of circulating red cells and plasma in dog 15 is evident in the result of —26% below the expected methemoglobin figure, while dog 13 shows plasma trapping in the result of +63% above the expected methemoglobin value. Thus, it is incorrect to accept the occurrence of hemodilution or hemoconcentration from determination of the hematocrit of blood in the great vessels. On the contrary, the results of these experiments indicated that different concentration of red cells can occur in the vessels; hemoconcentration in the great vessels with hemodilution in some of the capillaries and vice versa. Thus to explain the apparent hemoconcentration observed in the great vessels it is not necessary to assume extravasation of plasma or water nor to explain hemodilution is it necessary to assume loss of red cells from the circulatory system. To explain hemodilution in the great vessels by trapping, presupposes the stagnation of hemoconcentrated blood in the capillaries of some regions of the body and vice versa for hemoconcentration.

Summary. Total red blood cell volume (using red cells tagged with methemoglobin as a tracer) and plasma volume (using Evans blue dye T-1824) of dogs in different types of shock have been determined. Decreases in total red cell and plasma volumes have been observed in anti-platelet serum shock. The decrease in the red cell volume was greater than of the plasma volume, while in histamine shock the reverse was observed. Assumption has been made that trapping of red cells explains the apparent hemodilution in the great blood vessels in anti-platelet serum shock. The degree of trapping of the red cells, was determined by the proportion of circulating methemoglobin before and after shock. Analysis of the results shows the greater importance of the consideration of absolute values of red cell and plasma volumes rather than the acceptance of results indicated by the hematocrit of the blood from the great vessels, for the interpretation of the pathogenesis of shock.

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Experimental Porphyria. I. Isolation of Uroporphyrin I from Bone Marrow of Lead Poisoned Rabbits.* (19412)

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(Introduced by C. J. Watson.)

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It is generally believed that lead interferes with hemoglobin synthesis. Associated abnormalities in porphyrin metabolism, namely, the presence of increased erythrocyte protoporphyrin and of urinary coproporphyrin III have been repeatedly documented. More recently, studies in this laboratory have revealed the occurrence of marked increases in the concentration of coproporphyrin III in the bone marrow of lead-poisoned rabbits(1). In the course of these studies, large amounts of an ether-insoluble, uro-type porphyrin were found in the marrow of the same animals. The present report is concerned with the iso-

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lation and identification of this latter porphyrin.

Experimental. The effect of lead on bone marrow and blood porphyrin concentrations has been studied in 23 rabbits. Five of these (No. 60, 93, 94, 95, and 96) were given single subcutaneous injections of 100 mg of lead acetate (63.7 mg of lead) per kg of body weight, and were killed for tissue porphyrin analyses 25 to 44 days later. Rabbits No. 62, 64, 65, 77, and 88 were injected subcutaneously with 100 mg of lead acetate per kg 25 days prior to sacrifice and examination. For this period of 25 days, each rabbit received daily intravenous injections of an aqueous solution of 5 mg of crystalline riboflavin phosphate.‡ Rabbits No. 62 and 88 were also given daily intravenous injections of 500 mg of ascorbic acid. Two animals (No. 91 and 99) were repeatedly given small amounts of lead acetate intravenously over periods of several weeks prior to the examination. The total of the injected lead acetate amounted to 85 and 100 mg, respectively. Eleven rabbits were given repeated subcutaneous injections of phenylhydrazine (20-40 mg per injection) over periods of 6 to 45 days. Total dosage ranged from 110-400 mg. On the day of the last phenylhydrazine administration they were injected intravenously with 20 mg of lead acetate per kg of body weight. Four animals (No. 105, 106, 210, and 214) were sacrificed 3 days later for blood and bone marrow porphyrin studies. Seven (No. 212, 213, 216, 219, 220, 221, and 222) were sacrificed for study after 7 to 9 days. After bleeding the animals the bone marrow of the long bones was recovered and the copro- and protoporphyrin extracted according to the method previously described (1). The ethyl acetate and glacial acetic acid extract was washed 3 times with 3% sodium acetate. In all instances the sodium acetate solution contained an ether insoluble uro-type porphyrin which was also insoluble in ethyl acetate and butyl alcohol at a pH of approximately 6. At a pH of approximately 4 (gray to congo paper), however, the uroporphyrin was readily extracted from the aqueous solution by either of the latter two solvents. This difference in solubility was taken advantage of in further purification. The 3% sodium acetate extract containing the uro-type porphyrin was acidified with acetic and hydrochloric acids to a pH of approximately 4, and the porphyrin was extracted by ethyl acetate. For fluorimetry, the porphyrin was reextracted with 1.5 normal hydrochloric acid.

‡ Provided through the courtesy of Hoffmann-LaRoche Co.

Results. The important data are summarized in Tables I and II in terms of porphyrin concentrations in μg per 100 ml of packed cells. No correction factor has been applied for the non-erythropoietic cells contained in the marrow. In several of the animals the liver and kidneys were also examined for porphyrins. Since the amounts of uro- and coproporphyrin found in these organs were insignificant compared with the values for the bone marrow, they are not included in these tables. Fluoromicroscopic examination of liver sections revealed essentially no red fluorescence, whereas the immature erythropoietic cells of the bone marrow and the peripheral blood showed the typical bright red fluorescence of porphyrin(2-5). As seen in Tables I and II, marked elevation of uro-type porphyrins were observed in the bone marrow of all lead intoxicated rabbits. In the circulating red cells, however, this porphyrin was found in significant amounts only in those rabbits pretreated with phenylhydrazine and killed shortly after administration of the lead. The administration of riboflavin with or without ascorbic acid had no significant effect on the results obtained. The values obtained for erythrocyte and bone marrow coproporphyrin and protoporphyrin are essentially similar to those previously reported for lead poisoned rabbits(1). It is seen that elevation of erythrocyte coproporphyrin occurred only in those rabbits with acute lead poisoning.

As seen in Table I, the repeated administration of lead acetate intravenously resulted in porphyrin concentrations similar to those observed in the rabbits given only a single subcutaneous injection of lead acetate.

Isolation of uroporphyrin I from the bone marrow. The bone marrow uroporphyrin has

TABLE I. Erythrocyte and Bone Marrow Porphyrins in Rabbits with Chronic Lead Poisoning.

Rabbit No.	Erythrocytes Uro-	Erythrocytes Copro-porphyrin	Erythrocytes Proto-	Bone marrow Uro-	Bone marrow Copro-porphyrin	Bone marrow Proto-	Remarks
			In μg per 100 ml cells				
60	0	5.9	233	437	522	125	Subcutaneous lead acetate
94	0	.7	137	15	194	143	
93	0	1.2	157	28	246	105	
96	0	13.8	348	147	490	337	
95	0	2	165	69	121	100	
64	0	4.8	269	678	781	325	Riboflavin
65	0	3.4	168	423	334	105	
77	0	4.8	235	160	319	135	
88	0	34.6	237	375	392	128	Riboflavin and ascorbic acid
62	0	8.1	227	435	510	143	
91	0	2	404	75	248	176	Intrav. lead acetate
99	0	4.4	525	74	298	164	
*	0	.5 (.4-.6)	83.3 (58-106)	?tr	4.5 (1.7-8.6)	87.5 (54-137)	Normal control

* Mean values for 3 normal control rabbits (No. 44, 51, 57).

TABLE II. Erythrocyte and Bone Marrow Porphyrins in Rabbits with Acute Lead and Phenylhydrazine Poisoning.

Rabbit No.	Erythrocytes Uro-	Erythrocytes Copro-porphyrin	Erythrocytes Proto-	Bone marrow Uro-	Bone marrow Copro-porphyrin	Bone marrow Proto-	No. days animals killed after lead acetate inj.
			In μg per 100 ml cells				
105	44	274	1410	118	971	371	3
106	31	488	942	141	1410	299	
210	149	587	1135	117	1056	252	
214	292	594	450	844	1018	339	
212	6.4	26.2	785	376	1782	328	7-9
213	1.7	9.8	527	55	440	247	
216	0	1.4	376	79	396	355	
219	3.4	3.4	198	443	903	960	
220	3.9	4.1	411	482	1303	409	
221	1.5	4.4	360	139	594	138	
222	70.3	321	1153	114	416	521	
*	0	60.5±41.5	137±19	tr—4.1	68.5±25.5	110±44	Phenylhydrazine control

* Mean values for 2 control rabbits with phenylhydrazine anemia alone (No. 145 and 207).

been crystallized on two occasions. All available bone marrow from 3 rabbits with lead intoxication (No. 39, 71, and 74) was pooled and the uroporphyrin extracted and purified as previously described. It was esterified by mixing the final concentrate with approximately 20 volumes of a solution of methyl alcohol-concentrated sulfuric acid (19:1). The methyl ester was taken into chloroform and further purified by calcium carbonate chromatography as previously described(6). It was then crystallized from chloroform: methyl alcohol. The crystals had the characteristic hair-like form of uroporphyrin I methyl ester. The melting point was 278 to 282°C (corrected) with formation of droplets at the latter temperature. The Soret band absorption maximum in chloroform solution (4060 Å) was identical with that of purified uroporphyrin I methyl ester isolated from urine of a patient with congenital or erythropoietic porphyria. From this it is evident that at least as far as the formation of this porphyrin is concerned, the present findings deserve the designation of "experimental porphyria."

In a second experiment 8 rabbits with experimental lead intoxication were given 90 mg

of phenylhydrazine in 3 divided doses over a period of 8 days prior to the examination. A pool of 55 g of bone marrow was obtained. Isolation and estimation of the different porphyrins yielded the following values per 100 g of marrow: Uroporphyrin, 458 μg; coproporphyrin, 815 μg, and protoporphyrin, 300 μg.

Uroporphyrin I was again crystallized as the methyl ester. The melting point was 283 to 286°C (corrected). The absorption maximum in chloroform was again at 4,060 Å, and the fluorescence spectrum maximum as determined in a Steinheil analytical microspectroscope was identical with that of uroporphyrin I methyl ester.

The type III isomer was found to constitute 96% of the total coproporphyrin§ isolated from the bone marrow of these rabbits. It was crystallized in form of its methyl ester from chloroform:methyl alcohol. The characteristic rosette-form crystals were easily soluble in cold ether.

Discussion. The presence of traces of uroporphyrin has been reported in human urine (7,8) and tissues in post fetal life(9). The occurrence of larger amounts has generally been considered pathognomonic of porphyria. The normal occurrence in certain species such as the fox squirrel (*Sciurus niger*)(10), the turacos(11,12), and molluscs(13) has been well documented. The reported occurrence of traces of uro-type porphyrins in normal human urine and in a variety of experimental conditions will be considered in a separate communication.

It is believed that this is the first report of the isolation of crystalline uroporphyrin under experimental conditions. The crystallization of the type I isomer of uroporphyrin from the bone marrow of lead-poisoned rabbits is especially surprising, since the coproporphyrin isolated from the marrow and the urine of these rabbits is nearly all the type III isomer. The exact mechanisms responsible for this accumulation of two different isomer types are unknown. Further studies relating the above findings to human porphyria are described in the companion paper.

Summary and conclusions. 1. Crystalline uroporphyrin I has been isolated from the bone marrow of lead poisoned rabbits and found to be identical with uroporphyrin I isolated from the urine of a patient with photosensitive (erythropoietic) porphyria. 2. The bone marrow of rabbits with acute or chronic lead poisoning has been found to contain from 15 to 844 μg of uroporphyrin I per 100 ml. 3. In experimental lead poisoning, significant amounts of uroporphyrin are found in the circulating red blood cells only during the very acute stage. Erythrocyte coproporphyrin is likewise increased markedly only during the acute phase.

§ Determined by the fluorescence-quenching method (Schwartz, S. and co-workers, *J. Biol Chem.*, 1947, v168, 133).

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