Kinetics of lead interaction with human erythrocytes

D. BARLTROP
M.D., F.R.C.P.

A. M. SMITH*
Ph.D.

Paediatric Unit, St Mary's Hospital Medical School, London

Summary

The kinetics of the interaction of lead with human erythrocytes has been investigated *in vitro* by means of tracer studies with ^{203}Pb . The elution characteristics of lead from erythrocytes in contact with EDTA suggest the existence of two binding sites differing in their affinity for lead. The findings are consistent with the existence of two compartments for lead binding in the cell, and a model system based on this concept is proposed.

Introduction

Divalent inorganic lead ions are known to be associated with the erythrocyte rather than the plasma fraction of human blood (Clarkson and Kench, 1958). The significance of the lead concentration that occurs in the erythrocytes as a consequence of normal exposures has not been established and although the anaemia of plumbism has been attributed to impaired haemoglobin synthesis and decreased red cell life (Waldron, 1964), the mechanisms underlying these changes remains uncertain. It has been postulated that the cell membrane is the principal site for lead binding (Aub *et al.*, 1925) but recent work with red cell haemolysates has shown that haemoglobin has a greater affinity for lead than erythrocyte stroma or membrane material (Barltrop and Smith, 1971). Chelating agents such as calcium disodium EDTA are commonly used in the treatment of lead poisoning and are thought to enhance the removal of lead from the soft tissues although it has been reported that some chelated lead is skeletal in origin (Hammond, Aronson, and Olson, 1967). There are, however, inconsistencies in the *in vivo* response so that the blood lead concentration is not always reduced to normal values by chelation therapy and may increase after the cessation of treatment. Studies with erythrocytes containing lead added *in vitro* have shown that little lead is removed by EDTA in contacts of short duration (Passow, Rothstein and Clarkson, 1961) and there is evidence that neither EDTA nor the lead-EDTA complex enters the red cell (Barltrop and Smith,

1972). The apparent response to chelating agents in the treatment of clinical plumbism cannot therefore be explained in terms of the observed interaction of lead with erythrocytes *in vitro*. This paper reports kinetic studies which have been designed to explore the existence of erythrocyte binding sites for lead other than haemoglobin.

Materials and methods

Human whole blood was obtained by venepuncture and collected into lithium-heparin tubes. Stable lead solutions were prepared from PbCl_2 dissolved in 0.9% sodium chloride and labelled with $^{203}\text{PbCl}_2$ in saline. Addition of the prepared lead solution was made to blood to give a final concentration of 50 $\mu\text{g Pb}/100\text{ ml}$. CaNa_2EDTA solution in saline was added to blood to give a final concentration of 10 mol/l . Blood containing added lead was mixed continuously in stoppered syringes on a rotary mixer and sampled at intervals. The aliquots thus obtained were centrifuged at 2500 rpm and the distribution of added lead determined in aliquots of plasma and cells by counting at the photo peak for ^{203}Pb (0.279 MeV) with a Hewlett-Packard Auto Gamma Spectrometer. Packed cell volumes were determined by means of a Hawksley microhaematocrit centrifuge. The precision of replicate experiments was such that the erythrocyte lead content could be determined to $\pm 1\%$.

Results

The uptake of lead by erythrocytes from saline was rapid at ambient temperatures and under the experimental conditions 97% of the added lead was associated with the cell fraction within 15 min. The rate of lead uptake was decreased by cooling so that at 4°C the reaction was complete 30 min after initial contact (Fig. 1). The erythrocytes showed a marked affinity for lead and could accommodate concentrations greatly in excess of those encountered in clinical lead poisoning. Similar uptake characteristics were observed for lead at low concentrations added to plasma, however, at values exceeding 200 $\mu\text{g Pb}/100\text{ ml}$ blood an increasing proportion of lead remained in the plasma fraction which appeared to

* Present address: Department of Employment, Baynard House, Chepstow Place, London.

FIG. 1. The uptake of lead by erythrocytes from plasma at 22°C and 4°C. Final concentration of lead, 50 $\mu\text{g}/100\text{ ml}$.

FIG. 2. Residual lead in plasma after contact with erythrocytes as a function of initial plasma lead concentration. Haematocrit 45%.

be inaccessible to the erythrocytes (Fig. 2). This critical value is likely to have been exceeded in studies involving the intravenous administration of lead solutions owing to the local 'bolus' effect.

Initial investigations were designed to explore the kinetics of uptake by arresting the entry of lead into the cell by serial additions of EDTA to the system. Using this technique it was found that some lead escaped from the cells after initial entry thus invalidating this approach. Kinetic studies were therefore limited to the egress of lead from previously treated cells using the same technique of trapping with EDTA in the plasma phase.

Erythrocytes exposed to lead for 15 min gave a biphasic elution curve suggesting the existence of two binding sites differing in their affinity for lead. It would be expected therefore that with the lapse of time after initial contact with lead there would be

progressive transfer from the weakly to firmly bound states. This hypothesis was tested by studying the elution characteristics of lead from cells after varying intervals of contact with the metal. Each study was controlled with cells maintained under identical conditions but to which lead had been added only 15 min before contact with EDTA. The findings indicated that the lead elution curves for the red cells altered with time such that recently added lead was more easily recovered (Figs 3 and 4).

FIG. 3. Effect of duration of contact with lead on the rate of elution from erythrocytes by EDTA 10^{-4} mol/l . Cells 6 hr *in vitro* at 22°C. Time exposed to lead 6 hr (top line) and 15 min (bottom line).

FIG. 4. Effect of duration of contact with lead on the rate of elution from erythrocytes by EDTA 10^{-4} mol/l . Cells 24 hr *in vitro* at 22°C. Time exposed to lead 24 hr (top line) and 15 min (bottom line).

It appeared, therefore, that two variables (elution time and *in vitro* time) determined the residual concentration of lead in the erythrocytes of the short contact system and their interrelationship is illustrated by means of a three dimensional display (Fig. 5). By contrast, lead contacts of long duration gave elution curves which were essentially similar for contact periods of 1.5–18 hr although a slight increase in elution rate was observed after 24 hr (Fig. 6). Inspection of the data in Figs 5 and 6

FIG. 5. Three dimensional display relating residual erythrocyte lead to time of cells *in vitro* and duration of elution by EDTA 10^{-4} mol/l. Cells in contact with lead 15 min pre-elution.

FIG. 6. Three dimensional display relating residual erythrocyte lead to time of cells *in vitro* and duration of elution by EDTA 10^{-4} mol/l. Cells in contact with lead throughout *in vitro* time.

showed that the two planes tended to approximate at short *in vitro* times but this could not be explored experimentally because of the required interval of 15 min for completion of lead uptake from plasma.

Extrapolation of the data for both long and short contact systems to zero *in vitro* time (T_0) gave similar plots which did not differ significantly ($P = > 0.05$) and were linear after the first 10 min. The intercept of the T_0 plots on the ordinate (92%) was less than the total erythrocyte lead content (97%). This discrepancy, although small, could not be attributed to experimental error and is consistent with the existence of a second compartment in which Pb is less firmly bound.

The studies were repeated at 4°C to inhibit the metabolic activities of the cell at room temperature.

FIG. 7. Effect of duration of contact with lead on the rate of elution from erythrocytes by EDTA 10^{-4} mol/l. Cells *in vitro* 5 hr at 4°C. Contact time with lead 5 hr (top line) and 15 min (bottom line).

FIG. 8. Model system representing the interaction of lead with the erythrocyte. A and B represent compartments in which lead is bound loosely and firmly respectively.

A marked increase in the availability of lead was noted for the short contact system such that 30% of the added lead remained available for elution within 2 min of contact with EDTA and no further loss could be demonstrated with increasing elution time. By contrast, lead in the long contact system remained unavailable to EDTA (Fig. 7). A model based on the hypothesis of a double compartment system can be represented diagrammatically (Fig. 8).

Discussion

The elution curves observed with EDTA suggest that the erythrocyte lead is not as firmly bound as was previously thought. The rate of removal of lead from the cells is greater than the previously published *in vivo* data. Castellino and Aloj (1965) showed that the biological half-life of lead in erythrocytes in the presence of EDTA *in vivo* is in the order of 110 hr, whereas the present studies suggest a value of 120 min. However, the *in vivo* situation is complicated by redistribution and equilibration between the tissues.

The data presented appear to support the existence of a second erythrocyte-associated compartment for lead binding, but do not provide evidence of its nature or site. The findings could equally well be explained by a primary combination of lead at the red cell surface with subsequent transfer through the membrane to combine with haemoglobin. Alternatively, they could represent binding to intracellular non-haemoglobin fractions preceding subsequent redistribution. The latter view is supported by the uptake and elution curves at 4°C in which recently transferred lead remains more readily available to EDTA than in comparable studies at room temperature. The known characteristics of the lead-haemoglobin system are consistent with a reversible reaction in favour of complex formation which will thus be related to the duration of contact with lead. Cooling would tend to diminish dissociation so that complexed lead would be less available for elution by EDTA than at room temperature. It would appear, therefore, that the lead-haemoglobin complex is not directly accessible to EDTA and is situated in a second compartment.

It is suggested that compartment A binds lead

weakly while in B the lead is more firmly bound with the equilibrium in favour of the lead-haemoglobin complex. Transfer from the plasma to compartment A is little influenced by temperature whereas further transfer is critically temperature-dependent. Plasma lead is in equilibrium with compartment A and receives lead from B only under conditions favouring dissociation of the lead-haemoglobin complex with intermediate binding in compartment A.

An alternative explanation might be that the weakly bound component is represented by the plasma protein 'shell' at the erythrocyte membrane surface and that compartment B is represented by the total erythrocyte contents. This would require the concept of free circulation of haemoglobin within the cell to allow transport of newly formed lead haemoglobin complex from the inner surface of the erythrocyte membrane. However, this would seem to be unlikely in view of the available evidence concerning the internal structure of the erythrocyte (Perutz, 1948).

Acknowledgments

D.B. was a Wellcome Senior Fellow in Clinical Science. ²⁰³Pb was supplied by the M.R.C. Cyclotron Unit, Hammer-smith.

References

- AUB, J.A., FAIRHALL, L.T., MINOT, A.S. & REZNIKOFF, P. (1926) *Lead Poisoning*, Williams and Wilkins, Baltimore.
- BARLTROP, D. & SMITH, A.M. (1971) Interaction of lead with erythrocytes. *Experientia*, **27**, 92.
- BARLTROP, D. & SMITH, A.M. (1972) Lead binding to human haemoglobin, *Experientia*, **28**, 76.
- CASTELLINO, N. & ALOJ, S. (1965) Effects of calcium sodium ethylenediaminetetraacetate on the kinetics of distribution and excretion of lead in the rat. *British Journal of Industrial Medicine*, **22**, 172.
- CLARKSON, T.W. & KENCH, J.E. (1958) Uptake of lead by human erythrocytes *in vitro*. *Biochemical Journal*, **69**, 432.
- HAMMOND, P.B., ARONSON, A.L. & OLSON, W.C. (1967) The mechanism of mobilization of lead by ethylenediaminetetraacetate. *Journal of Pharmacology and Experimental Therapeutics*, **157**, 196.
- PASSOW, H., ROTHSTEIN, A. & CLARKSON, T.W. (1961) The general pharmacology of heavy metals. *Pharmacology*, **13**, 185.
- PERUTZ, M.F. (1948) Submicroscopic structure of the red cell, *Nature*, **161**, 204.
- WALDRON, H.A. (1964) The effect of lead on the fragility of the red cell incubated *in vitro*. *Journal of Clinical Pathology*, **17**, 405.