CCL. THE REACTIONS OF LEAD COMPOUNDS WITH SERUM AND SERUM-MODELS¹.

By MAURICE JOWETT.

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

(Received November 4th, 1932.)

THE reactions of lead compounds with serum are of interest, since they determine largely the fate of lead entering the body.

The first fairly direct evidence that lead ions react with the phosphate ions of serum was apparently obtained by Aub and his co-workers [1924], who found that haemolysis in hypotonic salt solutions was lessened by addition of soluble lead salts. Lead salts previously mixed with serum had no such effect. At the concentration present in serum, phosphates were the only substances found to have the same power of inhibiting the action of lead as had serum itself.

Scremin [1925] added solid lead iodide, carbonate and sulphate to serum, and found phosphate in the sedimented precipitates.

Brooks [1927] ultrafiltered serum and aqueous solutions after adding small quantities of lead acetate or of a suspension containing metallic lead and lead hydroxide. The lead in the mixtures was not filterable, and some of the phosphate had ceased to be filterable. The observed mean ratios of phosphorus atoms removed from solution to lead atoms added are given in Table I.

Table I. *Data of Brooks.*

	Filtered	P/Pb
Lead acetate added to phosphate + NaCl	At once	0.62
Lead suspension added to phosphate + NaCl	After 7 days	0.62
Lead acetate added to Ringer's solution	At once	0.69
Lead suspension added to Ringer's solution	{ After some days }	1.0
Lead acetate added to cow serum		1.1
Lead suspension added to cow serum		1.1

These results were interpreted by Brooks as indicating formation of PbHPO_4 or $\text{Pb}_3(\text{PO}_4)_2$, which would require ratios of 1.00 or 0.67.

Bischoff and Maxwell [1928] made similar experiments, including however analyses for calcium, and found that calcium also became unfilterable. Figures calculated from their experiments are given in Table II. They show that calcium precipitates with the lead, except in acid solutions.

¹ This investigation was undertaken on behalf of the Liverpool Medical Research Organisation (Director: Prof. W. Blair Bell).

Table II. *Data of Bischoff and Maxwell.*

	Ca/Pb	P/Pb	P/Ca + Pb
Lead acetate added to Ringer's solution	0.28†	0.67	0.52
Lead hydroxide added to Ringer's solution	0.73	0.90	0.52
Lead acetate added to phosphate + CaCl ₂ *	0	0.70, 0.63	—
Lead acetate added to rabbit serum	1.03	1.21	0.60
Lead acetate added to rabbit serum	0.92	1.39	0.72
Lead acetate added to rabbit serum	0.92	1.19	0.59
Lead acetate added to pig serum	0.94	1.37	0.71
Mean of serum experiments	0.95	1.29	0.655

* The final p_{H} was <6.0 . In the other experiments it was >7.0 .

† The low value is due to a shortage of calcium in the solution.

In serum the data, as interpreted by the authors, would indicate that probably $\text{Pb}_3(\text{PO}_4)_2 \cdot \text{Ca}_3(\text{PO}_4)_2$ is formed, since this would require the ratios in the table to be 1.00, 1.33 and 0.67. Bischoff and Maxwell assume, however, that only filterable calcium reacts with phosphate, although they themselves show that non-filterable calcium can disappear by reaction with oxalate. If we assume instead that the partition of calcium between the forms initially present is unchanged by addition of lead, we can derive from their data the ratios 1.33, 1.29 and 0.56, which no longer approximate to the requirements of $\text{Pb}_3(\text{PO}_4)_2 \cdot \text{Ca}_3(\text{PO}_4)_2$.

The exact product of the addition of lead salts to serum is therefore still uncertain, and the present work was undertaken to obtain further information on its nature, and on the rates of the reactions, which are of toxicological interest. It seemed improbable that the ultrafiltration method already used would yield much more of interest, and the author has employed a manometric method, which is less specific but more suited to measuring rates of reaction. The lead electrode has also been of value.

A less direct method of attack follows from the general tendency for the formation in precipitations of the least soluble salt. Measurements of the solubility of lead carbonate will be given before other salts are considered.

The solubility of lead carbonate.

Measurements of the solubility of lead carbonate in aqueous solutions at 37.5° were made by the manometric methods of Warburg [1926]. As the results are not very accurate, they are reported briefly.

2–4 cc. of dilute solutions of lead acetate, containing sometimes a little added HCl or NaHCO_3 , were shaken in manometric vessels in the presence of nitrogen containing about 5 % CO_2 . Lead carbonate precipitates according to the equation

After some hours excess of HCl is added from a separate compartment in the vessel; the lead carbonate redissolves, and the CO_2 previously absorbed is evolved. The increase in pressure tells us the amount of PbCO_3 that had precipitated—some 30–40 % of the total lead—and hence the amount of Pb^{++}

left in solution. We can also calculate the amount of H^+ that has been liberated; this reacts almost completely with acetate ion to give acetic acid. From the ratio of acetic acid to acetate in the system we obtain the p_H , which in turn regulates the concentration of CO_3^{--} . The solubility product of lead carbonate can therefore be calculated from one pressure change, provided that we know the initial composition of the solution, the CO_2 content of the gas mixture, and the various equilibrium constants and activity coefficients concerned. The presence of H^+ and HCO_3^- in the solutions has to be allowed for in the calculations, which are made by successive approximations.

Experiments were also made in the presence of a suspension of lead carbonate. After some hours' shaking $NaHCO_3$ is added instead of HCl , and the Pb^{++} left in solution is estimated by the gas change in the reaction:

Similar values were obtained by this method, with which equilibrium between solid and solution should be attained more rapidly. This indicates that equilibrium is probably attained by both methods.

The calculated solubility products are given in Table III. The solubility product S is defined by the relation

$$-\log_{10} S = pS = pPb^{++} + pCO_3^{--},$$

in which the ionic terms are activity terms¹.

Table III. *Solubility product of $PbCO_3$ at 37.5° .*

Ionic strength	pS
0.020	11.91
0.019	12.00, 11.88, 12.00, 12.00
0.017	12.00
0.012	12.18, 12.23, (12.02)
0.009	12.24, 12.23, (11.64), 12.23, 12.24
0.008	12.22, 12.24
0.005	(12.42)
0.004	12.16, 12.22

From the values at the lower ionic strengths we take a mean value 12.22 for pS , which may be in error by 0.2, since equilibration of the solutions with accurately known gas mixtures was not very exactly attained. No other measurements appear to have been made at 37.5° .

¹ The equilibrium constants assumed are as follows:

$$\begin{aligned} \frac{a_{H^+} \times a_{CH_3COO^-}}{C_{CH_3COOH}} &= 10^{-4.75}, \\ \frac{a_{H^+} \times a_{HCO_3^-}}{C_{CO_2}} &= 10^{-6.33} \text{ [Hastings and Sendroy, 1925].} \\ C_{CO_2} &= \frac{0.56}{22.3} \times \frac{P}{760} \text{ [P = partial pressure of } CO_2 \text{ in mm. Hg],} \\ a_{CO_3^{--}} &= 10^{-16.55} \frac{C_{CO_2}}{a_{H^+}^2} \text{ [Hastings and Sendroy, 1925].} \end{aligned}$$

Activity coefficients for Pb^{++} , CH_3COO^- and HCO_3^- are calculated by the simple Debye-Hückel equation. The equilibrium lead ion concentration is about $\frac{1}{3}$ the ionic strength.

Solubilities of certain lead salts in serum.

We shall now calculate the solubilities in human serum at 37.5° of certain lead salts, the anions of which are present in serum. An approximate value for the solubility product of lead carbonate has just been obtained. For secondary and tertiary lead phosphates, and pyromorphite, we make use of solubility products derived by Jowett and Price [1932] for aqueous solutions at 37.5°.

We take the p_H of serum to be 7.35, its phosphate concentration to be 0.0012 M , and the bicarbonate 0.025 M . The values in Table IV for the solubilities of the lead salts in serum can then be derived¹, in terms of the lead-ion concentrations at equilibrium.

Table IV. *Solubilities of lead salts in serum.*

Salt	Lead-ion concentration	Solubility ratio	P/Pb
PbCO ₃	2.6.10 ⁻⁷ M	1000	0
PbHPO ₄	1.7.10 ⁻⁷ M	660	1.00
Pb ₃ (PO ₄) ₂	1.2.10 ⁻⁸ M	50	0.67
Pb ₅ Cl(PO ₄) ₃	2.6.10 ⁻¹⁰ M	1	0.60

The least soluble salt by far is pyromorphite, Pb₅Cl(PO₄)₃ or PbCl₂. 3Pb₃(PO₄)₂. This is therefore, of these salts, the most likely to be formed when lead salts are added to serum or serum-like solutions, and will be the ultimate reaction product if these salts are the only possible resultants.

In pyromorphite the atomic ratio of phosphorus to lead is 0.60. Brooks found (Table I) a ratio of 0.62 for lead compounds added to a phosphate-chloride mixture, and hence the compound formed was probably pyromorphite, where Brooks believed it to be the tertiary phosphate of lead.

On adding lead chloride to a similar mixture containing also bicarbonate, the present author found a phosphorus-lead ratio of 0.56, by analysis after an hour or two. Probably some lead carbonate was precipitated, the ratio thus being lowered below 0.60.

When Ca⁺⁺ is present, the precipitate contains calcium, and the atomic ratio of calcium to lead is of the order unity. Carbonate may be present in the precipitates, since with Ringer's solution an atomic ratio of 0.52 for P/(Ca + Pb) is found (Table II). The precipitates however probably consist mainly of phosphorus-containing compounds, and we have to decide between compounds of the type (Pb, Ca)₃(PO₄)₂ and of the type (Pb, Ca)₅Cl(PO₄)₃. The following arguments favour the latter type of formula.

(1) The reaction product in solutions containing no calcium is probably mainly pyromorphite. Pyromorphite is much less soluble than Pb₃(PO₄)₂, and

¹ p_{K_2} and p_{K_3} for phosphoric acid are assigned the values 7.165 and 12.18, following Bjerrum and Unmack [1929], and p'_{K_2} for phosphoric acid in serum is assumed to be 6.72 (see later). $\gamma_{HPO_4^-}$ is taken to be 0.235. Hence $a_{HPO_4^-} = 10^{-3.64}$ and $a_{PO_4^{3-}} = 10^{-8.47}$, and the calculated figures may be obtained, taking $\gamma_{Pb^{++}}$ to be 0.11, the value used by Jowett and Price [1932] in 0.16 M NaCl.

the entry of calcium into the structures would probably leave the pyromorphite type of solid still the less soluble.

(2) The $P/(Ca + Pb)$ ratio recalculated by the author from the data of Bischoff and Maxwell in serum, 0.56, and the ratio found by these authors in Ringer's solution, 0.52, both favour a substance of ratio 0.60 rather than 0.67.

(3) Pyromorphite occurs in a number of minerals, lead being partly replaced by various metals, including calcium; $Pb_3(PO_4)_2$ is of very limited occurrence in nature. Lead-calcium compounds of the pyromorphite type are therefore known to exist, while those of the tertiary phosphate type are not.

(4) Chloro-apatite, $Ca_5Cl(PO_4)_3$, has also a wide distribution in nature while $Ca_3(PO_4)_2$ has not. On the other hand it seems likely that the calcium compound uniting with lead might be similar to one of those formed in ossification; chloro-apatite may be present in bones [Bassett, 1917] but the small chloride content of bones shows that it is not largely present.

The manometric method.

In most of the reactions which lead compounds are likely to undergo in serum or serum models acid-base changes occur. In systems buffered by CO_2 and HCO_3^- , these changes may be followed by measurements of the pressure of carbon dioxide.

Pressure changes have been followed by the type of manometers and vessels employed by Warburg [1926]. The vessel attached to the manometer has two compartments. Into the small compartment we measure 0.1–0.4 cc. of the lead-containing solution, into the larger one 1–4 cc. of serum or other liquid. The vessel is swept out with a gas-mixture containing some 5 % CO_2 in nitrogen, and is then shaken in a thermostat at 37.5° until the manometer level is steady. The contents of the two compartments are mixed by tilting the vessel to and fro, and pressure readings are then made at intervals, the vessels being shaken between readings.

The reactions of lead ion with serum-models.

A. In absence of calcium. When lead chloride is added to a neutral solution containing chloride, bicarbonate and phosphate, one of the following precipitations would be expected to occur:

- (1) $Pb^{++} + HPO_4^- \rightarrow PbHPO_4$,
- (2) $Pb^{++} + HCO_3^- \rightarrow PbCO_3 + H^+$,
- (3) $3Pb^{++} + 2HPO_4^- \rightarrow Pb_3(PO_4)_2 + 2H^+$,
- (4) $5Pb^{++} + 3HPO_4^- + Cl^- \rightarrow Pb_5Cl(PO_4)_2 + 3H^+$.

Owing to the buffering of the phosphate and carbonate-bicarbonate systems additional changes take place, but to a first approximation the H^+ released according to these equations would liberate an equivalent amount of carbon dioxide. Reaction (1) would liberate no gas, and the other reactions 1, 0.67 or 0.60 moles of CO_2 per mole of Pb^{++} .

The results obtained manometrically are indicated in Exps. 1-4 of Table V. It is assumed in the calculations that the evolution of carbon dioxide is due to combination of Pb^{++} with $\text{PO}_4^{=}$ according to such equations as (3) or (4), allowance being made for the buffering effects. The ratio of P disappearing

Table V. *Addition of lead chloride to aqueous solutions.*

Exp. No.	Initial concns. (mM/l.) of				Moles $\text{PbCl}_2 \times 10^6$	Vols. of solutions	mm. ³ CO_2	P/Pb
	Ca	Phosphate	NaHCO_3	p_{H}				
1	0	1.5	24.6	7.50	2.4	0.24, 4.0	36.9	0.68
2	0	1.5	26.1	7.31	3.0	0.2, 3.5	44.5	0.59
3	0	0.75	16.4	7.32	3.0	0.3, 4.0	48.0	0.62
4	0	1.5	24.6	7.50	4.0	0.4, 4.0	57.4	0.62
5	1.25	1.46	24.0	7.49	1.2	0.12, 4.0	35.6*	1.26*
6	1.25	1.46	24.0	7.49	1.2	0.12, 4.0	33.8	1.20
7	1.25	1.46	24.0	7.49	2.5	0.25, 4.0	55.5	0.95
8	2.5	1.42	23.4	7.48	2.5	0.25, 4.0	77.6	1.29
9	5.0	1.35	22.2	7.45	2.45	0.245, 4.0	116.0	1.90
10	1.12	1.47	25.7	7.30	3.0	0.20, 3.58	63.9	0.83
11	1.66	1.45	25.4	7.29	3.0	0.20, 3.62	67.2	0.86
12	3.21	1.40	24.6	7.28	3.0	0.20, 3.74	86.6	1.10

NaCl was also present, bringing the ionic strength to 160 mM/l.

* In Exps. 5-9 the values given are those obtained 5 hours after the addition, in Exps. 10-12 1 hour after.

to Pb added is found to be about 0.62, the same value as that of Brooks. Both values may be somewhat in error (the manometric value by perhaps 3-5 %) but they indicate that the product formed is probably mainly pyromorphite. The agreement of the two values rules out much formation of PbCO_3 , the probable formation of which in experiments referred to earlier may be due to conditions less favourable to attainment of equilibrium.

Fig. 1. Addition of lead chloride to Ringer's solutions.

The evolution of gas after addition of lead in terms of the P/Pb ratio is shown by Curve I, Fig. 1 (Exp. 4), and by Curve I, Fig. 2 (Exp. 2). It is complete in about 5 minutes, and takes place at about the same rate as when HCl is added to Ringer's solution. The precipitation must therefore be rapid—a

turbidity is visible immediately—the lag in evolution of gas being due to the limited surface and rate of shaking of the vessels.

B. In presence of calcium. When lead chloride is added to solutions of the Ringer type containing calcium, the reactions are more complicated. The results (Table V, Exps. 5–12) have been calculated as before.

The evolution of gas is rapid for about 5 minutes and continues at a diminishing rate for some hours. Curves II and III of Fig. 2 show the evolution

Fig. 2. Addition of lead chloride to Ringer's solution or serum.

of gas in Exps. 11 and 12, corrected for the lag of evolution on the assumption that the lag is the same as in similarly conducted experiments in absence of calcium, the chemical reaction being taken to be instantaneous in the latter case. The curves represent the extent to which Pb^{++} has reacted with $\text{PO}_4^{\equiv}$ at various times. After a very short time the P/Pb ratio exceeds 0.60, and is higher for the system in which the initial calcium ion concentration is greater. In Fig. 1 are shown gas evolutions, expressed in terms of P/Pb ratios, over greater time intervals: Curves II, III and IV illustrate Exps. 7, 8 and 9. The initial $[\text{Ca}^{++}]$ rises from Exp. 7 to 9, and so does the P/Pb ratio.

In Table VI the data are arranged to show that the initial $\text{Ca}^{++}/\text{Pb}^{++}$ ratio in the solution, before precipitation has begun, determines largely the value of P/Pb that is reached after some time.

Table VI.

Exp. No.	P/Pb	Initial $\text{Ca}^{++}/\text{Pb}^{++}$	Initial Ca^{++}	$0.60 + 0.155$ $\text{Ca}^{++}/\text{Pb}^{++}$
10	0.83	1.3	1.12	0.80
11	0.86	2	1.66	0.91
7	0.95	2	1.25	0.91
12	1.10	4	3.21	1.22
6	1.20	4.2	1.25	1.25
5	1.26	4.2	1.25	1.25
8	1.29	4	2.50	1.22
9	1.90	8.2	5.00	1.88

The findings may be interpreted as follows. As in the absence of calcium, pyromorphite, $\text{Pb}_5\text{Cl}(\text{PO}_4)_3$, precipitates rapidly. Calcium is however able to compete with lead for a place in the molecular structure, and the actual substance produced is $(\text{Pb}, \text{Ca})_5\text{Cl}(\text{PO}_4)_3$. The extent to which calcium can enter the precipitate by competition with lead depends on the Ca/Pb ratio in the solution. This ratio rises as precipitation proceeds, but the initial value serves as a measure of the effect. The ratio of phosphorus disappeared to lead added therefore reaches a value 0.60 or higher very rapidly: the extent to which the value is higher than 0.60 depends on the $\text{Ca}^{++}/\text{Pb}^{++}$ ratio, and is roughly proportional to it, as shown in the last column of Table VI. After the initial rapid precipitation of calcium and lead, the removal of phosphate from the solution continues, but at a much slower rate. Aggregation of the precipitate may make it more difficult for Ca^{++} to react with it: insolubility of the precipitate may tend to prevent replacement of Pb by Ca, and there must be a limit to the extent to which Ca can add on to the precipitate to form a layer containing little or no Pb.

The above view of the phenomena is not completely satisfactory. It does not explain why the reaction continues so much more rapidly in Exp. 9 (Curve IV, Fig. 1) than in the others.

An alternative explanation of the facts can be proposed. Calcium tends to add on to the precipitated $\text{Pb}_5\text{Cl}(\text{PO}_4)_3$ until it is converted into $\text{Pb}_5\text{Cl}(\text{PO}_4)_3 \cdot \text{Ca}_5\text{Cl}(\text{PO}_4)_3$. In support of this, the P/Pb ratio reaches values near 1.20 in four experiments.

The single very high value for P/Pb can be explained by supposing that in Exp. 9, where the $[\text{Ca}^{++}]$ is the highest of the series, precipitation of $\text{Ca}_3(\text{PO}_4)_2$ takes place. All the solutions are supersaturated with respect to $\text{Ca}_3(\text{PO}_4)_2$, that in Exp. 9 being most highly supersaturated. In Exp. 9 some 90 % of the phosphate is removed from solution, and hence shortage of phosphate may be the limiting factor to reaction.

We have hardly sufficient data to decide between the two possibilities raised: (1) that the Ca/Pb ratio in the precipitate can vary continuously, and

(2) that the Ca/Pb ratio tends to rise to unity and stop there, unless calcium phosphate should precipitate as well.

The second explanation appears rather more probable, taking into account also the results obtained with serum.

Calculation of results.

The details of the calculation for Exp. 4 are given below. The terminology of Warburg [1924; 1925] is employed. Pressures are expressed in such a unit that 10,000 mm. equal 1 atmosphere, and concentrations often in mm.³ per cc., where 22,300,000 mm.³ equal 1 mole¹.

An hour after adding 0.4 cc. of 0.01 *M* PbCl₂ to 4.0 cc. of solution, the increase in pressure was 50.2 mm., corresponding to an evolution of 57.4 mm.³ of CO₂ in the system:

$$\Delta h = \Delta p_{\text{CO}_2} = + 50.2. \quad k_{\text{CO}_2}^{\text{Ring.}} = 1.144, \quad x_{\text{CO}_2} = 57.4 \text{ (mm.}^3\text{)}.$$

The initial pressure of CO₂ was 420 mm. (approximately, from the also approximate composition of the gas-mixture), and the final pressure is therefore 470.2: $p_{\text{CO}_2} = 420$, $p'_{\text{CO}_2} = 470.2$.

The initial [HCO₃⁻] (0.0246 *M*) is given by:

$$x_{\text{HCO}_3^-} = 0.0246 \times 1.10^{-3} \times 2.23.10^7 = 550 \text{ (mm.}^3\text{/cc.)}.$$

When both dilution and evolution of CO₂ are taken into account, the final [HCO₃⁻] concentration is 487:

$$x'_{\text{HCO}_3^-} = \frac{550 \times 4 - 57.4}{4.4}.$$

The p_{H} values are now calculated by means of the equation²

$$p_{\text{H}} = 6.13 - \log \frac{0.056 p_{\text{CO}_2}}{x_{\text{HCO}_3^-}},$$

which yields the values 7.499 and 7.397. The p_{H} thus decreases during the reaction by about 0.102.

The ratio of HPO₄⁼ to H₂PO₄⁻ in the solution is altered by the change of p_{H} . The amounts of the ions are decreased by the precipitation, and to calculate a trial result we assume that the moles of phosphate disappearing are 0.60 times the g.-atoms of lead added. The phosphate initially in solution in the vessel is 6.10⁻⁶ moles, and finally (on our assumption) 3.6.10⁻⁶.

We calculate the moles of H₂PO₄⁻ and HPO₄⁼ initially and finally present by means of the relation³

$$p_{\text{H}} = 6.72 + \log \frac{C_{\text{HPO}_4^=}}{C_{\text{H}_2\text{PO}_4^-}},$$

¹ Warburg uses the value 22,400,000, but according to E. J. Warburg [1921] the lower value is more accurate for CO₂.

² The value 6.13 is that used by Hastings, Murray and Sendroy [1926-7].

³ The data of Bjerrum and Unmack [1929] yield the value 6.726 for solutions of ionic strength 0.16, which however do not consist mainly of sodium chloride. Using the p_{K_2} value of these authors, namely 7.165, and taking a rather inaccurate value 0.458 for Δp_{K_2} for isotonic salt solutions from the data of Jowett and Millet [1929], we derive a value 6.707. Sendroy and Hastings [1926-7] use the value 6.66 for serum, but the value 6.72 is preferred here for serum also.

and obtain the values $8.56 \cdot 10^{-7}$ and $6.26 \cdot 10^{-7}$ for H_2PO_4^- , and $51.44 \cdot 10^{-7}$ and $29.74 \cdot 10^{-7}$ moles for HPO_4^- . Hence $2.3 \cdot 10^{-7}$ moles of H_2PO_4^- disappear and $21.7 \cdot 10^{-7}$ of HPO_4^- . Each mole of H_2PO_4^- reacting with lead releases 2 moles of CO_2 , and each mole of HPO_4^- 1 mole of CO_2 .

The CO_2 appearing in the system should therefore be given by

$$= 2.23 \cdot 10^{-7} \times (21.7 \cdot 10^{-7} + 2 \times 2.3 \cdot 10^{-7}) = 58.7.$$

A correction must be applied to this value, since a small amount of CO_3^- is present in the solution, and by the dilution and acidification some of this is transformed to HCO_3^- with absorption of CO_2 .

The concentration of CO_3^- is given by¹

$$x_{\text{CO}_3^-} = \frac{(x_{\text{HCO}_3^-})^2}{227 p_{\text{CO}_2}}.$$

Hence $x_{\text{CO}_3^-}$ is initially 3.17, and finally 2.22; the amount of CO_3^- (and CO_2) disappearing from the system is therefore 2.9 mm.³

Hence the calculated formation of CO_2 in the system is 55.8, while the observed value is 57.4. The observed value for P/Pb is therefore not 0.60, but 0.62.

The other experiments have been calculated similarly; the trial value for P/Pb must be near the observed.

The reaction of lead ion with serum.

When lead chloride is added to cow-serum, the results are very similar to those which follow its addition to Ringer's solution containing calcium. The evolution of gas becomes slow after 5 minutes², but continues for perhaps an hour. The rate of the slow process is of the same order as in Ringer's solution, although the particles formed precipitate in Ringer's solution after a few minutes, and do not in serum. The favouring influence of small particle size in serum may be offset by protein adsorption hindering reaction.

The buffering of serum results in formation of CO_2 from only about a half of the H^+ developed in the system. The calculation of the acid formed (x_a) has been made by the methods of Warburg [1925], using the constants for carbonic acid of Hastings, Sendroy and Van Slyke [1928]. The ratio of H_2PO_4^- to HPO_4^- reacting with lead is determined by the final p_{H} of the system. As before, the data have been interpreted on the assumption that Pb^{++} reacts with $\text{PO}_4^{=}$, and the ratios of P/Pb derived from the gas evolved after 60–90 minutes are shown in Table VII. Three different samples of serum were used, the last one being kept in a refrigerator for 3 days before use.

The observed values of P/Pb may be in error by some 10 %, and the variations shown in Table VII may not be real. The mean observed value is

¹ The value used for the equilibrium constant has been interpolated from the data of Walker, Bray and Johnston [1927]; it is 102 in these authors' units, 227 in ours.

² Shown in Curve IV, Fig. 2, which illustrates Exp. 3a; the rate of evolution is corrected to that due to chemical reaction by taking lag due to physical causes to be the same as when HC is added to serum.

1.19. By direct analysis Brooks found a value 1.1, and Bischoff and Maxwell 1.29. As the manometric value lies between these, we have evidence that the main process actually occurring is disappearance of $\text{PO}_4^{\equiv}$, and the value is in agreement with the view that the product of reaction is the compound $\text{Pb}_5\text{Cl}(\text{PO}_4)_3 \cdot \text{Ca}_5\text{Cl}(\text{PO}_4)_3$.

Table VII. *Addition of lead chloride to cow-serum.*

Exp. No.	Moles PbCl_2 $\times 10^6$	Vol. of solutions	x_s	Final p_{H}	P/Pb
1a	2.5	0.25, 3.0	74.6	7.04	1.01
1b	2.5	0.25, 3.0	79.3	7.04	1.07
2a	2.93	0.195, 3.5	94.4	7.17	1.15
2b	3.0	0.20, 3.5	102.2	7.17	1.21
3a	3.0	0.20, 3.5	115.0	7.19	1.37
3b	3.0	0.20, 3.5	110.9	7.19	1.32

The calcium contents of the sera were not estimated. The phosphate concentration in one case was 0.0023 *M*. Both calcium and phosphate concentrations were probably similar to those in the aqueous solutions in which P/Pb reached values near 1.2, and our experiments do not show evidence of any differences between the reaction in Ringer's solution and serum, except that in serum there are no values which can be attributed to formation of calcium phosphate. This agrees with the fact that injections of lead preparations do not cause cardiac symptoms similar to those produced by injections of colloidal calcium phosphate.

The reactions of other lead compounds.

Experiments were made with the colloidal preparation S_7 , described by Brooks [1927], and with similar preparations containing no calcium. The colloids, which contain mainly metallic lead and lead hydroxide, were treated with oxygen to oxidise the lead, centrifuged slightly, and added (as before) to phosphate-containing solutions in absence or presence of calcium chloride, or to serum.

Some carbon dioxide from the gas mixture was however absorbed by the alkaline colloids before they were mixed with the solutions (although the colloid was covered with liquid paraffin) and as the absorption was variable the manometric data can only be interpreted semi-quantitatively. As far as they go, they show that the products of reaction are the same as when lead chloride reacts.

In the absence of calcium the manometric change is about 90 % complete in 5 minutes: the proportion of lead that has reacted will be less than this, since lead carbonate in reacting brings about less gas exchange than does lead hydroxide, which is likely to react more rapidly. The reaction when calcium is present is also rapid. The rapidity of the reactions must be due to the small size of the particles. It is intelligible that calcium should react well with the particles: the $[\text{Pb}^{++}]$ at the reacting surface has as an upper limit the

solubility of lead hydroxide (about $0.0005 M$), while the $[Ca^{++}]$ in the solution is $0.0025 M$.

Lead carbonate when finely divided—prepared by adding oxidised S_7 to a solution containing bicarbonate—also reacts rapidly with phosphate, the reaction being about half complete in 5 minutes. Brooks found the reaction to be slow when a coarse suspension was used.

The slow completion of the reaction of S_7 with phosphate, which Brooks attributed to slow reaction of lead carbonate with phosphate, is probably due to a slow completion of the reaction of calcium.

Experiments have been made with two further preparations of lead, (1) a colloidal solution of the lead salt of benzenesulphonylglycine¹, and (2) a soluble compound² known as R 232, in solutions of which a very low concentration of Pb^{++} is maintained in equilibrium with a lead compound. These compounds react with phosphate in absence and presence of calcium in a very similar manner to lead chloride, the rates of reaction being similar.

Summing up, we may say that Pb^{++} reacts almost instantaneously with phosphate, and so do fairly soluble lead salts (lead hydroxide and lead benzenesulphonylaminoacetate). The less soluble lead carbonate reacts rapidly if finely dispersed, and the very insoluble lead sulphide reacts very slowly [Brooks, 1928]. The reactivity of lead compounds evidently depends on the rate at which they can form Pb^{++} , solubility and surface both being of importance. No other factors have yet manifested themselves.

Lead ion concentrations during the reactions.

Lead ion concentrations have been measured after addition of lead compounds to solutions of the Ringer type. Measurements were made by the technique of Millet [1929], standardisation in terms of $[Pb^{++}]$ being effected by use of a standard solution³.

1 cc. or less of S_7 or dilute lead chloride is added to some 25 cc. of solution (containing $0.025 M NaHCO_3$, and of p_H 7.55–7.6), and after the solutions are mixed E.M.F. measurements are begun at once. The solutions mixed are indicated in Table VIII, and the Pb^{++} concentrations during the first half-hour are shown in Fig 3.

Precipitation of Pb^{++} progresses so rapidly that before measurements are begun the Pb^{++} concentrations have fallen in every case below $1 \cdot 10^{-7} M$: they continue to fall, but at a decreasing rate.

¹ This preparation, which contained gum acacia and sodium thiosulphate, was made by Prof. I. M. Heilbron and Dr B. Beilensohn in the Department of Organic Chemistry, University of Liverpool.

² Supplied to Prof. W. Blair Bell by Dr W. Collier, of the Koch Institute, Berlin.

³ We assume here for simplicity that all the lead is present as lead ion. Probably association of Pb^{++} to $PbCl^+$ takes place, but its extent will be fairly constant in the solutions concerned. The lead amalgam electrode apparently functions correctly at these low Pb^{++} concentrations, owing to its regulation by a dispersed lead salt, and in the case of S_7 to removal of oxygen by metallic lead.

Table VIII. *Addition of lead compounds to various solutions.*

Curve	Compound added	<i>mM</i>		Lowest lead ion concentrations observed		Compound being formed
		Phosphate	Ca	$pC_{Pb^{++}}$	Time (mins.)	
VI	S_7	0	0	7.32	27	$PbCO_3$
V	$PbCl_2$	1	1	8.59	72	
	$PbCl_2$	1	1	9.00	280	$Pb_5Cl(PO_4)_3$
IV	S_7	1.5	1	8.96	42	
III	S_7	1.5	1	9.46	170	$Pb_5Cl(PO_4)_3$
II	S_7	1.5	0	9.54	66	
I	S_7	1.5	0	9.57	30	

Fig. 3. Lead ion concentrations during the precipitations.

The experimental curves are widely spread out in Fig. 3, indicating that different reactions are occurring, since the $[Pb^{++}]$ must tend towards the solubility of the compound which has been formed.

The experiments were made at 25° , and our solubility data relate to 37.5° . The calculated equilibrium Pb^{++} concentrations (at 37.5°) for our experimental conditions are for $PbCO_3$ $10^{-6.8}$, for $Pb_3(PO_4)_2$ $10^{-8.1}$, and for $Pb_5Cl(PO_4)_3$ $10^{-9.8}$. These figures are fortunately widely different.

When no phosphate is present we should expect formation of $PbCO_3$, and this apparently takes place, for Curve VI lies at a Pb^{++} level of about 10^{-7} .

In no case is $Pb_3(PO_4)_2$ the final product of reaction, unless the temperature coefficient of the solubility of this salt is extremely high. Curves I-V are tending towards values below $10^{-9.5}$, and the reaction product is therefore

either $\text{Pb}_5\text{Cl}(\text{PO}_4)_3$ or a compound of similar solubility. This deduction is made from E.M.F. measurements alone, and is an independent confirmation of the conclusion already reached.

The slope of the curves is greater when calcium is present (III-V) than when it is absent (I, II). It might therefore be inferred from this alone that calcium takes some part in the reaction. With our previous knowledge, we may note in the curves a confirmation of the view already advanced that calcium and lead ions compete for places in the solid phase. So far we have regarded the effects on the calcium; now we see that the lead ion is affected, in that its concentration does not reach a low level as rapidly in the presence of calcium as in its absence.

The data do not tell us whether the lead-calcium compound is more or less soluble in our solutions than pyromorphite, since measurements were not pursued to attainment of equilibrium. On theoretical grounds, if the lead calcium compound is the stable one in presence of calcium, it must also be the less soluble in terms of lead ion, but need not be much less soluble.

SUMMARY.

The reactions of lead compounds, and in particular Pb^{++} , with serum and serum-models have been studied by a manometric method, with the lead electrode and with the help of solubility data. The manometric method is not specific, but is valuable for measuring rates of reaction.

When CO_3^- is the only precipitant ion, lead carbonate is formed. In the presence of phosphate (and chloride) the product is pyromorphite, $\text{Pb}_5\text{Cl}(\text{PO}_4)_3$. When calcium also is present, as in serum, the product approximates to $\text{Pb}_5\text{Cl}(\text{PO}_4)_3 \cdot \text{Ca}_5\text{Cl}(\text{PO}_4)_3$, an equimolecular compound of pyromorphite and chloro-apatite. Lead ion reacts very rapidly, but the union of calcium is less rapid than that of lead and may be incomplete under unfavourable conditions. Solubility and extent of surface determine the rate of reaction of solid lead compounds with phosphate.

An approximate value is obtained for the solubility product of lead carbonate at 37.5° . The solubilities in serum of PbCO_3 , PbHPO_4 , $\text{Pb}_3(\text{PO}_4)_2$ and $\text{Pb}_5\text{Cl}(\text{PO}_4)_3$ are calculated, pyromorphite being the least soluble of the four salts.

REFERENCES.

- Aub, Reznikoff and Smith (1924). *J. Exp. Med.* **40**, 151.
—— and Reznikoff (1924). *J. Exp. Med.* **40**, 189.
Bassett (1917). *J. Chem. Soc.* **111**, 620.
Bischoff and Maxwell (1928). *J. Biol. Chem.* **79**, 5.
Bjerrum and Unmack (1929). *Det. Kgl. Danske Videnskabernes. Selskab. Mathematisk-fysiske Meddelester.* **9**, 1.
Brooks (1927). *Biochem. J.* **21**, 766.
—— (1928). *J. Phys. Chem.* **32**, 1717.
Hastings, Murray and Sendroy (1926-7). *J. Biol. Chem.* **71**, 723.
—— and Sendroy (1925). *J. Biol. Chem.* **65**, 445.
—— ——— and Van Slyke (1928). *J. Biol. Chem.* **79**, 183.
Jowett and Millet (1929). *J. Amer. Chem. Soc.* **51**, 1004.
—— and Price (1932). *Trans. Faraday Soc.* **28**, 668.
Millet (1929). *Trans. Faraday Soc.* **25**, 147.
Scremin (1925). *Arch. exp. Path. Pharm.* **105**, 49.
Sendroy and Hastings (1926-7). *J. Biol. Chem.* **71**, 783.
Walker, Bray and Johnston (1927). *J. Amer. Chem. Soc.* **49**, 1235.
Warburg (1924). *Biochem. Z.* **152**, 51.
—— (1925). *Biochem. Z.* **164**, 481.
—— (1926). *Über den Stoffwechsel der Tumoren*, Berlin, 1-7, 159.
Warburg, E. J. (1921). *Biochem. J.* **15**, 1.