Lead and haemoglobin synthesis: a review

J. M. WHITE

M.D.

Department of Haematology, Royal Postgraduate Medical School, Du Cane Road, London

It has been known for many years that lead poisoning is associated with both abnormal haemoglobinization of the red cell and red cell production. Thus Gould, Kullman and Shecket noted in 1937 that the treatment of cancer patients with lead resulted over several weeks in a progressive anaemia, reticulocytosis and erythroblastosis. Basophilic stippling of red cells was a prominent feature. Recently it has been realized that lead exposure does not invariably lead to anaemia in the adult, although in children it may be marked (Albahary, Guillaume and Martin, 1965; Boyett and Butterworth, 1962; Hutchinson and Stark, 1961; Watson, Deckers and Lichtman, 1962). The bone marrow morphology shows normoblastic hyperplasia and the presence of abnormal ringed sideroblasts, the last of which indicates that the controlled synthesis of haemoglobin within the cell is abnormal. This has now been confirmed by the finding that the synthesis of both globin and haem were inhibited by inorganic lead *in vitro*.

Much of the early work concerned the effect of lead on haem synthesis. As early as 1895, Stokvis showed that in lead-poisoned rabbits there was an increased excretion of porphyrins. Later, increased excretion of coproporphyrin and δ -aminolaevulinic acid were found in man (Grotepass, 1932; Haeger, 1957). Bessis and Breton Gorius (1959) pointed out that lead produced morphological changes of the mitochondrion in that the cristae were swollen and disorganised. It is now known that lead *in vitro* inhibits ALA dehydratase, ferrochelatase and coproporphyrinogen oxidase (Dresel and Faulk, 1956; Goldberg *et al.*, 1956). The mechanism of inhibition is thought to be due to blockade of -SH groups which these enzymes possess. However, there is no evidence that the -SH groups are near the active site of the enzyme, nor that -SH blockade would result in their inactivation.

Present data, however, indicate that these enzymes are inactivated and this results in a deficiency of haem within the cell and an increased accumulation and excretion of haem intermediates. There are also data which indicate that total globin synthesis is inhibited but it is not known whether this is a primary abnormality or a secondary effect caused by haem deficiency. (Kassenaar, Morell and London,

1957). To answer these questions a systematic study has been carried out on the effect of lead on globin, and α and β chain synthesis *in vitro* with and without the addition of haemin. The methodology and results have been reported in detail elsewhere (Piddington and White, 1974) and only an outline of the experiments and implication of the findings will be given here.

In vitro, using reticulocytes from patients with haemolytic anaemia, it was found that total globin synthesis was markedly inhibited by lead and the degree of inhibition was dependent on the concentration over a range of 25-500 μ g (Table 1). Similarly, there was a depression of α and β chain synthesis ($\alpha : \beta < 1.0$) (Table 2). Also α , chain synthesis was significantly more depressed than β chain synthesis at least until the concentration of lead reached 200 μ g/100 ml. The addition of haemin to the system at concentrations of 10^{-3} mol/l corrected total globin chain synthesis but only partially corrected

TABLE 1. The effect of lead on total globin synthesis with and without added haemin

Lead conc. (μ g/dl)	-haemin (% inhibition)	+haemin (% inhibition)
50	20	3
100	40	5
200	57	9
400	74	7

The results given are the mean values of seven experiments. The lack of inhibition in the presence of haemin is highly significant.

TABLE 2. The effect of lead on the relative synthesis of α and β chains with and without added haemin

Lead conc. (μ g/dl)	-haemin $\alpha : \beta$	+haemin $\alpha : \beta$
0	1.06	1.07
50	0.99	1.05
100	0.91	1.03
200	0.86	1.00
400	0.80	0.92

The results given are the mean values of five experiments. The difference in the ratios, with and without haemin, is probably insignificant.

the imbalance between α and β chains (Tables I and II). Studies also showed that the transport of the isotope label used (^3H leucine) into the reticulocyte, was markedly inhibited. Although this finding may partially explain the inhibition of total globin synthesis, it would not account for the relatively greater inhibition of α chains, in that both chains have the same number of leucine residues. Using an isotope of lead (^{203}Pb), a significant, yet small amount was found associated with the ribosomal fraction of the cell. These *in vitro* findings indicate that the inhibitory effect of lead on globin synthesis acts at two levels—the first, and major effect, is secondary to haem deficiency, and the second is probably due to the effect of lead *per se*. With regard to the first effect, the pattern of inhibition of globin synthesis is strikingly similar to that found in other haem deficiency states, namely iron deficiency (White and Hoffbrand, 1974) and sideroblastic anaemia (White, Brain and Ali, 1971). In these disorders, not only was globin synthesis subnormal, but there was a relatively greater inhibition of α chain synthesis than β chain synthesis. At present the most likely explanation is that when haem is deficient a soluble inhibitor to globin chain initiation is formed, which is removed by the presence of exogenous haemin (Hunt, Vanderhoff and London, 1972). Since the inhibitory effect of lead can be largely overcome by haem, it is considered that the major effect of lead is an inhibition of haem synthesis which, via the formation of an inhibitor, affects globin synthesis. The evidence that lead has a direct effect upon globin synthesis is at present speculative and based on the fact that haemin did not completely correct the relatively greater inhibition of α chain synthesis, and also that a small, yet significant, amount of lead was bound to the ribosome fraction of the cell. Since in this study only reticulocytes were used, one can only postulate that lead has a direct effect at the level of translation of globin messenger RNA. However, it is also possible that lead may cross, or disturb, the nuclear membrane and affect transcription of the messenger.

These *in vitro* findings, especially the decrease in the $\alpha : \beta$ ratio, prompted a study into the *in vivo* synthesis of globin in lead workers as compared with a control group. Twenty-eight lead workers were studied, all of whom had elevated blood lead levels. None, however, had any evidence of lead toxicity. It was found that the $\alpha : \beta$ ratio of this group (mean 1.24) was not statistically different ($P = 0.027$) from the control group (mean 1.041). However, six subjects had ratios greater than 1.2, a finding which was reproducible.

From these findings it is concluded that the supply

of haem is not defective in the red cell precursors of these subjects, although from the data of other workers the levels of blood lead are high enough to cause disturbances in the haem pathway. Also, the finding that some workers had an increased rate of α chain synthesis may indicate that during chronic exposure compensation of α chain synthesis takes place and in some instances 'overcompensation' occurs.

There are no data available regarding the changes of the MCH of lead workers at the beginning of their work, but such data should easily be obtained and this is crucial to the hypothesis which has been suggested.

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