7. THE PLASMA-CELL PARTITION OF BLOOD LEAD

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THE distribution of lead between the cells and plasma of blood has been the subject of various reports. According to Aub *et al.* [1926], in experimental lead poisoning 80 % of the lead is in the plasma; Blumberg & Scott [1935] find 90 % in the cells in lead poisoning in man and at least half and usually more in the cells in normal blood; Teisinger [1936] finds that the lead is divided between the cells and plasma in a variable ratio, while Willoughby & Wilkins [1938] could detect no lead in the serum of 90 % of samples of normal blood.

In view of these different findings a further investigation of the plasma-cell partition was undertaken and the results are reported here.

EXPERIMENTAL

For the determination of lead in fresh human blood approximately 40 ml. blood were withdrawn from a vein and mixed with a minimal amount of potassium oxalate Analar; one portion was then centrifuged and the plasma separated. Lead was estimated in the whole blood and plasma by the method described previously [Tompsett & Anderson, 1935; 1939; Tompsett, 1939]. In two cases a determination of cell volume was made by means of the haematocrit and the actual lead concentration in the cells calculated. The results obtained with blood from one normal laboratory worker, two hospital patients with a history of possible exposure to lead but with no obvious poisoning, and three cases of lead poisoning, are given in Table 1. It will be seen that the ratio of whole blood lead to plasma lead is fairly constant, irrespective of the amount of lead present. The concentration in whole fresh blood is approximately three times that in the plasma. Case No. 6 is exceptional in having a ratio of only 1.6 : 1.

A supply of normal stored blood was available in bottles discarded from a blood bank at the end of the period of storage. It was possible to use large volumes of this blood, and 100 ml. of whole blood and 100 ml. of plasma were taken from each bottle for analysis. A correction was made for the dilution due to the sodium citrate and saline anticoagulant solution in the bottles and for the small amount of lead in this solution. The results of the analyses of eight samples, which are given in Table 1, are thus calculated for the original blood, and show a distribution of lead between the cells and plasma similar to that found for fresh blood, but with a slightly higher ratio of lead in whole blood.

Two young male rabbits were given lead by mouth in the form of lead acetate mixed with a diet of crushed oats and bran. They received approximately 30 mg. of lead per kg. per day, the one for 8 and the other for 24 days. Blood films were examined for punctate basophilia at intervals, and at the end of the periods of lead administration the animals were anaesthetized with ether, bled by heart

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Table 1. *Lead concentration in $\mu\text{g. per 100 ml. whole blood, plasma or cells}$*

Disease	Whole blood	Plasma	Cells	Whole blood	
				Plasma	
1. Normal	69	28	—		2.5
2. Lead poisoning	136	46	—		2.9
3. Lead poisoning	366	118	—		3.1
4. Neuritis	40	15	—		2.7
5. Lead poisoning	128	45	258		2.8
6. Undiagnosed	46	28	73		1.6

Stored normal human blood

	Whole blood	Plasma	Whole blood
			Plasma
	76	27	2.8
	24	7	3.4
	43	12	3.6
	31	9	3.4
	48	14	3.4
	20	6	3.3
	20	5	4.0
	58	18	3.2

puncture and killed. The blood was allowed to clot and lead was estimated in the separated serum and clot. Two other young males were each given a single dose of 0.5 g. lead acetate in solution by stomach tube, and blood was taken as before, in the one 20 hr. and in the other 48 hr. after lead administration, and lead was again estimated in the serum and clot. Blood lead was also estimated in a control litter-mate and in two stock female rabbits. The concentration of lead in the whole blood was found by adding the total amount in the serum to the total in the clot and correcting for volume. The results are shown in Table 2. The distribution in normal rabbit blood was of the same order as in blood from human subjects. Rabbits nos. 2 and 3, receiving lead in the food, had a raised blood lead, no. 2, which received lead for 8 days, showing a slight relative increase in the serum lead, while in no. 3, which received lead for 24 days, the serum lead concentration was the same as that of the whole blood. Administration of lead in one large dose by stomach tube caused a greater rise in whole blood lead; in no. 4, 20 hr. after administration of lead, the concentration in the serum was nearly equal to that in the whole blood, while in no. 5 the serum lead concentration was 50 % greater than that of the whole blood.

Table 2. *Rabbit blood: lead concentration in $\mu\text{g. per 100 ml. whole blood or serum}$*

	Whole blood	Serum	Whole blood
			Serum
1. Control	37	17	2.2
2. 30 mg. lead per kg. for 8 days	79	41	1.9
3. 30 mg. lead per kg. for 24 days	85	86	1.0
4. 0.5 g. lead acetate by stomach tube, bled 20 hr. afterwards	102	96	1.0
5. 0.5 g. lead acetate by stomach tube, bled 48 hr. afterwards	112	153	0.7
6. Normal female	11	4	2.75
7. Normal female	53	21	2.5

The blood films of these animals all showed a great increase in polychromatic cells after lead administration, but no. 2 was the only one which had a definite punctate basophilia.

In view of the possibility that lead might be present in the cells as a soluble soap, e.g. lead oleate, a sample of 20 ml. of fresh normal human blood was extracted with alcohol-ether mixture, as in Bloor's method for the extraction of the total lipoids of blood, and then lead was estimated in the extract and residue. No appreciable amount of lead was found in the extract, while the residue contained a normal amount.

DISCUSSION

The accuracy of the analytical method employed is of paramount importance in investigations of this nature, and faults in the methods used may account for some of the widely different distributions of lead in the blood which have been reported. We have criticized some of these methods in a previous paper, in which we first described the method at present in use [Tompsett & Anderson, 1935]. This method consists essentially in the extraction and separation of the lead as an ether-soluble complex with sodium diethyldithiocarbamate and its subsequent estimation colorimetrically with dithizone. Kench [1940] has used this method with slight modifications for the estimation of lead in urine and obtained accurate results. Unfortunately he is wrong in stating: 'Tompsett & Anderson [1935; 1939] have extracted lead from biological materials as a complex either with sodium diethyldithiocarbamate or with dithizone, both substances being stable in the presence of ammoniacal cyanide.' One of the objects of our first paper was to advocate the use of sodium diethyldithiocarbamate instead of dithizone for the extraction of lead. Speaking of the dithizone method we said, 'we have found that in practice, when used to separate lead from solutions of the ash of urine, liver etc., the method gave erratic results'.

The relatively constant ratio between the concentrations of lead in human whole blood and in plasma, which is the same in normal bloods as in those from cases of lead poisoning, suggests that an equilibrium is reached in the distribution between cells and plasma. By contrast, when in the rabbits active absorption of lead was taking place this equilibrium was not reached and lead was present in greater concentration in the plasma. Thus it is possible to reconcile Aub's finding of 80 % of lead in plasma in experimental lead poisoning with the later reports that most of the lead is in the cells in clinical lead poisoning.

The form in which lead is present in the blood is undecided. Most of the investigations of the question have been made on serum or blood to which lead salts have been added *in vitro*, or on the blood after intravenous injection of lead salts. These experiments can all be criticized on the grounds that they are unphysiological. The findings reported here do not provide any data as to the nature of blood lead, but exclude the possibility of an ether-soluble form.

SUMMARY

1. The concentration of lead in human whole blood was approximately three times that in the plasma of normal subjects and of patients with lead poisoning.
2. In stored blood the ratio of lead concentration in whole blood to that in plasma was slightly higher than in fresh blood.
3. In rabbits absorbing lead by mouth the concentration of lead in the plasma was equal to or greater than that in whole blood.
4. The lead in normal human blood was not extracted by ether.

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