

# Interaction of calcium and lead in human erythrocytes

C N O N G \* AND W R L E E

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**ABSTRACT** The interactions of calcium and lead on the human erythrocytes have been studied in vitro using  $^{45}\text{Ca}$  and  $^{203}\text{Pb}$  as tracers. The chemical groups binding calcium and lead on the erythrocytes were also investigated. Calcium ions in the plasma were shown to be capable of replacing the  $^{203}\text{Pb}$  on the red cells. More than 85% of the  $^{203}\text{Pb}$  in the erythrocyte was associated with the cytoplasmic components, and the rest was bound to the stromal membrane. About 90% of  $^{45}\text{Ca}$  was attached to erythrocyte membrane. Extraction of  $^{45}\text{Ca}$  and  $^{203}\text{Pb}$ -labelled erythrocyte membranes with chloroform/methanol mixture showed that the distribution patterns of these two nuclides are similar, with over 88% protein bound, less than 10% lipid bound, and traces in the aqueous phase. Chemical modification of erythrocyte membrane proteins with carbodi-imide, p-chloromercuribenzoate (PCMB), and maleic anhydride suggested that the carboxyl groups are responsible for binding lead and calcium to the red cell membrane. The SH groups may have a minor role in the binding for both cations. Amino groups did not appear to affect the binding of these cations. Gel chromatography of  $^{45}\text{Ca}$ -labelled erythrocyte membrane indicated that  $\text{Ca}^{++}$  bound to the same fraction of membrane proteins as  $^{203}\text{Pb}$ , corresponding to a molecular weight of about 130 000 to 230 000. A possible implication of these findings is that lead and calcium may compete for the same binding site(s) on the erythrocyte.

The exact mechanism by which calcium influences the metabolism of lead is not known. Several experiments, however, indicate that lead may affect the physiological functions of calcium.

Goyer and Rhyne<sup>1</sup> suggested that the absorption of lead and the partitioning of lead in various body compartments are regulated by the same mechanism that controls the metabolism of calcium. Using experimental animals, Meredith *et al*<sup>2</sup> showed that doubling of dietary calcium caused a significant depression of oral lead absorption. Sorell *et al*<sup>3</sup> showed that children with blood lead greater than 60  $\mu\text{g}/100\text{ ml}$  (3  $\mu\text{mol}/\text{l}$ ) had a lower mean daily uptake of calcium and vitamin D than those with a normal range of blood lead. It has also been shown in bone tissue cultures that an increase in  $\text{Ca}^{++}$  concentration in the culture medium decreases the efflux of lead from bone cells.<sup>4</sup>

Kostial and Vouk<sup>5</sup> observed that lead blocked the transmission of nerve impulses in the perfused

cervical ganglion. A similar phenomenon was also observed in frog sympathetic ganglia by Kober and Copper,<sup>6</sup> who postulated that lead competes with the calcium in the presynaptic nerve terminals. In-vitro experiments have suggested that  $\text{PbCl}_2$  also exerts an effect on the neuromuscular junction.<sup>7</sup>

The present investigations were undertaken to evaluate the relation between lead and calcium in human erythrocytes. Attempts were also made to identify the calcium and lead binding sites in the erythrocytes.

## Materials

Molecular weight markers for gel filtration were purchased from BDH Chemicals Ltd. Sephadex G-200 was purchased from Pharmacia (UK) Ltd.

$^{45}\text{Ca}$  was a gift from the Radiation Protection Service, University of Manchester. The isotope is a  $\beta$ -emitter with a half-life of 165 days.  $^{203}\text{Pb}$  is prepared in the cyclotron by bombardment of a thallium target and is obtained carrier free. It was supplied by the Medical Research Council Cyclotron Unit, Hammersmith Hospital. The radioactive half-life is 52 hours.

Fresh human blood was obtained from volunteers

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by venepuncture. Heparin was used as an anti-coagulant. Double glass distilled water was used in all of the experiments using calcium.

All reactions were carried out in plastic or polypropylene tubes to minimise the absorptions of  $\text{Ca}^{++}$  or  $\text{Pb}^{++}$  which occur with glass and also to avoid the haemolytic effect of glass.

## Methods

### DETERMINATION OF RADIOACTIVITY

#### *Gamma activity counting of $^{203}\text{Pb}$ samples*

$^{203}\text{Pb}$  radioactivity was measured by a LKB-Wallace 1280 ultragamma automatic counter connected to a teletype dynamic-printer. Measurements were made using a Na (Tl) crystal to detect the activity at 279 KeV, which is specific for  $^{203}\text{Pb}$ . Normal precautions and checking of background samples eliminated contamination.

#### *Aqueous solution of $^{45}\text{Ca}$*

A LKB 1210 ultrabeta well-type liquid scintillation counter was used to detect the  $\beta$ -emitting calcium-45. The Scintillant used was that of Whyman,<sup>8</sup> which is 0.6% PPO in toluene mixed with Triton X-100 (5:3, v/v).

### PROTEIN

The concentration of protein was measured by the method of Lowry *et al.*<sup>9</sup> Bovine serum albumin was used for calibration.

### EXTRACTION OF PROTEIN AND LIPID FROM ERYTHROCYTES

Lipid and protein were extracted from erythrocytes by the method of Mitchell and Hanahan,<sup>10</sup> which consists of extracting the  $^{203}\text{Pb}$ - or  $^{45}\text{Ca}$ -containing samples on two successive occasions with a chloroform/methanol mixture.

Blood samples were incubated with  $^{203}\text{Pb}$  at 37°C for two hours in a shaking water bath. Isolation and fractionation into plasma and erythrocyte were achieved using the method described later. A mixture of 1 ml aliquot with 1 ml of chloroform and 1 ml of methanol was then shaken for 30 seconds. After the addition of another millilitre of chloroform, 1 ml of distilled water was added and shaking was continued for 10 minutes. The sample was then centrifuged and separated into three distinct layers; the organic lower phase was the chloroform layer containing the phospholipid while the second aqueous upper phase was the methanolic layer of non-bound free ions. The insoluble protein residue was removed and the procedure was repeated once

more. Each fraction was analysed for lead by radioactivity counting.

### DISPLACEMENT OF ERYTHROCYTE LEAD BY CALCIUM

Erythrocytes were incorporated with  $^{203}\text{Pb}$  at a concentration of 2.45  $\mu\text{mol/l}$  (50  $\mu\text{g}/100\text{ ml}$ ) for two hours. They were then washed twice with saline buffer and reincubated with fresh plasma containing  $\text{CaCl}_2$  in concentrations ranging from 20 to 200  $\mu\text{mol/l}$  at 37°C for four hours.

The displacement of  $^{203}\text{Pb}$  was observed by measuring the radioactivity of  $^{203}\text{Pb}$  in the plasma.

### DISTRIBUTION OF $^{45}\text{Ca}$ AND $^{203}\text{Pb}$ IN THE ERYTHROCYTES

Twenty millilitres of fresh blood were collected and centrifuged at 2500 rpm for 10 minutes, after which the buffy layer was carefully aspirated. The supernatant and erythrocytes were mixed and incubated for one hour in the presence of 20  $\mu\text{Ci}$  of  $^{45}\text{Ca}$  or  $^{203}\text{Pb}$ . After incubation the cells were spun down at 3000 rpm, and the plasma fraction was aspirated. The red cells were washed four times with 10 volumes of cold isotonic saline and were then lysed with five volumes of water. The total volume of the lysate was measured and a one-millilitre fraction was removed for radioactivity estimation. The stroma (ghosts) was separated from the rest of the lysate by centrifugation at 30 000 g for 40 minutes and then freed from haemoglobin according to the method of Maddy.<sup>11</sup> The amount of radioactivity in the entire amount of the stroma was measured. All the separations were done at 4°C.

The radioactivity in whole blood, plasma, lysate, and the stroma was determined by  $\beta + \gamma$  - counting, and the percentage of activity in each fraction was also calculated.

### PREPARATION OF ERYTHROCYTE MEMBRANES

The stepwise osmotic haemolysis method of Dodge *et al.*<sup>12</sup> was used for human RBC membrane preparation. This method was reported by the authors to cause minimal loss of membrane proteins and maximal loss of haemoglobin.<sup>13</sup>

The use of EDTA in the method, however, creates some problems. Using this reagent more haemoglobin is released from the membrane but some of the loosely bound protein probably accompanied it. In addition, the lead would also be chelated and thus make the results difficult to interpret. Therefore this reagent was not used.

Samples of fresh human blood were used in this study. The blood was preincorporated with  $^{203}\text{PbCl}_2$  (final concentration 2.45  $\mu\text{mol/l}$ ) for 90 minutes at 37°C. The plasma and buffy layer were carefully

removed by aspiration. Particular attention was directed to eliminating the white blood cells as much as possible. For this reason there was considerable loss of red cells.

#### CHEMICAL MODIFICATION OF ERYTHROCYTE MEMBRANES

Chemical reagents were used to identify the principal binding molecule(s) in the membrane. The methods of introducing these group specific agents were as follows.

##### *Reaction with carbodi-imide: modification of carboxyl groups*

The procedure was based on the method of Carraway and Koshland.<sup>14</sup> Membranes were suspended in 0.5 M NH<sub>4</sub>Cl and carbodi-imide was added to give a final concentration of 0.1 M. The final volume was 2 ml, containing 10 mg membrane protein. The pH was adjusted to 4.75 by HCl, and the mixture was incubated at 37°C for one hour: 0.1 M HCl was added through the incubation time to maintain a constant pH of 4.75. The reaction was terminated by the addition of 10 ml ice-cold 0.5 mM histidine-imidazole buffer (pH 7.0), and the membranes were centrifuged at 0-4°C for 20 minutes at 6000 g. The membranes were washed twice more with this buffer before lead binding was measured.

To determine the effect of cations released from the membrane, <sup>45</sup>Ca or <sup>203</sup>Pb-containing membranes were incubated with 0.1 M carbodi-imide (pH 7.0) at 37°C for one hour.

Controls in both experiments contained 0.1 M histidine-imidazole buffer (pH 7.0). The reaction was terminated, and membranes were washed as described above.

##### *Reaction with PCMB: modification of thiol groups*

A stock solution of 5 mM p-chloromercuribenzoate (PCMB) was prepared in 10 mM phosphate buffer (pH 7.0) by the method of Riordan and Vallee.<sup>15</sup> Sufficient of this stock solution was added to 10 mg of membrane protein to give a final concentration of 1 mM PCMB in a final volume of 2 ml. Incubation was carried out at 37°C for one hour. Controls contained 10 mM of phosphate buffer. Since PCMB solubilised 24% membrane protein, the released cation was removed by dialysis against 0.5 mM histidine-imidazole buffer (pH 7.0) overnight at 0-4°C.

##### *Reaction with maleic anhydride: modification of amino groups*

Reaction of amino groups with maleic anhydride was based on the procedure of Klapper and Klotz.<sup>16</sup> Solid maleic anhydride was slowly added to 10 mg of

membrane protein in 2 ml of histidine-imidazole buffer. The final concentration of maleic anhydride was 0.1 M and the acylation was carried out at 4°C for 30 minutes. The pH was maintained at 7.0 by the addition of 0.2 M NaOH. The control contained 0.1 M histidine-imidazole buffer, pH 7.0. Unreacted maleic anhydride and free cations were removed by dialysis as previously described for the thiol modification procedure.

#### FRACTIONATION OF ERYTHROCYTE MEMBRANE BY GEL CHROMATOGRAPHY

The gel filtration technique used for fractionation of membrane protein was that described by Andrews.<sup>17</sup> Cross-linked dextran sephadex-G with a water regain of 19 g/g of gel and particle size of 120-400 mesh was used. Filtration was performed in a plexi-glass column (120 × 3.2 cm).

The column was predetermined and standardised with commercially available proteins of known molecular weight. The protein standards used were: human gammaglobulin, conalbumin, cytochrome-C, myoglobin, and trypsin.

Protein separations were performed with a column equilibrated with 0.1 M Tris-HCl (pH 8.0) containing 1 M NaCl and 0.02% sodium azide as a preservative. The flow rate was maintained at 24 ml/hr by adjusting the hydrostatic pressure. All experiments were done at room temperature. Erythrocyte membranes labelled with <sup>45</sup>Ca or <sup>203</sup>Pb were dissolved in the equilibration buffer to a total volume of about 10 ml, and the solution was carefully applied to the top of the column by layering the buffer already present. The column effluent was collected with a LKB Radi-Rac-rotator.

Proteins were estimated spectrophotometrically using a Pye Unicam SP-600 with a 1 cm light cuvette and transmission at 280 nm.

## Results

### EFFECTS OF CaCl<sub>2</sub> ON THE RELEASE OF <sup>203</sup>Pb FROM ERYTHROCYTES

In control experiments, when leaded erythrocytes were incubated in 0.9% saline buffer, only 4% of the total <sup>203</sup>Pb eluted. This <sup>203</sup>Pb found in the supernatant may be due either to a small degree of passive exchange of Pb<sup>++</sup> with other cations, or to some haemolysis and subsequent loss of haemoglobin during the washing of red cells, whereas for leaded erythrocytes incubated in fresh plasma (without addition of CaCl<sub>2</sub>), about 14% of the <sup>203</sup>Pb was released. That a higher percentage of <sup>203</sup>Pb appeared in the plasma than in the saline buffer can probably be attributed to an affinity the plasma or its constituents have for <sup>203</sup>Pb, so that those lead ions not

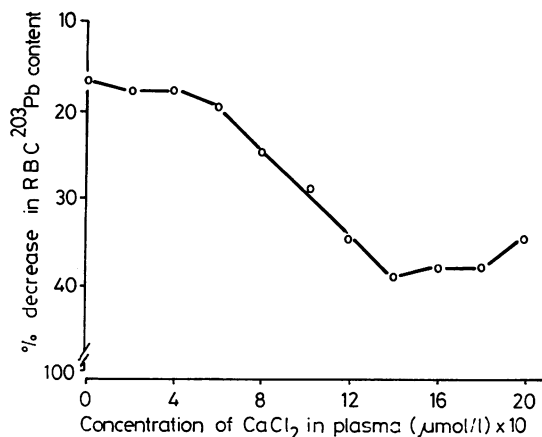


Fig 1 Effects of CaCl<sub>2</sub> on release of <sup>203</sup>Pb.

firmly bound to the red cells may have moved out of the erythrocytes and bound to the plasma.

Figure 1 shows the displacement of <sup>203</sup>Pb from human erythrocytes after four hours incubation in plasma with various concentrations of calcium chloride (in each case as a mean of two experiments). The increase in lead displacement was not obvious until the added calcium chloride concentration reached 60 μmol/l. When the added calcium chloride concentration was above 120 μmol/l, however, no further significant effect on the red cells was observed. This probably occurred because the high concentration of calcium chloride caused agglutination of red blood cells.

#### DISTRIBUTION OF Ca<sup>++</sup> AND Pb<sup>++</sup> IN THE ERYTHROCYTES

Table 1 shows the percentage distribution of <sup>45</sup>Ca and <sup>203</sup>Pb in haemolysate and stromal membranes determined from three experiments each carried out in duplicate. The results showed a significant difference in the binding behaviour; 85% of <sup>203</sup>Pb had penetrated into the red cells to bind with the cytoplasmic components. On the other hand, only 10.8% of <sup>45</sup>Ca was associated with the cytoplasmic components—that is, the main binding fraction for <sup>45</sup>Ca was the stromal membrane. This high percentage

Table 1 Distribution of <sup>45</sup>Ca and <sup>203</sup>Pb in the erythrocyte

Nuclide	Percent distribution (Mean ± SEM)	
	Membrane	Cytosol
<sup>45</sup> Ca	89.2 ± 9.9	10.8 ± 1.6
<sup>203</sup> Pb	14.9 ± 1.7	85.1 ± 8.9

Table 2 Distribution of Ca<sup>++</sup> and Pb<sup>++</sup> in the erythrocyte membrane

Nuclide	Fraction (percent recovered) (Mean ± SEM)		
	Protein	Lipid	Aqueous
<sup>45</sup> Ca	88.4 ± 1.76	9.0 ± 0.95	2.6 ± 0.65
<sup>203</sup> Pb	92.3 ± 1.84	6.7 ± 1.4	1.3 ± 0.20

of calcium found in the erythrocyte membrane confirms the observations reported by Schatzmann and Vincenzi.<sup>18</sup>

#### DISTRIBUTION OF Ca<sup>++</sup> AND Pb<sup>++</sup> AT MOLECULAR LEVEL

Table 2 shows the results of <sup>45</sup>Ca and <sup>203</sup>Pb distribution in the erythrocyte membranes as determined from three experiments, each carried out in duplicate. The radioactivity recovery was 92%. There is no significant difference in the distribution of these two cations at molecular level. The protein fraction that contained most of both nuclides accounted for 88.4% of <sup>45</sup>Ca and 92% of <sup>203</sup>Pb. About 9.0% of the <sup>45</sup>Ca and 6.7% of <sup>203</sup>Pb were found in the lipid-containing phase. Only traces of the labelled materials were recovered in aqueous form, probably released from either protein or lipid molecules during extraction. The lead distribution pattern in the present investigation confirms the low affinity of inorganic lead for phospholipid reported by Beattie *et al.*<sup>19</sup>

#### GROUPS BINDING <sup>203</sup>Pb AND <sup>45</sup>Ca

The effects of chemicals affecting different binding groups on the binding and release of <sup>45</sup>Ca and <sup>203</sup>Pb are shown in tables 3 and 4, which present the results of three experiments, each carried out in duplicate.

The activity of the membrane carboxyl group when treated with 1-cyclohexyl-3-c (morpholinoethyl) carbodi-imide methotoluene-p-sulphonate significantly decreased. The capacity of the membrane to bind both <sup>45</sup>Ca and <sup>203</sup>Pb—that is, the change as a percentage of the control is about 68% both for calcium and for lead. Treatment of nuclide-containing erythrocyte membrane with this chemical also released considerable amounts of <sup>45</sup>Ca and <sup>203</sup>Pb, about 52% in both cases.

On the other hand, blocking the erythrocyte membrane SH groups with PCMB did not appreciably change the binding pattern. The capacity of the membrane to bind <sup>45</sup>Ca and <sup>203</sup>Pb was reduced by about 18.4%. When erythrocyte membranes containing <sup>45</sup>Ca and <sup>203</sup>Pb were treated with this agent, about 16% of <sup>45</sup>Ca and a similar amount of <sup>203</sup>Pb were released.

Table 3 *Effects of chemicals on membrane <sup>45</sup>Ca uptake and release*

Chemical	nmoles of Ca <sup>++</sup> bound/mg membrane protein (Mean ± SEM)					
	Treatment before binding			Treatment after binding		
	Control	Treated	% change	Control	Treated	% change
Carbodi-imide	72.3 ± 4.8	22.78 ± 2.8	68.5	290.56 ± 45	137.12 ± 23.2	52.8
p-chloromercuribenzoate	141.51 ± 23	115.47 ± 24	18.4	214.9 ± 42.4	180.4 ± 43.5	16.1
Maleic anhydride	152.54 ± 41	137.76 ± 29.3	9.7	242.3 ± 70.9	236.2 ± 69.0	2.5

Table 4 *Effects of chemicals on membrane <sup>203</sup>Pb uptake and release*

Chemical	nmoles of Pb <sup>++</sup> bound/mg membrane protein (Mean ± SEM)					
	Treatment before binding			Treatment after binding		
	Control	Treated	% change	Control	Treated	% change
Carbodi-imide	42.3 ± 3.2	13.4 ± 0.8	68.3	181.6 ± 14.7	85.7 ± 6.3	52.8
p-chloromercuribenzoate	91.3 ± 4.7	74.5 ± 4.6	18.4	153.5 ± 11.7	128.9 ± 10.8	16.0
Maleic anhydride	105.2 ± 6.7	98.7 ± 6.3	6.2	170.3 ± 9.4	166.3 ± 11.3	2.3

Inhibition of the membrane amino groups of the erythrocyte membrane proteins with maleic anhydride showed that the capacity to bind both <sup>45</sup>Ca and <sup>203</sup>Pb was only slightly reduced.

These results suggest that the binding groups in the red cell membrane for calcium and lead are similar—that is, the carboxyl groups are primarily responsible for the binding, other groups playing only a minor part.

#### DISTRIBUTION OF CALCIUM AND LEAD IN THE ERYTHROCYTE MEMBRANES

Lead and calcium have been shown to be associated with erythrocyte membrane protein (table 2). Attempts were made to define more closely the principal binding groups of these two cations in the stromal membrane by gel chromatography.

Erythrocytes that had been incorporated with either <sup>45</sup>Ca or <sup>203</sup>Pb were isolated from the plasma, and the stromal membranes were prepared according to the method of Dodge *et al.*<sup>12</sup> The membrane (ghosts) pellet was then washed three times to remove the cations loosely associated with the membrane, leaving the nuclides that were firmly bound. The labelled stromal membranes were then dissolved in Triton-X-100, and the polypeptides were denatured and separated in Sephadex G-200.

Figure 2 is the chromatogram of the membranes before removal of lipids. Most of the recovered <sup>45</sup>Ca was associated with fraction 43-45, corresponding to a high molecular weight component of molecular weight 130 000 to 230 000.

Evidence that <sup>203</sup>Pb is probably bound to the same molecular groups as <sup>45</sup>Ca comes from the finding that <sup>203</sup>Pb also appeared in the fraction of similar molecular weight (fig 3).

#### Discussion

In this investigation a high proportion (85%) of lead in the erythrocyte was associated with the intracellular constituents, and only a relatively small amount (less than 15%) was present in the stromal membrane. This distribution pattern may explain the contradictory published reports with regard to the site of lead binding in the erythrocytes. Earlier workers<sup>20-23</sup> reported that lead was associated with the erythrocyte membrane, whereas Barltrop and Smith<sup>24,25</sup> reported that lead was firmly bound to the cytoplasmic constituents of the erythrocyte. These groups of investigators apparently did not differentiate the two fractions.

The finding that 90% <sup>45</sup>Ca was attached to the erythrocyte membrane is in agreement with Forster and Manery,<sup>26</sup> who showed that the binding of Ca<sup>++</sup> within the erythrocyte was of minor importance. The limited uptake of <sup>203</sup>Pb by the erythrocyte membrane is in accordance with the finding of Bruenger *et al.*<sup>27</sup> who used <sup>210</sup>Pb as a tracer.

The stepwise osmotic haemolysis<sup>12</sup> technique used throughout these experiments was chosen because three washes of ghosts (membrane) pellet would be expected to remove the cations loosely associated with the membrane and leave only the cations that were firmly bound. Extraction experiments by chloroform/methanol show that both calcium and lead cations were firmly bound, as about 90% of the total ghosts Ca<sup>++</sup> and Pb<sup>++</sup> were recovered bound firmly to membrane proteins, whereas less than 3% was present in the aqueous phase (table 2).

It is difficult, however, to draw an overall conclusion from these findings, as the exact composition

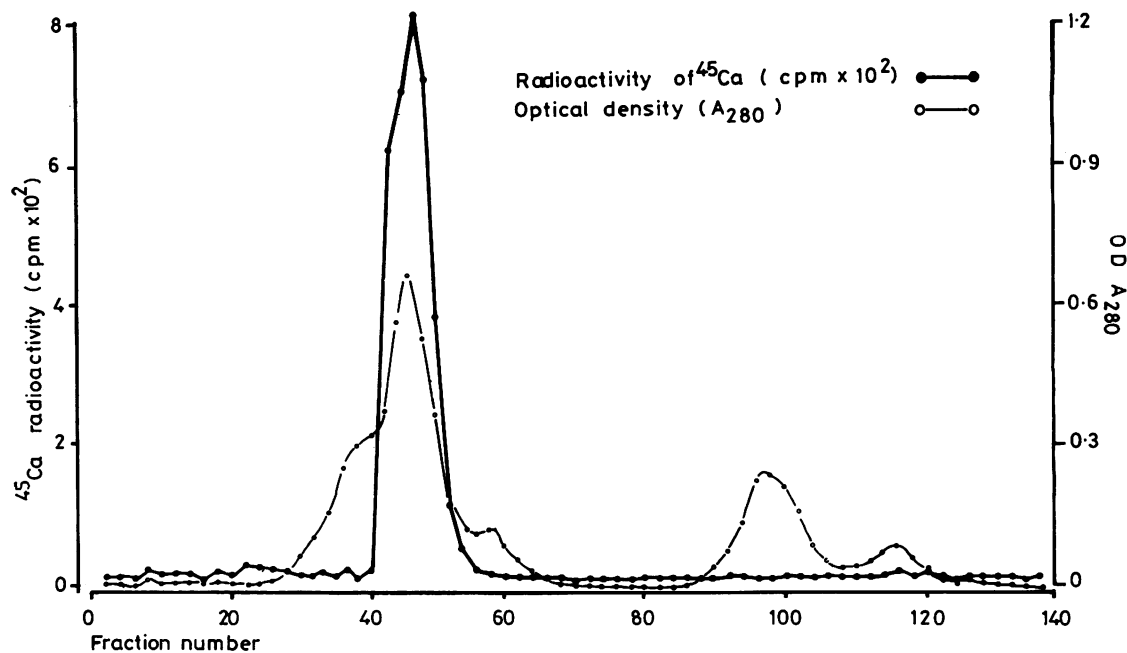


Fig 2 G-200 Sephadex chromatography of <sup>45</sup>Ca-labelled erythrocyte membrane.

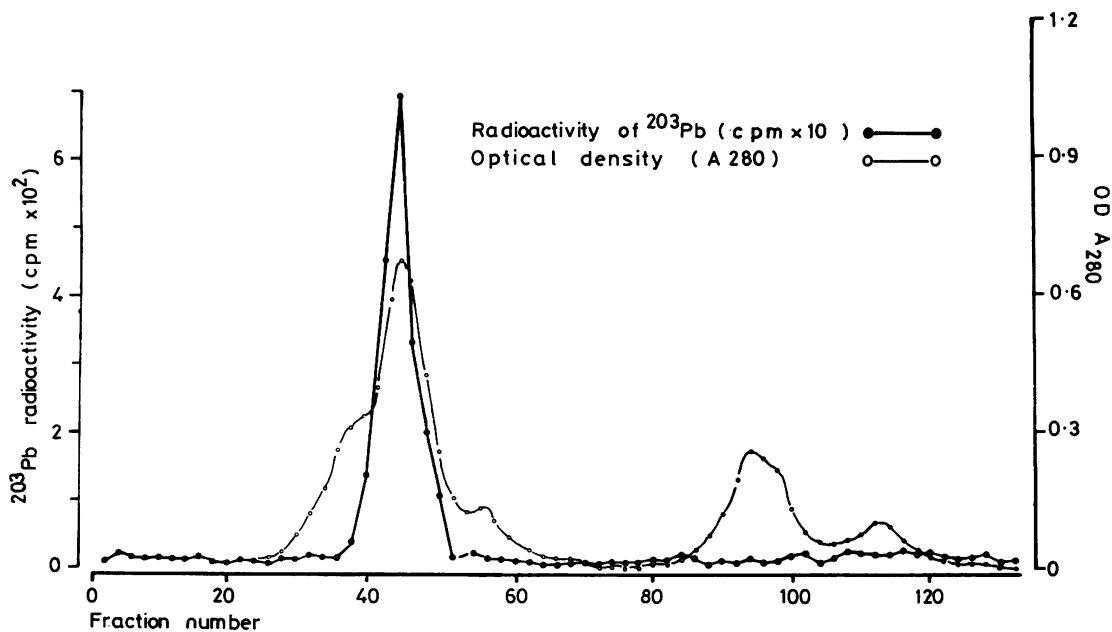


Fig 3 G-200 Sephadex chromatography of <sup>203</sup>Pb-labelled erythrocyte membrane.

of the membrane in the isolated state may differ from that in the intact erythrocyte. In the process of membrane isolation certain proteins or lipids may be washed off. If the membrane composition does differ in the isolated state then possibly some of the binding molecules may have been missing in the present investigation.

It is also possible that in washing the membranes the protein may experience conformational changes. If such changes do occur in the transition from the native to the isolated state then the calcium and lead-binding properties could also alter.

Although the distribution patterns of  $^{45}\text{Ca}$  and  $^{203}\text{Pb}$  in the erythrocytes were different (table 1), lead appeared to have a high affinity for intracellular constituents of RBC and less for stromal membrane, whereas calcium was found mainly attached to the RBC membrane (89.2%). But detailed examination of lead binding sites in the stromal membrane indicated that both cations have a mutual binding site on the erythrocyte membrane, presumably of identical molecular group—that is, carboxyl group (figs 2 and 3 and table 2).

The results of exposing leaded erythrocytes to calcium in our in-vitro system, indicate that post-treatment with calcium reduces the lead content of the red cells. This observation suggests that calcium may influence the transportation of lead from cell to plasma, and that the concentration of calcium in plasma or extracellular fluid may influence the activity of lead. These in-vitro observations confirm the in-vivo experiments noted by Rosen,<sup>28</sup> who showed that in lead-intoxicated rats challenging with calcium above a specific concentration threshold produces a rapid decrease in lead content of red cells.

The finding that lead interacts with the carboxyl group is noteworthy because it may explain the mechanism by which lead inhibits the activity of  $\text{Na}^+/\text{K}^+$ -ATPase,<sup>29-30</sup> as membrane carboxyl groups are known to be involved in the formation of the intermediate  $\text{Na}^+/\text{K}^+$ -ATPase.<sup>31</sup> If lead combines with carboxyl groups at the active site of  $\text{Na}^+/\text{K}^+$ -ATPase the phosphorylation activity of the enzyme would be prevented.

The evidence that calcium and lead interact on the same site(s) on the erythrocyte can be summarised as follows.

(1) More than 90% of the  $^{203}\text{Pb}$  in the erythrocyte membrane was associated with membrane protein (table 2). Extraction of  $\text{Ca}^{++}$  has shown that a very high proportion (88%) of this cation in red cells membrane was also protein bound.

(2) Sephadex G-200 chromatography of  $^{45}\text{Ca}$ - and  $^{203}\text{Pb}$ -labelled erythrocyte membranes indicated that the calcium binding fraction is also the lead binding fraction (figs 2 and 3), which is of molecular weight

130 000 to 230 000. Use of group specific agents to identify the binding molecule(s) suggested that carboxyl groups are primarily responsible for the binding of both lead and calcium in the erythrocyte membrane.

(3) Calcium ions in the plasma were shown to be capable of replacing the  $^{203}\text{Pb}$  on the erythrocytes.

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