Active transport of lead by human red blood cells

T.J.B. Simons

Department of Physiology, King's College London, Strand, London WC2R 2LS, England

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Human red cells suspended in lead–citrate buffers (2.6 μM Pb^{2+}) take up much less Pb than predicted from studies of equilibrium binding of Pb to haemolysates. Pb uptake is increased by ATP depletion, or by loading at 0°C. Tracer studies with ^{203}Pb indicate that the low uptake at 37°C in the presence of substrate is not due to membrane impermeability to Pb. Cold-loaded cells extrude Pb against a concentration gradient at 37°C when glucose is present. These results suggest that the cellular loading of Pb is dependent on the balance between an inward leak and an outward pump. The extrusion of Pb from the cells is possibly brought by the Ca pump.

Pb *Erythrocyte* *Ca pump* *(Ca^{2+} + Mg^{2+})-ATPase*

1. INTRODUCTION

Red blood cells are known to take up most of the Pb in blood [1–4] but there has been little quantitative work on the interaction between Pb and red blood cells. Recently, I measured the rate of Pb uptake into resealed ghosts made from human red cells, over a range of Pb^{2+} concentrations [5]. The results were consistent with a passive membrane permeability to Pb^{2+} of 1.3×10^{-5} cm/s. Now I show that there are two components of Pb uptake by intact red cells. One can be rapidly removed by washing the cells in EDTA, and is only seen when the cells contain phosphate. It is probably a lead phosphate precipitate adhering to the outer surface of the cells. The second component is retained by the cells after washing in EDTA. This is identified as intracellular Pb. Experiments reported here suggest that intracellular Pb depends upon a balance between a passive, inward leak, and an active, energy-dependent extrusion of Pb.

2. MATERIALS AND METHODS

Human red cells from 4–14-day-old blood-bank blood were used. Washed cells were suspended in a solution based on 100 mM KCl, 130 mM sucrose and 5 mM Hepes(K) (pH 7.4) at 37°C (basic medium). The relatively high sucrose and low salt content of this medium was needed to avoid colloid–osmotic haemolysis, as the cells become very permeable to monovalent cations when exposed to Pb [6,7]. This medium was supplemented with Pb or 1 mM EDTA, as required. For Pb uptake, 1.5 mM $Pb(NO_3)_2$ and 3 mM (K) citrate were added. This buffer mixture gives a free Pb^{2+} concentration of 2.6 μM, measured with the Orion 94-82A Pb ion-sensitive electrode, calibrated as in [8]. The Pb content of the cells was determined either after centrifuging a portion of suspension through Dow Corning 550 silicone oil in a 1.5 ml plastic centrifuge tube and cutting off the cell pellet (experiment in fig.1) or after centrifuging the cells and washing the cell pellet twice with 10 ml ice-cold EDTA medium (experiments in fig.1,2,4,5). In both cases the cell pellets (100–200 μl) were extracted with 2–3 ml of 100 mM KCl and 200–300 μl of 15% $HClO_4$, Pb determined by atomic absorption flame photometry at 217 nm with a Pye Unicam SP9 spectrophotometer and Pb standards made up in KCl–$HClO_4$ mixture. In trials with cell suspensions, this procedure gave 91–98% extraction efficiency.

Phosphate was assayed as in [9].

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The binding of Pb to cell constituents was studied by titration with $Pb(NO_3)_2$ solution at room temperature, measuring the free Pb^{2+} concentration with the Orion Pb ion-sensitive electrode. Haemolysates were prepared by freeze thawing cells suspended in a solution containing 80 mM KCl, 110 mM sucrose and 30 mM (K)Hepes and separated from membranes by centrifuging. They were then dialysed against the same solution for 20 h at 4°C.

All cell concentrations are expressed in relation to a fixed number of cells, 10^{13}. This is roughly equivalent to 1 l packed cells. The cell count was measured with a ZF Coulter counter.

^{203}Pb was supplied by the MRC Cyclotron Unit, Hammersmith Hospital, London, and counted in a well-type scintillation counter.

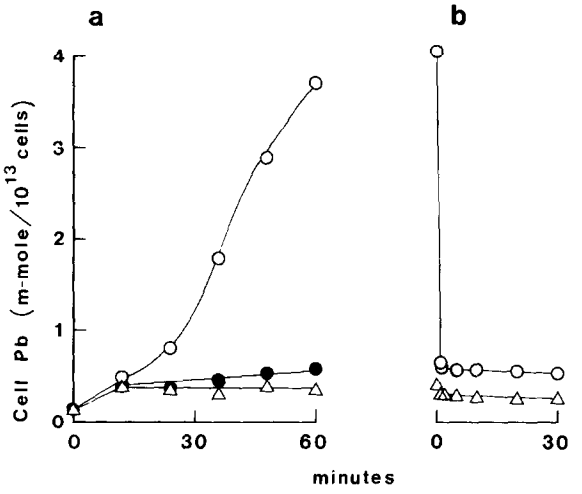

Fig.1. (a) Net uptake of Pb by cells in a Pb buffer at 37°C and (b) dissociation of Pb from the same cells in EDTA at 0°C. (a) Cells were suspended in 2.6 μM Pb^{2+}-buffered medium at 37°C, about 10% haematocrit, and sampled at the times shown either by centrifuging through silicone oil (○, △) or by centrifuging and washing with EDTA-supplemented medium at 0°C (●). Cells were pre-incubated for 2 h at 37°C either in basic medium (lacking Pb) (○,●) or with 5 mM inosine, to lower phosphate levels (△). In the last case, inosine was also added in the incubation with Pb. After the Pb incubation (a), the cells were centrifuged, washed twice by resuspension and centrifuging with basic medium (lacking Pb or EDTA), then resuspended at 0°C in medium supplemented with 1 mM EDTA. (b) Pb content of the cells at differing times, measured by centrifuging the cells through silicone oil. One of 3 similar experiments.

3. RESULTS

The uptake of Pb by red blood cells incubated in phosphate-free media containing Pb buffers depends on the phosphate concentration in the cells, and the method of measurement (fig.1a). If cells were separated from medium by centrifuging through silicone oil, control cells, with 2 mM internal phosphate, took up much more Pb than cells pre-treated with inosine [10], which lowered internal phosphate to 0.11 mM. Cells pre-loaded with phosphate took up even more Pb than control cells (not shown). Much of the Pb taken up by phosphate-containing cells can readily be removed by washing the cells at 0°C in solutions containing 1 mM EDTA (fig.1a). Fig.1b shows the time course of removal of Pb from cells by EDTA. Over 80% of the Pb taken up by control cells dissociates within 2 min, but the rest is firmly bound. Very little Pb is removed from the low-phosphate cells by EDTA. The fraction of cell-associated Pb removed rapidly by EDTA is identified as extracellular lead phosphate. The remainder is considered to

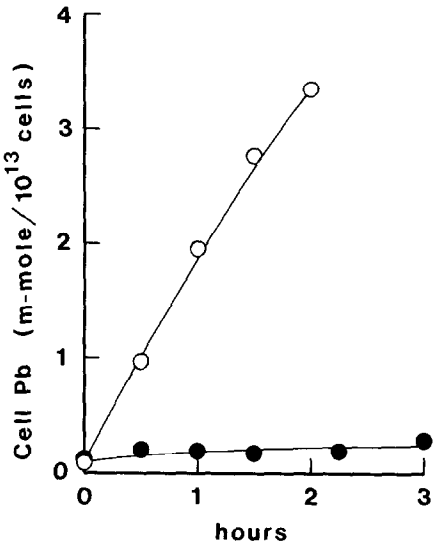

Fig.2. Net uptake of Pb by inosine-fed (●) or ATP-depleted cells (○). Pb uptake was measured with the cells suspended in a 2.6 μM Pb^{2+}-buffered medium at 37°C, about 9% haematocrit, either with the medium supplemented with 5 mM inosine (●) or with cells pre-incubated for 20 h at 37°C in basic medium with 1 mM EGTA, then washed (○). This lowers cell ATP levels to 5–10 μM [12]. One of 2 similar experiments.

be intracellular and/or bound tightly to the cell membrane. Cells were washed with solutions containing 1 mM EDTA before analysis for Pb in all the remaining experiments in this paper.

The uptake of Pb into the intracellular compartment depends on the metabolic state of the cells (fig.2). Cells incubated at 37°C with inosine or glucose, which both act as metabolic substrates (inosine alone lowering phosphate levels), take up around 0.3 mmol $Pb/10^{13}$ cells in 2.6μM Pb^{2+} [mean uptake between 10 and 60 min in 12 experiments (±SE) 0.34 ± 0.05 mmol $Pb/10^{13}$ cells]. By contrast cells depleted of ATP take up 10-times as much in 2 h. Pb uptake in cells provided with substrates is very much less than would be expected if Pb binds passively to cell constituents. Fig.3 shows the equilibrium binding of Pb to dialysed cell lysate (essentially haemoglobin). At 2.6μM Pb^{2+}, haemoglobin binds about 20μmol Pb/g, corresponding to about 6 mmol/l cells for cells containing 300 g Hb/1. The passive binding of Pb by cells would actually be greater than this, because of binding to dialysable components and the plasmalemma.

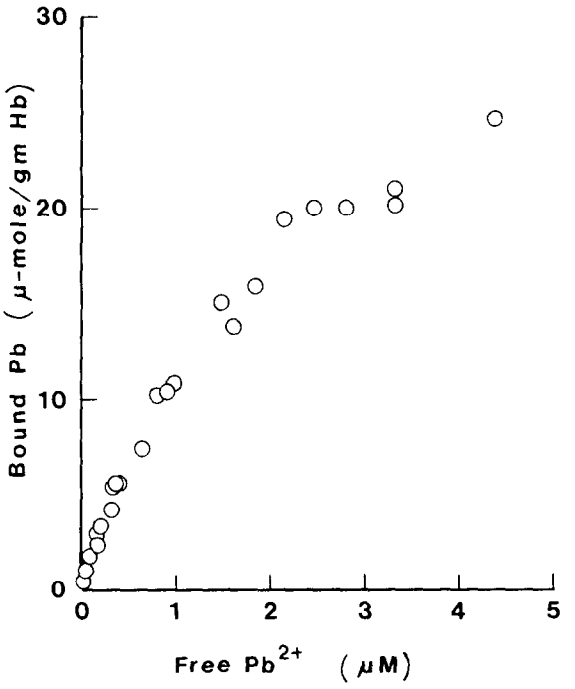

Fig.3. Titration of haemoglobin solutions with Pb. Haemoglobin solutions (25–50 g/l) were prepared as described in section 2, and titrated with Pb by making successive additions of $Pb(NO_3)_2$ solutions and measuring free Pb^{2+} with the Orion electrode, waiting 5 min after each addition. Bound Pb was calculated as the difference between the total Pb in haemoglobin solutions and in control solutions. Combined results of 3 experiments.

It is clear that the cell Pb content is maintained far below the equilibrium Pb level in the presence of metabolic substrates. This could arise from membrane impermeability to Pb, or the presence of an active extrusion mechanism. Fig.4 shows, using tracer ^{203}Pb, that the majority of the Pb associated with the cells in the presence of glucose is in a rapidly exchangeable compartment. The rate of exchange is comparable with the rate of uptake of Pb into depleted cells (fig.2). These observations suggest that the membrane is permeable to Pb, and the low concentration of Pb in the cells is due to active extrusion.

This is confirmed in fig.5, where cells are first loaded with Pb by suspension in 2.6μM Pb^{2+} buffer for 18 h at 0°C. They gain about 1.6 mmol $Pb/10^{13}$ cells, which is still far below the equilibrium level. Upon resuspension in fresh media at 0°C, they continue to gain Pb slowly in 2.6μM

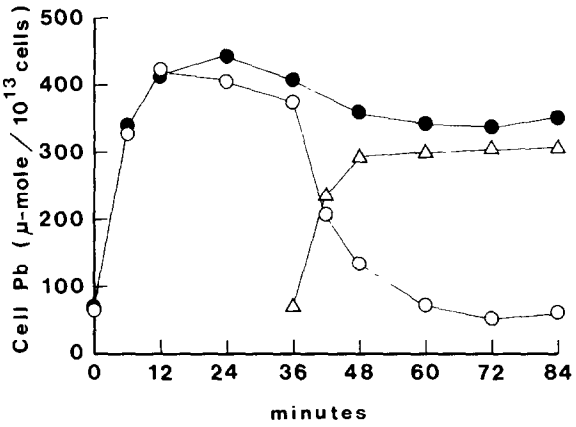

Fig.4. Exchanges of ^{203}Pb with red cells. Cells were incubated at 37°C in 2.6μM Pb^{2+}-buffered solution with 5 mM glucose (6% haematocrit). The Pb content of the cells was measured either by atomic absorption (●), or with tracer ^{203}Pb (○, △). In this case, the tracer with either (○) present from 0–36 min, after which the cells were washed and resuspended in unlabelled Pb solution, or (△) present from 36–84 min, with the cells in unlabelled Pb from 0–36 min. The phosphate content of the cells rose from 1.1 to 2.0 mM in the course of the experiment. One of 3 similar experiments.

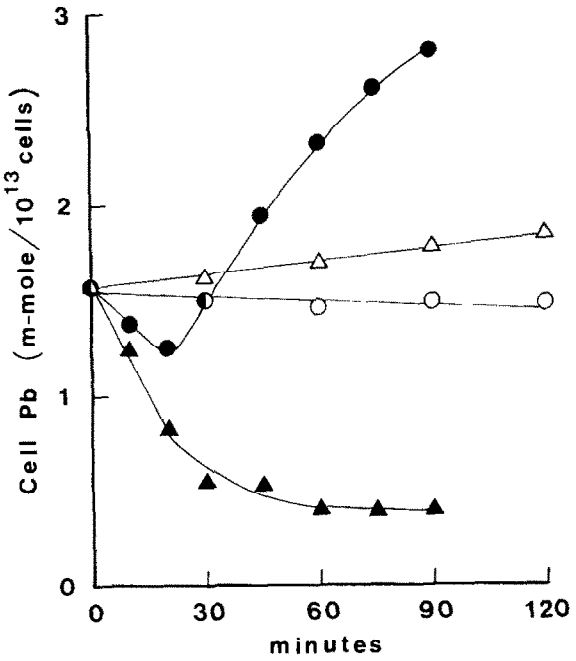

Fig.5. Net Pb movements in cells loaded with Pb at 0°C. Cells were loaded with Pb by suspension in 2.6 μM Pb^{2+}-buffered medium at 0°C for 18 h, then washed in basic medium, and finally resuspended at 0°C (○, △) or at 37°C (●, ▲) (7% haematocrit). The cells at 0°C were in 1 mM EDTA-supplemented medium (○) or 2.6 μM Pb^{2+} medium (△). The 37°C cells were all in 2.6 μM Pb^{2+} medium, supplemented with either 5 mM glucose (▲) or 5 mM inosine and 5 mM iodoacetamide (●). The 37°C results are corrected for lysis, which was about 7% after 30 min and 15% after 90 min. The phosphate content of the cells at 37°C was 0.4 mM initially, rising over 90 min to 1.9 mM with glucose, and 0.8 mM with inosine and iodoacetamide. One of 3 similar experiments.

Pb^{2+} buffer, and lose Pb at a similar rate in 1 mM EDTA. Low temperature blocks active transport, and the slow rate of Pb gain or loss represents the passive membrane permeability to Pb. The intracellular location of the Pb is also confirmed by the slow rate of loss in EDTA (cf. fig.1b). When the Pb-loaded cells are resuspended in Pb buffer at 37°C with glucose, they rapidly lose Pb, down to a steady level of about 0.4 mmol/10^{13} cells. When metabolism is poisoned with inosine and iodoacetamide [11], the cells gain Pb rapidly, after an initial transient Pb loss. These results, taken together, imply that Pb is extruded from red cells against a concentration gradient, i.e., there is active transport of Pb.

4. DISCUSSION

These experiments demonstrate that the uptake of Pb by red blood cells is determined by a balance between an inward leak and an outward pump. The inward leak can be measured in ATP-depleted cells, and is similar in size to the rate of Pb uptake into resealed ghosts containing EGTA [5]. The outward extrusion of Pb occurs against a concentration gradient, can be prevented by cooling to 0°C and probably depends on ATP. The red cell membrane contains two active cation transport mechanisms – the Na and Ca pumps. Authors in [13] have shown that Pb^{2+} can substitute for Ca^{2+} in activating the red cell membrane (Ca^{2+} + Mg^{2+})-ATPase. They report a V_{max} relative to Ca^{2+} of 70%, and a K_d for Pb^{2+} of 2 pM. The V_{max} of the Ca pump is probably about 30 mmol/l cells per h at 37°C [14], so the Ca pump would easily be able to support the rates of Pb extrusion reported here. Clearly, further experiments would be needed to confirm that the Ca pump can transport Pb. If it does, it has obvious relevance for studies of the distribution and metabolism of Pb in other tissues and in whole animals.

It is well established that around 99% of the Pb in human blood is bound to the red cells [15]. This level of binding can occur after allowing for active extrusion of Pb. Red cells contain around 0.3 mmol Pb/l cells in the steady state in 2.6 μM Pb^{2+} solution, in the presence of substrates. If one can extrapolate this to the much lower concentrations occurring in vivo (1–3 μmol/l cells), it corresponds roughly to a cell : plasma ratio of 100 : 1. Other factors, apart from the pump and leak, may also be involved. Cells and plasma contain millimolar concentrations of phosphate, so lead phosphate sols may be present [2], and some Pb may be bound to plasma proteins.

The present work also raises the possibility that Pb may contribute to useless pump/leak cycles and thereby lower cell ATP levels, although rough calculations indicate this is unlikely.

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