CIII. THE INTERACTION OF A FINELY DIVIDED LEAD SUSPENSION WITH BLOOD-SERUM, RINGER SOLUTION, AND AQUEOUS PHOSPHATE SOLUTION.¹

BY JOHN BROOKS.

*From the Muspratt Laboratory of Physical Chemistry and Electrochemistry,
University of Liverpool.*

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THE present paper deals with the changes which take place when lead in a fine state of subdivision in a stabilising medium (as used in the treatment of malignant disease) is added to blood-serum.

The chief points on which information was desired were:

- (i) The possible production of the toxic ionic lead after injection of the lead suspension into the blood-stream.
- (ii) The chemical reactions between the constituents of the suspension and those of the blood.

In the literature dealing with lead poisoning there are several references to the form which lead compounds assume in the blood, but no very definite conclusions have been reached.

The question has been investigated by Dr M. Jowett in this laboratory from the standpoint of the solubility of inorganic lead salts in water and in serum respectively. Of these salts the most important are the tertiary and secondary phosphates and the carbonate. Jowett has calculated that the tertiary phosphate $\text{Pb}_3(\text{PO}_4)_2$ is, under body conditions, the least soluble. Lead salts when introduced into the blood should therefore ultimately pass into this compound since there are phosphate ions present in serum. This has been suggested by Fairhall *et al.* [1926].

Though the transformation into the phosphate would probably be rapid when soluble lead salts were introduced into the blood, the same might not be the case when the lead was in a colloidal form. Various measurements of the rate of combination have therefore been made.

Because of the complexity of both lead suspension and serum some work has also been done with aqueous solutions of sodium phosphate and with a modified Locke-Ringer solution.

¹ This investigation was undertaken on behalf of the Liverpool Medical Research Organisation.

INTERACTION OF LEAD SUSPENSION WITH SERUM 767

EXPERIMENTAL.

The lead suspension. This is prepared by Bredig's electrical sparking method using lead electrodes in a 0.5 % gelatin solution containing calcium chloride (*N*/200) as stabilising medium. The average diameter of the particles in the black suspension after centrifuging is 0.2μ , these particles being negatively charged. The freshly-prepared suspension contains approximately 0.5 % total lead; a typical suspension had the composition: 0.51 g. total Pb per 100 cc. This contains

65.6 % Pb as metallic lead,
8.9 % Pb as lead carbonate,
25.5 % Pb as lead hydroxide¹ (by difference).

The concentration of ionic lead (determined by colorimetric estimation of lead in the ultra-filtrate from the suspension) is *ca.* 0.009 %. This corresponds approximately with the value expected from the solubility of lead hydroxide². The chemical composition of the particles varies with the time and extent of exposure of the suspension to the atmosphere, and the suspension eventually turns white on complete oxidation of the lead to lead hydroxide. The total lead content of each fresh suspension used was estimated by the iodometric method described by Fairhall *et al.* [1926].

The aqueous phosphate solution. The solution used had the following composition: 0.00156 *M* Na₂HPO₄, 0.00095 *M* HCl, 0.9 g. NaCl per 100 cc. The *p*_H of this solution was 7.4 and 1 cc. contained 0.0484 mg. phosphorus, which is within the range of the inorganic phosphorus content of blood-serum.

*Ringer solution*³. This solution was prepared by mixing isotonic solutions of the various salts and had the following composition:

Salt	Moies per litre
NaCl	0.137
KCl	0.00280
CaCl ₂	0.00197
NaHCO ₃	0.0264 (unless otherwise stated)
Na ₂ HPO ₄ †	0.00176 „ „
KH ₂ PO ₄ †	

This solution is used in conjunction with a gas mixture of 95 % oxygen and 5 % carbon dioxide. When saturated with this mixture the *p*_H at 37.5° is 7.49. The *p*_H is given by the Henderson-Hasselbalch equation [1917]

$$p_H = p_K + \log \delta + \log \frac{[\text{NaHCO}_3]}{[\text{CO}_2]},$$

¹ For the sake of convenience the compound formed by the oxidation of metallic lead in contact with water is called lead hydroxide and written Pb(OH)₂, although according to Pleissner [1907] it has the composition Pb₃O₂(OH)₂; Glasstone [1922] gives a more complex composition to this substance.

² Pleissner [1907] gives 100.5 mg. PbO per litre at 18° for the solubility of lead hydroxide in water.

³ This solution (with the exception of the phosphate present) as also the method of its *p*_H regulation is similar to that used by Warburg [1924] in his work on glycolysis.

where $[\text{NaHCO}_3]$ and $[\text{CO}_2]$ are the concentrations in moles per litre of (sodium) bicarbonate and dissolved carbon dioxide respectively. In the present case at 37.5°

$$[\text{CO}_2] = 1.18 \times 10^{-3},$$

$$[\text{NaHCO}_3] = 2.64 \times 10^{-2},$$

whence

$$p_{\text{H}} = 7.49,$$

$$p_{\text{K}} + \log \delta = 6.14.$$

Serum. Fresh cow's serum was used, thymol being added as preservative. In order to maintain a physiological p_{H} the serum was saturated with the gas mixture referred to above and subsequent operations were conducted in presence of this mixture. The p_{H} was then known when the bicarbonate concentration of the serum was determined.

Fig. 1. Apparatus for ultra-filtration.

When the lead suspension was added to serum or to the other two solutions mentioned above there was usually no immediate aggregation of the particles. To estimate the amount of phosphate ion which had disappeared from solution it was necessary to remove the suspended particles. This was done by ultra-filtration under a pressure of 200–300 mm. Hg through collodion bags (prepared from a 6 % collodion solution). The arrangement represented in Fig. 1 permitted ultra-filtration in an atmosphere of the gas mixture and also at different temperatures. The collodion bag was secured to the glass tube by a strip of parchment and tightly-wound linen thread. The air space *A* was necessary to prevent a too rapid fall of pressure during filtration. If necessary the air was displaced by the gas mixture from the apparatus. The first 1.5 cc. of filtrate were rejected. With serum the rate of filtration was 3–5 cc. per hour, with Ringer solution this rate was approximately doubled.

Inorganic phosphorus was estimated by Briggs' modification [1924] of the Bell-Doisy colorimetric method. Bicarbonate concentrations were determined

INTERACTION OF LEAD SUSPENSION WITH SERUM 769

(at 37.5° and in presence of the gas mixture) with Warburg's apparatus and technique [Negelein, 1925].

The experimental procedure was as follows. The lead suspension was added to serum or to Ringer solution previously saturated with gas mixture in such amounts that excess phosphate was present. The gas mixture was bubbled through the liquid for 3–4 minutes, ultra-filtered after a given time, and the uncombined phosphate in 1–3 cc. of the filtrate estimated. The gas mixture was not used with the aqueous phosphate solution.

The combination of the metallic lead present with phosphate is necessarily preceded by its transformation into the hydroxide¹. Rough measurements had previously shown that the rate of this change in the suspension itself when exposed to air is dependent on the rate of stirring and the area of the air-liquid interface. The reaction is rapid given a sufficient supply of oxygen—as can be seen by the immediate whitening which takes place on adding the suspension to large enough volumes of water. With the amounts of suspension and serum, etc., used in the present experiments, the reaction was complete in less than 5 minutes².

Table I gives the results obtained when the lead suspension was added to aqueous phosphate solution. The mixtures were allowed to stand for 7 days before being filtered. The p_H of the mixtures varied from 8.2 to 8.8³.

Table I. *Lead suspension contains 0.517 % total lead.*

cc. phosphate solution	cc. suspension	g. atoms phosphorus bound to lead $\times 10^4$	$\frac{\text{g. atoms P bound}}{\text{g. atoms Pb}}$
80	1	0.156	0.62
40	1	0.158	0.63
40	2	0.321	0.64
40	3	0.470	0.63

The last column gives the ratio of g. atoms of phosphorus, which as phosphate had disappeared from solution, to g. atoms of total lead present. The value for the ratio does not differ greatly from that for tertiary lead phosphate, $Pb_3(PO_4)_2$, i.e. 0.67. The similarity of the results at different final concentrations of phosphate and also the large amounts of the latter which have disappeared do not favour the view that the disappearance is due to adsorption alone. It seems probable therefore that the particles are converted into particles of lead phosphate.

Before the interaction of the lead suspension with serum was investigated, a few filtration experiments with serum alone were carried out. The ultra-filtrate was colourless and protein-free and contained the same concentration

¹ Sackur [1905] has shown that pure lead is only attacked by dilute acids in the presence of oxygen. The velocity of solution is practically independent of the strength and concentration of the acid but dependent on the concentration of dissolved oxygen and the rate of stirring.

² Measured approximately by a manometric method.

³ The change of p_H in the very weakly buffered system is apparently due to the following reaction:

of bicarbonate and inorganic phosphorus as the serum itself. Further, the concentration of inorganic phosphorus in the filtrate was unaltered after the serum had stood for 14 days.

Table II summarises the results obtained on adding the suspension to phosphate and Ringer solution and to serum. For comparison are added the values obtained when a solution of lead acetate (5 mg. Pb per cc.) was used in place of the suspension. In the latter cases the mixtures were usually filtered immediately; in the former after standing for several days. Similar results were obtained at room temperature and at 37.5°.

Table II.

	<u>g. atoms P bound</u> <u>g. atoms Pb</u>		
	Phosphate solution	Ringer solution	Blood- serum
Lead suspension	0.62	1.0	1.1
Lead acetate	0.62	0.69	1.1

These values are each the average of several experiments¹ (3-7). The addition of small volumes of lead suspension to Ringer solution or serum does not change the p_H appreciably (the p_H of the mixture being calculated from the bicarbonate concentration of the ultra-filtrate)². The decrease in bicarbonate concentration due to dilution by the suspension is balanced by the bicarbonate formed in the reaction, *e.g.*

The ratios in Table II fall into two classes which correspond approximately with the ratios for the secondary phosphate PbHPO_4 and the tertiary phosphate $\text{Pb}_3(\text{PO}_4)_2$, *i.e.* 1.0 and 0.67 respectively. Assuming that the results represent the formation of these compounds, it follows that the secondary phosphate is formed when lead acetate or lead suspension is added to serum, whilst with Ringer solution lead suspension gives the secondary compound and lead acetate the tertiary. The tertiary phosphate is formed when either the suspension or lead acetate is added to aqueous phosphate solution. These differences can hardly be due to the effect of p_H since with the phosphate solution a ratio 0.62 was obtained both with lead acetate (p_H of mixture *ca.* 6.0) and lead suspension (p_H of mixture *ca.* 8.5), whereas with Ringer solution or serum (p_H of mixture *ca.* 7.5)³ the two ratios, 1.0 and 0.67, were obtained. It seems preferable to attribute the formation of the secondary phos-

¹ The values of a given series (*e.g.* Table I) agreed well between themselves. Greater deviations (5 %) were obtained between the different series. This may have been due to the slight differences in tint sometimes obtained between the standard and the experimental solutions in the colorimetric method.

² With the mixture in Table IV, $[\text{NaHCO}_3]$ of mixture ultra-filtrate = 0.0266.

³ Similar results were obtained with Ringer solution and serum in the absence of the gas mixture (p_H of mixture > 8.5).

INTERACTION OF LEAD SUSPENSION WITH SERUM 771

phate under certain conditions to delayed equilibrium¹ even though the same ratios as those quoted above were obtained after the mixtures had stood for 14 days. It may be noted that the greater the complexity of the system the greater is the tendency to give the secondary phosphate.

Rate of combination of phosphate ions with the lead suspension.

The rate of combination was measured by filtering portions of the mixture at various time intervals and estimating the uncombined phosphate in 2 cc. of filtrate. The amount of suspension used was such as to combine with approximately two-thirds of the phosphate. Under the conditions employed the preliminary oxidation of metallic lead to lead hydroxide was complete before filtration was commenced.

Two series of experiments were carried out with Ringer solution and serum at 20° and 37.5°. The filter (with the exception of the manometer) was immersed in a thermostat. The results are given in Tables III and IV. The times are reckoned from the time of mixing to the mid-time of filtration.

Table III.

75 cc. Ringer solution.		
4.5 cc. lead suspension = 20.7 mg. total Pb.		
	g. atoms P bound	
	g. atoms Pb	
Time (hours)	20°	37.5°
0.5	0.92	0.96
1.5	0.93	0.96
3.0	0.95	0.98
5.0	0.95	0.97
8.3	0.98	0.98

Table IV.

50 cc. serum (0.00196 M phosphate).		
3.0 cc. lead suspension = 13.8 mg. total Pb.		
	g. atoms P bound	
	g. atoms Pb	
Time (hours)	20°	37.5°
2.0	0.83	0.95
6.0	0.86	0.98
25.0	0.95	0.99
50.0	0.97	1.04
73.0	1.04	1.00

When these values are plotted (cf. Fig. 2) the curves show a rapid rise in the amount of combined phosphate up to approximately 90 % of the final amount, followed abruptly by a much slower combination of the particles with the remaining 10 % of phosphate. The greater portion of the reaction is

¹ Holt, La Mer and Chown [1925], who have studied the solubility of the calcium phosphates in water, found under certain conditions where the tertiary salt was the least soluble that the secondary phosphate was initially formed and that the subsequent transformation into $\text{Ca}_3(\text{PO}_4)_2$ was slow.

too rapid to be investigated by the present method. It can be seen, however, that at 20° the rate of combination in serum is somewhat slower than in Ringer solution. At 37·5°, although the rates are similar, the degree of dispersion of the suspension in the two liquids is very different (see below).

Fig. 2.

- ▲ Serum and lead suspension at 37·5°. ● Serum and lead suspension at 20°.
 ■ Ringer solution and lead suspension at 20°. ◇ + Serum and lead carbonate suspension at room temperature.

The rates in serum and Ringer solution were compared under more comparable conditions by preparing a Ringer solution containing the same concentrations of bicarbonate and of phosphate as were contained in the serum. These were 0·0312 and 0·00204 *M* respectively. The results are given in Table V.

Table V.

Room temperature.
 50 cc. serum or Ringer solution.
 3 cc. lead suspension = 16·2 mg. total Pb.

Time (hours)	$\frac{\text{g. atoms P bound}}{\text{g. atoms Pb}}$	
	Ringer	Serum
1·5	0·92	0·80
7·0	0·92	0·83
8·0	0·93	0·86
26·0	0·96	0·90

The slower rate in serum is probably due to the presence of a film of serum proteins round the particles of the suspension.

It was thought that the rapid decrease in the rate of combination might be accounted for by the decrease in the area of reacting surface with time and also possibly to a decreasing rate of penetration of the phosphate ions through the layer of lead phosphate. An attempt was made to test these points using a series of results obtained with the ultra-filter used by Giemsa¹ [1922]. It

¹ The rate of filtration with this type of ultra-filter was high using aqueous phosphate or Ringer solution.

was assumed that the velocity of chemical reaction at the surface was rapid compared with the rate of diffusion of phosphate ion. The calculated values of the velocity constant were found to fall rapidly with time. These values suggested that the process, instead of being governed throughout by the rate of diffusion, consisted of a reaction accompanied and eventually superseded by a much slower reaction, which was therefore presumably slow compared to the rate of diffusion of phosphate ion. The following facts support the view that the rapid reaction is a combination of phosphate and lead hydroxide and the slow reaction the combination of phosphate and lead carbonate.

(1) In the rapid reaction 90 % approximately of the combining phosphate disappears from solution. Approximately 10 % of the lead is actually present at the beginning as carbonate.

(2) It was found that the reaction proceeded at the same rate whether the mixture was vigorously stirred or maintained at rest. (The method could probably not detect any influence of stirring on the rapid reaction if such were present.)

(3) The very small solubility of lead carbonate in Ringer solution and serum (Jowett).

To obtain further information regarding the behaviour of lead carbonate with regard to phosphate ion the rate of combination of a coarse suspension of lead carbonate¹ with the phosphate ions of serum was measured. The results are given in Table VI and Fig. 2. The difficulty of obtaining a uniform sample of the suspension makes the absolute values in column 2 somewhat uncertain. The particles of the suspension used in (A) were smaller than those in (B); this probably accounts for the faster rate in the former case.

Table VI.

Room temperature.		
(A) 50 cc. serum (0.00203 <i>M</i> phosphate). 3.0 cc. lead carbonate suspension = 1.2 % Pb.		
Time (hours)	g. atoms P bound	
	g. atoms Pb	<i>k</i>
2	0.04	0.038
20	0.27	0.028
44	0.58	0.032
(B) 50 cc. serum (0.00203 <i>M</i> phosphate). 3.0 cc. lead carbonate suspension = 1.08 % Pb.		
Time (hours)	g. atoms P bound	
	g. atoms Pb	<i>k</i>
7	0.08	0.021
12	0.11	0.017
30	0.30	0.020
77	0.65	0.021

In both cases excess lead carbonate was present which combined finally with all the phosphate present. In spite of the decrease in phosphate concentration the velocity remains practically constant. Assuming that the

¹ Prepared by mixing solutions of lead acetate and sodium bicarbonate both containing 0.5 % gelatin. The suspension settled almost completely in 60 minutes.

velocity is proportional only to the reacting surface (*i.e.* assuming that the chemical change is slow compared with the rate of arrival of phosphate ions at the surface) and that PbHPO_4 is formed, we would write

$$\frac{dx}{dt} = k(a - x)^{\frac{2}{3}},$$

a = initial g. mol. PbCO_3 , x = g. mol. phosphate disappeared in time t , whence

$$k = \frac{1}{t} [a^{\frac{3}{2}} - (a - x)^{\frac{3}{2}}].$$

The calculated values of k are given in the third column, and are reasonably constant. The velocity of combination is also not inconsistent with the assumption that in the case of the experiments in Tables III–V the later slow disappearance of phosphate is due to the lead carbonate present in the lead suspension.

The lead ion content.

No detectable amount of ionic lead was found in the small volume of ultrafiltrates from the mixtures of serum (or Ringer solution) and the lead suspension. This would be expected, for however slowly the lead particles were converted into phosphate the lead ion concentration in the bulk of the serum would be governed by the solubility product of the phosphate and hence would be very small.

It may be noted that the values sometimes given for the so-called solubility of metallic lead in serum bear no relation to the resulting lead ion concentration. Such a solubility would depend chiefly on the amount of oxygen available for the oxidation of the lead to lead hydroxide and the peptising action of the serum on this compound or on lead phosphate formed therefrom.

The physical state of the lead particles in serum, etc.

The lead suspension was added to water, the phosphate solution, Ringer solution, and serum and the rate of settling at room temperature and at 37.5° observed. The rates were in the following order:

Ringer solution > water, serum > phosphate solution.

With the exception of Ringer solution at 37.5° (where precipitation occurs within 5 minutes) and to a lesser degree at room temperature, there is no marked acceleration of the slow rate of settling which takes place in the lead suspension itself.

The particles of the lead suspension are negatively charged. The increased stability in the phosphate solution may be due to an increase in this charge on conversion into lead phosphate and the decreased stability in Ringer solution may be due to the presence of the calcium ion. In serum there is the protective effect of the proteins to counterbalance any precipitating action of the various cations present. It may be noted in this connection that the addition of small amounts of lead acetate solution to serum gives a finely dispersed sol of lead phosphate [cf. Fairhall *et al.*, 1926, p. 24].

CONCLUSIONS.

It can be concluded from the results obtained that when the lead suspension is injected into the blood-stream the transformation of lead to lead hydroxide, and hydroxide to phosphate is rapid if there is rapid mixing with a sufficiently large volume of blood. In view of the speed of distribution of a dye-stuff throughout the blood (in about 5 minutes; cf. Lindhard [1926]) it is not improbable that a sufficient degree of mixing is attained to ensure the maximum velocity of chemical changes. Consequently the concentration of ionic lead in serum will fall almost immediately to that corresponding to the solubility of secondary lead phosphate. If, however, the particles temporarily reduced the phosphate concentration in the admixed serum to a very low value¹ the formation of lead carbonate would take place owing to the large amounts of bicarbonate ions present. Further mixing with more blood would again reduce the lead ion concentration to the value characteristic of lead phosphate.

Since, however, any given amount of a soluble lead salt under similar conditions would be transformed into lead phosphate (or carbonate) more rapidly than an equal amount of the lead suspension itself, it is necessary to explain the greater toxic effect of injection of the simple lead salt. It might be suggested that with the soluble salt a faster temporary local reduction of phosphate and bicarbonate concentration to a very low value would leave enough ionic lead in circulation for a sufficient time to cause injury. With the lead suspension the concentration of ionic lead could never be greater than that corresponding to lead hydroxide. An alternative and more probable view is that the fine sol of lead phosphate which would be formed from the soluble lead salt would be more toxic (greater number of smaller particles facilitating distribution, penetration, etc.) than the coarser suspension resulting from the introduction of the lead in the form of the lead suspension.

SUMMARY.

1. The changes which take place when a colloidal lead suspension is added to an aqueous solution of sodium phosphate, to a Ringer solution containing sodium bicarbonate and phosphate, and to cow's serum have been investigated.

2. Ionic lead is not produced in any detectable amount on the addition of the lead suspension to these solutions. The phosphate ions unite with the particles which remain in suspension (except in the case of Ringer solution at 37.5°).

3. The final values obtained for the ratio g. atoms phosphorus bound/g. atoms lead present correspond with the formation of the tertiary phosphate $\text{Pb}_3(\text{PO}_4)_2$ in the aqueous phosphate solution. On the other hand, the secondary

¹ Assuming the plasma volume to be 1.515 l. [Lindhard, 1926] and an inorganic phosphorus content of 3.72 mg. per 100 cc. there is sufficient phosphate present to combine with 0.38 g. Pb as PbHPO_4 . The maximum amount of lead suspension injected is 20 cc., i.e. 0.1 g. Pb.

phosphate PbHPO_4 is formed in Ringer solution and in serum. Using lead acetate in place of the suspension, the secondary phosphate is formed in serum and the tertiary salt in the other two cases. The formation of the secondary phosphate is attributed to delayed equilibrium.

4. The combination of phosphate ions with the particles of the lead suspension is preceded by the rapid oxidation of the metallic lead to lead hydroxide. The disappearance of the phosphate is due to two simultaneous reactions: (a) a rapid combination with the lead hydroxide, (b) a slow combination with the lead carbonate of the suspension. The results obtained with a suspension of lead carbonate alone show that the velocity of the latter reaction is not governed by the rate of diffusion of the phosphate ions to the reaction surface of the particles but by the slow chemical reaction at the surface.

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