).

If one postulates that the iron incorporation into protoporphyrin is one of the major points of attack of lead, the excretion of excessive quantities of aminolaevulinic acid becomes intelligible. It is generally assumed that the control of the biosynthesis of heme is regulated largely through the activity of the first enzyme of the pathway, namely ALA synthetase. This enzyme is repressed or inhibited by heme or a heme-like compound. If therefore the rate of formation of heme is reduced, ALA synthetase becomes de-repressed and increased amounts of ALA are formed, at least in the liver, and are excreted. Thus, disturbance of the iron incorporation into heme might lead to a reduced rate of formation of liver heme, and be responsible for the presence of ALA in the urine. This inhibition of incorporation of iron into protoporphyrin by Pb^{2+} might also lead to an inhibition of the formation of various cytochromes, and this might, at least in part, explain the generally toxic symptoms of lead observed in a variety of body systems.

The mature red cell contains no significant amounts of ALA synthetase, but the next enzyme on the pathway, i. e. ALAD, is present. This ALAD in the mature erythrocyte has no discernible biological function, since its substrate, i. e. ALA, cannot be formed in the mature red cell. It has been claimed by several workers (e. g. 10, 11), that the reduction in the activity of the ALAD is a general indicator of lead poisoning, and that the extent of its reduction can be used as quantitative measurement of the extent of lead intoxication. This belief can be criticised on several counts.

There is no doubt that this method is useful as a screening procedure. However, the biological significance of the reduction in the activity of

this enzyme is not immediately obvious. For a few types of cells, in which the activity of both ALA synthetase and ALAD have been measured, it is clear that the latter enzyme shows a very much higher activity. Both enzymes have K_M values of the order of $10^{-3}M$. Thus, in order to get the maximum rate of porphobilinogen formation, the concentration of ALA has to be greater than $10^{-3}M$. In other words, the relatively high activity of ALAD in relation to that of the synthetase is necessary in order to ensure a reasonably high rate of porphobilinogen formation. In order to assess the effect, for example of a 50 percent inhibition of the ALAD in a particular cell, one would have to know the activities of the two enzymes and their K_M values, and the concentrations of the various substrates and co-factors. Such information is at present unavailable, and it is therefore impossible to assess the significance of such reduction in ALAD in mature red cells which have been observed.

Another major point is the question whether the reduction in the activity of the ALAD is specific for lead poisoning. It is remarkable that the specificity of this decrease has by no means been established. Systematic examination of the enzyme's activity in the normal population and the large number of general medical disorders has not been carried out, although this is standard practice in establishing diagnostic laboratory tests. *Vallee* and his colleagues (*B. L. Vallee, D. D. Ulmer, P. Bodlaender* and *W. Branch*, in preparation) have recently carried out a thorough investigation of the level of ALAD in cases of lead poisoning and in a great variety of other clinical conditions. They have used a method which is more satisfactory than the techniques used by some others, who have worked in this field. Their results can be summarized in the following manner. In a group comprising 36 patients with severe hepatic insufficiency due to post-alcoholic cirrhosis, as indicated by various clinical and biochemical parameters, there was observed a very large reduction in the activity of ALAD in the mature erythrocytes. In all these cases there was a history of alcoholism and clinical findings were consistent with liver disease; all these patients were diagnosed clinically to be suffering from post-alcoholic cirrhosis of the liver, as established by independent observers. In almost all these patients blood lead levels were within accepted normal limits, and there was no history of exposure to lead. We must therefore assume that post-alcoholic cirrhosis as such is associated with reduction in the activity of ALAD. In less severe cases of post-alcoholic cirrhosis there was also a marked tendency for a reduction in the activity of ALAD, but the variability in individual cases tended to be somewhat greater. There is therefore little doubt that conditions other than lead poisoning are associated with reduction in the ALAD. These patients were but one group out of several hundred so investigated, and a number of other disorders were found to be associated with a decreased ALAD activity. Incidentally, it might be mentioned that an acute effect of ingestion of ethanol on the activity of the blood ALAD has been observed a few years previously (12). However, there is no indication that the effect observed by *Vallee* and his colleagues, in

their cases of hepatic cirrhosis was in any way due to the acute ingestion of ethanol.

It is thus quite likely that other conditions, unrelated to lead intoxication, will be discovered, which are associated with a reduction in activity of ALAD. It is thus clear that great care must be taken in interpreting the results obtained with the assay of this enzyme which cannot be attributed uniquely to any one condition without the customary benefit of multiple, mutually supportive diagnostic findings.

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Sažetak

UČINAK OLOVA NA SINTEZU HEMA

Olovo se nalazi posvuda u prirodi, čak i u onim krajevima i ljudima gdje nema nikakve potrošnje tog elementa. Nađeno je i u kostima pretpovijesnog čovjeka i prema tome moramo smatrati da je u tim količinama bezopasno ili da se čovjek privikao na takve malene koncentracije.

Po svemu se čini da toksično djelovanje olova treba pripisati interakciji s enzimima, posebice s onima koji imaju SH skupine. Razmjerno niske koncentracije olova inhibiraju lipoamin dehidrogenazu, enzim koji ima važnu ulogu u oksidaciji pirogroždane i alfa-oksoglutaridne kiseline u mitohondrijama. Olovo također inhibira i neke enzime iz skupine ATP-aza, posebice one koje sudjeluju u transportu Na^+ i K^+ . To onda ima za posljedicu remećenje nerвне transmisije u živčanim vlaknima i barem dijelom izaziva kliničku sliku olovne neuropatije i encefalopatije. I aktivnost dehidrataze delta-aminolevulinske kiseline ovisi u velikoj mjeri o njezinim SH skupinama, a inhibirana je razmjerno malim koncentracijama olova. Za inhibiciju sintetaze delta-aminolevulinske kiseline, međutim, potrebne su znatno veće koncentracije olova. Povećane količine delta-aminolevulinske kiseline u urinu olovom otrovanih osoba upućuju na to da sinteza ovog metabolita nije značajnije poremećena.

Već je pokazano da olovo inhibira dekarboksilaciju uroporfirogena i time interferira s dekarboksilacijom koproporfirogena. Čini se da je jedan od najvažnijih učinaka olova njegovo antagonističko djelovanje prema drugim metalima, naročito prema željezu. Uz ovaj uvjet čini se jasnim da će poremetnja inkorporacije željeza u hem smanjiti brzinu stvaranja jetrenog hema i biti odgovorna za prisutnost delta-aminolevulinske kiseline u mokraći. Ova olovom uzrokovana inhibicija inkorporacije željeza u protoporfirin može dovesti i do inhibicije stvaranja različitih citokroma pa to može, barem djelomično, objasniti opće toksične simptome otrovanja olovom.

S obzirom na dijagnostičku vrijednost, utvrđivanje aktivnosti dehidrataze delta-aminolevulinske kiseline jest nedostatno bez istodobnog utvrđivanja aktivnosti sintetaze delta-aminolevulinske kiseline, K_M vrijednosti za oba enzima kao i koncentracija različitih supstrata i kofaktora.

Osim toga iskrsava važno pitanje u kolikoj je mjeri inhibicija dehidrataze delta-aminolevulinske kiseline specifična za otrovanje olovom. Utvrđeno je naime da je u pacijenata koji su patili od insuficijencije jetre nakon alkoholne ciroze aktivnost dehidrataze delta-aminolevulinske kiseline u zrelih eritrocitima znatno smanjena a uz istodobno normalne koncentracije olova u krvi i bez podataka o ekspoziciji olovu. Prema tome čini se vjerojatnim da ima i drugih činilaca mimo intoksikacije olovom koji mogu utjecati na aktivnost dehidrataze delta-aminolevulinske kiseline pa u tom svjetlu treba i interpretirati rezultate mjerenja aktivnosti ovog enzima.

DISCUSSION FOLLOWING THE PAPER

COLE: I'm sure Dr Neuberger would be pleased to answer some questions if there are any. Does anyone have any questions or comments concerning his work?

I would just like to say that from my understanding of the work of Dr Vallee these patients had not necessarily ingested alcohol immediately before their hospitalization, that this was something that was chronic.

NEUBERGER: I think he had some evidence that most of the patients who had these relatively low ALA dehydratase values had not had access to alcohol for 3 or 4 days at least because they had been in hospital. Information given by patients about their alcohol consumption is not unreliable, but all the same it seems highly probable that the low level of ALA-dehydratase noted in many of the patients suffering from liver disease in Boston could not be explained by high intake of ethanol immediately before the test was done.

COLE: Fine, thank you, very much. We're certainly indebted to you for this fine presentation and for filling in so admirably on such short notice. Thank you, once again.

MAJIC: When talking about alcoholism I would like to add some results of the investigations carried out at the Institute for Medical Research and Occupational Health in Zagreb in patients with chronic liver lesions and in chronic alcoholics. There were approximately 60—65 patients in each group. Decreased ALAD activity was found in either group though lead blood values were not increased, thus our findings tend to support the results of Dr Neuberger.

NEUBERGER: It is clear that ALA-dehydratase in the circulating mature red cell has no physiological significance so far as we are aware. Many of the other enzymes concerned with the biosynthesis of haem have disappeared during the maturation of the cell, and it seems quite likely that the ALA-dehydratase in the erythroblast is higher than in the circulating red cell. The effect of lead may thus be much less marked in the erythroblast.

However, in order to get the facts clear, a study of the effect of lead should be done on the enzymes of immature red cells in the bone marrow at various stages of development. In addition, the circulating red cells are a mixed population comprising cells just recently released from the bone marrow and cells of all ages up to 120 days old. It would be of interest to establish how the ALA-dehydratase varies with the ageing of the mature red cell, and what the effect of lead is on this process.

CHISOLM: Your speculation that the ALA-dehydratase activity in circulating red blood cells decreases as these cells age is most interesting. In that regard, I thought you might be interested in the following observation: I am aware of studies in three children with sickle cell disease (hemoglobin Type SS) who had blood lead concentrations in the 70 to 100 $\mu\text{g}/100$ ml whole blood range and other evidences of lead toxicity. These three patients had the 20 to 30 percent reticulocyte counts in their peripheral blood, which is a customary finding in sickle cell disease. These three patients showed normal ALA-dehydratase activity, as measured *in vitro* in peripheral blood. This is certainly consistent with your speculation, and might be of clinical importance if one were to depend entirely on *in vitro* measurements of ALA-dehydratase activity in populations in whom sickle cell disease is found.

BARLTROP: With some deference, I wonder if I might remind Professor Neuberger of some work carried out in another department of his former hospital, in which it was shown that lead in red cells is bound principally to the hemoglobin component, and furthermore, that it did not appear to be bound to free sulphhydryl groups; mole for mole, perhaps four times as much lead is bound to hemoglobin as mercury. Could he perhaps comment on this, particularly with regard to what effect this might have on ALAD inhibition?

NEUBERGER: I am aware of this work, but I would like to ask Dr Barltrop how he accepts this in the light of his data?

BARLTROP: I cannot explain it, Sir; I was hoping you would.

NEUBERGER: I would say that probably some of the lead is bound to the ALA-dehydratase inactivating it and the lead which is bound to the hemoglobin may not have any direct significance for the ALA-dehydratase.

BARLTROP: In other words, it would not be sensible to relate ALAD inhibition to blood lead concentrations.

COLE: That has some obvious repercussions to, at least, some of the more recent proposals I've seen here in Europe.