The effect of lead on the fragility of the red cell incubated *in vitro*

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SYNOPSIS Osmotic and mechanical fragilities of the red cell were determined before and after incubation with varying amounts of lead *in vitro*.

Lead caused a very marked increase in the resistance of the red cells to hypotonic saline solutions, although it did not increase the mechanical fragility index of the cells after incubation significantly more than in the control samples of blood which were incubated without lead.

Observations by Aub, Fairhall, Minot, and Reznikoff (1926) indicated that when lead was added to red cells *in vitro* they became more resistant to haemolysis in hypotonic saline solutions, a finding subsequently confirmed by Pearse (1926) and by Clarkson and Kench (1958b). As a result of their investigations, Aub and his colleagues suggested that lead altered the red cell membrane in such a way as to render it more susceptible to trauma during passage through the capillaries, thus causing the anaemia frequently associated with lead poisoning. A recent study, however (de Kretser and Waldron, 1963), has failed to substantiate this hypothesis.

It was felt that it would be of some interest to study the effect of lead on the osmotic and mechanical fragilities of the red cell after sterile incubation with lead *in vitro* for 24 hours.

METHODS AND MATERIALS

Blood was collected by venepuncture into sterile heparin bottles from male volunteers who had had no industrial exposure to lead, nor any clinical or laboratory evidence of haematological disorders, past or present.

The osmotic fragility tests were performed by the method of Dacie (1956) using the buffered saline solution of Parpart, Lorenz, Parpart, Gregg, and Chase (1947). The results were expressed in the form of haemolytic increments (Suess, Limentani, Damashek, and Dolloff, 1948).

The mechanical fragility index was determined as described by de Kretser and Waldron (1963).

Volumes of a standard lead acetate solution (100 µg. lead/ml.) were added to tubes sufficient to give final concentrations of 10 and 20 µg. lead/ml. blood. The tubes were dried and sterilized by autoclaving. Whole blood, 3 ml., was added to the dried tubes and mixed by gentle inversion before incubating for 24 hours at 37°C. Then 3 ml. of whole blood was incubated in sterile tubes without lead, as a control, and osmotic and mechanical

fragilities were determined at the end of the incubation time. The results obtained were compared with those recorded before incubation.

RESULTS

The results of the incubated osmotic fragility tests are represented in Figures 1 and 2. Figures are expressed as the mean of the results in each group, group I being comprised of samples of blood incubated with 10 µg. lead/ml., and group II, those incubated with 20 µg. lead/ml. Twenty results were obtained from each group.

From the figures it will be noted that incubating whole blood without lead causes the cells to become more fragile, as one would expect, but the addition of lead causes a marked resistance to hypotonic saline solutions, a feature particularly noticeable in group II.

The results of the mechanical fragility experiments are given in Table I. It will be noted that incubation causes the mechanical fragility index of the red cells to increase appreciably, a finding previously reported by Bakker-v Aardenne, Verloop, and van Boetzelaer (1957). The mechanical fragility index of the blood incubated with lead was raised only slightly above that of the controls. This finding was consistent in both groups.

TABLE I
MECHANICAL FRAGILITY INDEX OF RED CELLS
BEFORE AND AFTER INCUBATION WITH AND WITHOUT
LEAD ADDED *IN VITRO*

	Before Incubation	Control after Incubation	Test after Incubation
Group I	9.9	21.9	24.0
Group II	9.1	23.1	27.3
Whole group	9.5	22.5	25.6

Received for publication 16 December 1963.

FIG. 1. Haemolytic increment curves, A, before incubation, B, after incubation, and C, after incubation with lead ($10 \mu\text{g./ml.}$) added in vitro.

DISCUSSION

As a result of their studies on the effect of lead on red cells *in vitro*, Aub *et al.* (1926) postulated that precipitation of lead tri-phosphate took place on the red cell membrane by the reaction of lead salt with inorganic phosphate groups. As a result of this reaction hydrochloric acid was released and the points of localized acidity which were created were responsible for the increased fragility of the red cell. The increase in mechanical fragility of the cell would give rise, *in vivo*, to the anaemia associated with lead poisoning.

Detailed kinetic studies by Clarkson and Kench (1958a), however, suggested a quite different mechanism for lead uptake. These authors considered that a peptized lead phosphate sol was first formed which then reacted on the cell membrane to form a precipitate of lead phosphate. Clarkson and Kench (1958b) found that the addition of lead to red cells *in vitro* caused a marked increase in resistance to haemolysis in hypotonic saline solutions. They concluded, however, that, *in vivo*, plasma lead will cause only minimal changes in the fragility of the circulating red cell.

The findings reported in the present paper indicate that incubating red cells with lead *in vitro* causes a marked decrease in osmotic fragility. It is known that lead can affect the permeability of the red cell membrane (Pranker, 1961) and it may be possible to explain the increased resistance to hypotonic saline solutions in these terms. More work

FIG. 2. Haemolytic increment curves, A, before incubation, B, after incubation, and C, after incubation with lead ($20 \mu\text{g./ml.}$) added in vitro.

would be necessary, however, before this could be stated with any degree of certainty.

The experiments reported here on the mechanical fragility of the erythrocyte suggest that lead causes only minimal changes in the mechanical fragility of the red cell. Although incubation with lead did increase the mechanical fragility index of the cell slightly more than in the control groups, the difference between the sets of results was by no means great. This finding is in keeping with that of de Kretser and Waldron (1963), who found that the mechanical fragility index in a group of lead workers was not greater than that of a control group of workers with no industrial exposure to lead. It seems likely, therefore, that an increase in the mechanical fragility index of the red cell is not a major factor in the production of lead anaemia.

My thanks are due to Dr. A. R. Thompson and the Management of Vauxhall Motors for permission to publish this paper.

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