43. Haemopoiesis in Lead Poisoning

By **J. E. Kench,* A. E. Gillam** and **R. E. Lane**, *From the University of Manchester*

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The marked changes in blood resulting from lead poisoning were ascribed by Aub *et al.* [1924] to increased destruction of the red blood corpuscles. From *in vitro* experiments these workers concluded that the cells became more fragile when exposed to traces of lead and consequently were less able to withstand the trauma to which they were exposed during circulation. This view receives support from Key [1924] and Brookfield [1928], and from the interesting observations of Clowes [1916] and of Bhatnagar [1921] on the effect of electrolytes on lipoid-water emulsions. Small ionic concentrations of heavy metals produced remarkable changes in the stability of such emulsions, which Clowes has discussed in detail from the viewpoint of the cell wall and its permeability.

In the last few years an additional approach has been possible through our increased knowledge of the porphyrin pigments and their relation to haemoglobin. Differences between lead anaemia and acute haemolytic anaemia have thus been brought to light, and Rimington [1938] has suggested that lead inhibits haemoglobin synthesis by preventing the incorporation of iron into the protoporphyrin nucleus. The pigment so set free appears in the urine as coproporphyrin III. This latter pigment may be found in the urine in lead poisoning when there is no evidence of increased blood destruction (i.e. the faecal urobilinogen is not increased).

Increase of blood protoporphyrin in lead poisoning has been reported by Vigliani & Waldenström [1937], and of non-haemoglobin iron by Vannotti & Imholz [1939], which induced these authors to conclude that the blocking theory had been substantiated. It is readily apparent, however, that the increase of non-haemoglobin iron observed by these workers (approximately 1 mg./100 ml.) is inadequate to account for the 25 % reduction in haemoglobin which occurred, for which the iron supply to the bone marrow would be 13 mg./100 ml. Similarly, the quantities of free protoporphyrin and coproporphyrin III reported as produced by lead intoxication (of the order 0·0003 g.) are extremely small compared with the normal daily production of protoporphyrin (0·5 g.) necessary to replace the haemoglobin changed into and excreted as bile pigments.

The investigations described in this paper were undertaken to obtain more information on the changes in haemopoiesis caused by lead, especial attention being paid to the blood and urinary porphyrins, and their relation to cell production.

Experimental

Specimens of urine were collected from lead workers over a period of 3 weeks. 100 ml. aliquots were taken for determination of lead by a modified dithizone method [Kench, 1940], and the ether-soluble porphyrins in the remainder of each specimen were separated during 12 hr. continuous extraction by the ether-acetic acid method [Rimington & Hemmings, 1939].

Venous blood samples were also taken and protoporphyrin extracted from separated cells and plasma by the method of Van den Bergh & Hyman [1928]. The relative volumes of cells and plasma were found by the haematocrit, whilst haemoglobin values (taking as the normal or 100 %, 14·5 g. haemoglobin/100 ml.) were obtained photometrically after conversion into globin haemochromogen (checked against iron and Haldane gasometric methods). Blood lead values were determined on 5–10 ml. of each specimen by drying

* Sir Halley Stewart Research Fellow.

and charring on the hot plate, and digesting with the minimum volume of lead-free H_2SO_4 (usually 10–20 ml.) required to produce a colourless digest. Lead was separated as its complex with sodium diethyl dithiocarbamate in ether, and finally determined as dithizonate in chloroform, using a Hilger Spekker micro-absorptiometer and a green filter (transparent 520–540 mμ).

Blood films were stained by alkaline methylene blue, and red cells showing punctate basophilia were enumerated by Seller's technique, using transmitted light, as described by Lane [1931].

For the final determination of porphyrins a Hilger-Nutting spectrophotometer was employed, the absorption measurements on the acidified extracts being carried out at 549 mμ for coproporphyrin and 558 mμ for protoporphyrin, at which wave-lengths these pigments exhibit intense and highly selective absorption bands.

The relative values were calculated to absolute concentrations of porphyrin from absorption data first obtained on standard solutions of the pigments. Coproporphyrin I standard in 0·5 % HCl was kindly supplied by Dr C. Rimington; it contained 10 μg./ml. and showed an intensity of absorption in a 4 cm. cell $E = \log I_0/I = 1{\cdot}047$, giving a standard value of $E^{1\%}_{1\,\mathrm{cm.}}$ (549 mμ) = 262.

Protoporphyrin was prepared from pyridine haemochromogen by reduction with hydrazine hydrate and treatment with conc. HCl, according to the method of Fischer & Pützer [1926]. The crude pigment was converted into the dimethyl ester by dissolving in methyl alcohol and saturating the solution at 0° with dry hydrogen chloride. After standing for 24 hr. at 0–4°, the methyl ester was extracted with chloroform and purified by fractional crystallization from dry benzene, the purity of each fraction being tested with the Hilger-Nutting visual spectrophotometer. The purest crystals dissolved in 20 % HCl had $E^{1\%}_{1\,\mathrm{cm.}}$ (558 mμ) = 240, and this value was used as standard.

After the quantitative analyses were completed the urinary coproporphyrin concentrates were pooled for the preparation of the corresponding methyl ester. This proved to have the crystalline appearance, solubility and double melting point (149°, remelt 172°) characteristic of the series III isomer.

Results and discussion

The first investigation was made on groups of lead workers thought likely to provide information on the relationship between haemoglobin concentration and urinary coproporphyrin excretion. Although it was found impracticable to examine faecal coproporphyrin, the findings of Watson [1937] show that coproporphyrin III excretion is confined almost entirely to the urine. We have assumed therefore that the urinary coproporphyrin values represent practically the total loss of series III porphyrin.

The data are presented in Table 1. The first group includes men who at the time of examination were exposed to moderately heavy lead absorption, e.g. the first subject had developed anaemia to the extent of 13 % in 6 months. The punctate count was the lowest and coproporphyrin excretion the highest of the group. On the other hand, the second subject, with a high punctate count and low urinary coproporphyrin level, showed no change in circulating haemoglobin. Subject no. 12 had long-established anaemia with a very low rate of regeneration, and it has been found impossible to raise the haemoglobin level by iron therapy.

If anaemia were due entirely to prevention of iron-porphyrin formation in accordance with Rimington's hypothesis we should expect approximately one-quarter of the protoporphyrin produced, viz. 0·12 g. to be excreted. Actually, we find only 0·00024 g., and in all cases coproporphyrin excretion represents but an extremely small and variable fraction of the total pigment formed, and its magnitude is independent both of the number of stippled red cells and the degree of anaemia. Moreover, this is true also of the free protoporphyrin found in the blood during active absorption of lead.

Table 1. *Haemoglobin values, punctate counts and urinary coproporphyrin excretion in lead workers*

Lead worker	Duration of exposure yr.	Av. Hb % (Haldane)	Hb changes %	Av. punctate count per 10^6 R.B.C.	Urinary lead μg./l.	Av. urinary coproporphyrin III μg./1500 ml.
1	½	84	−13	4000	295	515
2		91	0	7000	182	25
3		93	− 6	6500	324	164
4		91	−10	5500	354	86
5	10	100	None	1000	303	51
6		95		2000	234	181
7		86		3000	262	62
8		85		1500	197	24
9		94		5000	296	111
10		96		3000	340	60
11		91		1000	287	82
12	20	76	None	Free	436	238

Table 2. *Blood protoporphyrin during oral administration of lead acetate*

Duration of exp. days	Lead μg./100 ml.		Protoporphyrin μg./100 ml.		Punctate basophilia per 10^6 R.B.C.	Urinary coproporphyrin μg./day
	Cells	Plasma	Cells	Plasma		
1	50	47	56	52	1000	22
8	84	56	40	139	2500	134
17	32	159	56	54	2000	268
23	50	128	56	140	4000	—
28	207	157	56	130	2000	232
33	34	178	50	170	3000	—
38	86	75	65	68	1500	—

Table 3. *Lead and protoporphyrin in the blood of lead workers*

Case	Duration of lead exposure yr.	Blood lead μg./100 ml.		Protoporphyrin μg./100 ml.		Urinary coproporphyrin III μg./l.	Hb % (Haldane)	Punctate count per 10^6 R.B.C.
				Cells	Plasma			
13	½	204		271		646	70	5,000 S, M, L
14		70		75		413	80	5,000 S, M
15		67		122		426	89	Free
16	4	74		428		81	100	1,500 S, M
17	16	70		48		356	85	2,500 S, M
18	17	58		95		60	97	1,000 S
19	21	76		103		308	101	Free
20	28	188		216		526	92	1,500 S, M
21	26	130		232		245	86	4,000 S, M, L
				Cells	Plasma			
22	2	268		33	44	—	105	2,000
23	2	305		24	34	—	81	15,500 S, M, L
		Cells	Plasma					
24	2	31	22	34	30	—	77	15,000 S, M, L
25	17	57	23	65	38	—	97	12,000 S, M, L
26	10	98	29	90	14	—	86	Free

S, M, and L denote small, medium and large granules respectively.

Blood porphyrin changes in J. E. K. were observed during oral administration of 20 mg. of lead acetate daily (Table 2) and can be summarized briefly as follows:

(1) The concentration of protoporphyrin was generally greater in the plasma than in the cells.

(2) The basophilic substance of reticulocytes has been identified by Watson & Clarke [1937] as protoporphyrin; but intracellular protoporphyrin in this and other experiments was independent of the number of reticulocytes and of erythrocytes showing stippling.

(3) No change in haemoglobin concentration in the blood could be detected throughout, but urinary coproporphyrin III excretion reached a daily average value of 268 μg.

Finally, we have examined the possible relationship between the concentration of lead in the blood and the protoporphyrin content. The results, which appear in Table 3, indicate that (1) the number of stippled red cells gives no indication of the protoporphyrin content of the whole blood; (2) the concentration of the porphyrin in blood is greater than that of the coproporphyrin in the urine, but there is no constant numerical relationship between the pigment levels; (3) the ratio free/combined protoporphyrin in the blood is extremely small in all cases.

Consideration of these results leads to the following conclusions: (I) The physiological action of small amounts of lead cannot be explained in terms of partial prevention of the iron-porphyrin complex formation. (II) The occurrence of protoporphyrin and non-haemoglobin iron to the extent observed in these and other experiments appears to be only incidental to generally restricted cellular activity. Erythrocytes are hypochromic and reduced in numbers according to the degree of inhibition, in contrast to the findings in uncomplicated haemolysis in which no cellular poisons are present.

In the light of these results it seems to us that the following explanation is a more acceptable one. The immediate action of lead after its passage into the blood stream appears to be adsorption on the lipoid-protein film which constitutes the red cell membrane. Ørskov [1935] observed rapid shrinkage of cells exposed to lead and rapid loss of potassium into the surrounding medium. At *p*H 6·3–6·6 and bicarbonate ion 0·007 *N* a concentration of lead of 1 in 25,000,000 caused a marked increase in the permeability of red cells to potassium. These changes could only be observed in those mammalian species in which anaemia and stippling are predominant signs of lead poisoning and are of fundamental importance in the injurious action produced by lead.

In the early stages of lead exposure the destruction of cells in the peripheral blood leads to augmented formation of breakdown products which serve to stimulate the bone marrow. This activity, however, may, after continued lead absorption, be considerably depressed by the presence of a comparatively high concentration of lead in the bone marrow, and sternal puncture as carried out by one of us (R. E. L.) has confirmed this. Vannotti & Siegrist [1940] have shown recently that in bone marrow cultures taken from lead-intoxicated animals, haemoglobin production was inhibited, but returned to normal after addition of lactoflavin. The findings of Holmes *et al.* [1939] suggest that diminution of haemopoiesis in many cases may be explained by a deficiency of available ascorbic acid. During chronic lead poisoning an increased intake of ascorbic acid was required in order to overcome the inhibiting effect of traces of lead on intracellular reactions. This may be due to complex ion formation, investigated recently by Kety [1942] by potentiometric methods. Citrate, lactate, acetate and ascorbate ions all combine with lead to form soluble complexes whose dissociation was measured. The citrate complex is the least dissociated, and administration of citrate has been recommended, therefore, to increase mobilization and excretion of lead without increasing the concentration of toxic lead ions.

Summary

A study has been made of blood and urinary porphyrins in lead workers in order to ascertain how lead affects haemopoiesis. Our conclusions are:

I. The physiological action of small amounts of lead cannot be explained in terms of partial prevention of iron-porphyrin complex formation.

II. The occurrence of protoporphyrin and non-haemoglobin iron to the extent observed in these and other experiments appears to be incidental to restricted cellular activity.

III. Diminution of haemoglobin in lead poisoning is a consequence, not of non-utilization of protoporphyrin, but of restricted formation of this vital pigment.

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