tinin content and are excellent sources of protective antigen. These observations strongly suggest that the hemagglutinating and protective activities of the extracts reside in distinct chemical structures. Attempts are now being made to elute the protective antigen from stromata. It is hoped that such experiments may help to clarify this problem.

The stromata-antigen complex is a highly potent protective agent as judged by the Kendrick test(2); it is free of pertussal toxin and represents only a very minute portion of the supersonic extracts. The complex may therefore be an improved prophylactic agent against whooping cough. Since group O-Rh negative red cell stromata may be used for the adsorption of the protective antigen, this carrier should offer no clinical disadvantage, but may serve as an important stabilizing adjuvant. The clinical efficacy of the stromata-antigen complex will need to be proven by extensive field trials which are now being planned.

Summary. The protective antigen of *Hemophilus pertussis* has been adsorbed quantitatively, very selectively, and without loss of potency on human red cell stromata. The protective antigen and the hemagglutinin of *Hemophilus pertussis* are not identical. Clinical application of the stromata-protective antigen complex is discussed.

The author wishes to acknowledge the able technical assistance of Mrs. Livia Blum and Mrs. Eleanor Brown. Thanks are due to Dr. J. N. Adam, Jr., and Miss Frances L. Clapp for supplying some of the bacterial suspensions employed in this study and for carrying out the intracerebral challenge tests.

Received October 27, 1950. P.S.E.B.M., 1950, v75.

Porphyrins in the Bone Marrow and Circulating Erythrocytes in Experimental Anemias.* (18312)

RUDI SCHMID, SAMUEL SCHWARTZ, AND C. J. WATSON.
From the Department of Medicine, University of Minnesota Hospital, Minneapolis.

Recent reports(1-3) have described the occurrence of coproporphyrin as well as protoporphyrin in the circulating red blood cells, and have pointed to the correlation of the former compound with erythropoietic activity as revealed by reticulocyte percentage. These studies have induced us to examine the porphyrin content of the bone marrow in certain experimental anemias in rabbits, and to compare it with that of the circulating erythrocytes when examined simultaneously. Very little has been reported in the previous literature bearing on this matter. Vannotti(4) was able to extract considerable amounts of protoporphyrin from the bone marrow of rabbits with experimental hemorrhagic anemia. He also remarked upon the presence of coproporphyrin in the bone marrow of lead poisoned rabbits. He thought that this was derived from protoporphyrin, although there was no supporting evidence for this belief. DeLangen and Grotepass(5) observed small amounts of copro-and larger amounts of protoporphyrin in the bone marrow of a horse poisoned with lead. Stasney and McCord(6) found increased amounts of porphyrin (type not identified) in the bone marrow of cases of iron deficiency anemia and

* Aided by grants from the U. S. Public Health Service, Division of Research Grants and Fellowships (RG-345), and the Atomic Energy Commission, Contract Nos. AT (11-1)-100 and AT (11-1)-108.

1. Schwartz, S., Glickman, M., Hunter, R., and Wallace, J. Plutonium Project Report CH-3720, 1945 (AECD 2109).

2. Schwartz, S., Wikoff, H. M., to be published (*J. Biol. Chem.*)

3. Watson, C. J., a. *Trans. Assoc. Am. Phys.*, 1950, in press. b. *Arch. Int. Med.*, 1950, v86, 797.

4. Vanotti, A., and Siegrist, Th., Z. f. d. gesamte exp. Med., 1940, v108, 336.

5. DeLangen, C. D., and Grotepass, W., *Acta Med. Scand.*, 1938, v94, Fasc. III.

TABLE I.

Rabbit Nos.	Details of experiments
51, 44, 57	Normal controls
5, 7	100 mg lead acetate 15 and 4 days (No. 5) and 28 and 7 days (No. 7) prior to examination*
13, 15, 16, 18, 21	A single inj. of 100 mg lead acetate/kg body wt 4 wk (No. 13, 15, 16) and 5 wk (No. 18, 21) prior to examination*
6, 8	225 ml (No. 6) and 212 ml (No. 8) of blood removed in repeated bleedings over a period of 5 wk (No. 6) and 4 wk (No. 8)
22	Exposed continually in air to atmospheric pressure of 320 mm Hg (corresponding to 23,300 feet altitude) for 5 days prior to examination
9, 10, 23	130 mg (No. 9), 180 mg (No. 10), 160 mg (No. 23) phenylhydrazine in 3 or 4 divided doses over 8 to 10 days prior to examination†

* The lead acetate was dissolved in water with a few drops of buffered glacial acetic acid to bring the pH to approximately 5.5. The final sol. containing 25 mg of lead acetate per ml was administered by subcut. inj.

† Phenylhydrazine hydrochloride, dissolved in isotonic sodium citrate sol. was given in each case by subcut. inj.

pernicious anemia in remission, while minimal amounts were observed in pernicious anemia in relapse, aplastic anemia, leukemia, and sprue. The method of estimating amount was simply visual, depending on intensity of red fluorescence in the extracts.

The purpose of the present communication is to record the results of determinations of copro- and protoporphyrin in bone marrow and circulating red blood cells of presumably normal rabbits and rabbits subjected to 1) lead poisoning, 2) bleeding, 3) phenylhydrazine intoxication, and 4) reduced oxygen tension.

Methods. Adult rabbits of either sex were used. Seven were given lead, 3 phenylhydrazine, 2 were bled, and one was exposed to reduced oxygen tension. Further details of these experiments are given in Table I. At the conclusion of each experiment the rabbit was bled to death by cardiac puncture, after intravenous injection of 2 cc of heparin. This blood was employed for porphyrin determinations. Immediately after death both femora and humeri were freed and removed, the marrow cavities opened, and the bone marrow spooned out and suspended in isotonic sodium citrate solution. This was shaken for 1 minute to reduce trapping of cells between the marrow fat particles. The tube was then centrifuged for 8-10 minutes at 1500 r.p.m. after which the supernatant layer, including the fat was pipetted off. The packed cells were then resuspended in sodium citrate solution and small portions of this suspension were used for morphologic study, differential counts, and determinations of the percentage of nucleated red blood cells. The suspension was then spun down at 2000 r.p.m. for 30 minutes, the packed cell volume measured, and the copro- and protoporphyrin determined according to the method of Schwartz and Wikoff(2).

Results. The important data are shown in Table II. It is seen that in the normal rabbits the concentration of BMCP (bone marrow coproporphyrin) is only slightly greater than that of the ECP (erythrocyte coproporphyrin), and that protoporphyrin concentration is approximately the same in the bone marrow and circulating erythrocytes. In all four groups of experimental rabbits, on the other hand, BMCP values are markedly elevated, while levels of BMP (bone marrow protoporphyrin) are nearly all lower than those of the corresponding EP (erythrocyte protoporphyrin).

Certain differences between the experimental groups are apparent in the relative concentrations of the two porphyrins in bone marrow and peripheral blood as summarized in Table II. Thus the lead-poisoned rabbits show the greatest relative increase of BMCP as compared to ECP, while both BMP and EP are markedly elevated. The data of the

6. Stasney, T., and McCord, W. M., PROC. SOC. EXP. BIOL. AND MED., 1943, v51, 340.

TABLE II. Summary of Hematological and Porphyrin Data.

Group	Rabbit No.	% of nucleated RBC in BM*	% of non-nucleated RBC in BM†	BMCP γ%	BMP γ%	ECP γ%	EP γ%	% of reticulocytes in peripheral blood	Hb in g per 100 ml blood
Normal	51	31	—	1.7	71.4	0.6	58	1.2	12.7
	44	49	—	3.2	54	0.4	106	2.1	13.1
	57	49	—	8.6	137	0.4	86	2.5	12.3
Low O_2 tension	22	67	—	42	90	2.1	246	7.6	16.2
Bleeding	6	41	—	52	186	4.1	237	4.4	8.7
	8	55	23	67	148	15.9	393	15.8§	7.1
Phenyl-hydrazine	9	73	9	75	111	167	221	85 §	6.5
	10	87	—	99	163	102	441	55 §	6.5
	23‡	54	—	89	96	3.9	88	87 §	7.9
Lead	5	62	17	189	450	5.8	615	12.6	7.2
	7	49	—	194	226	6.0	647	17.4	8.1
	13‡	62	36	32	63	1.0	425	3.0	12.3
	15	39	43	409	454	3.1	402	2.4	8.6
	16	45	38	240	1296	0.3	534	1.0	12.5
	18	38	62	54.5	712	1.1	361	1.3	10.6
	21	35	24	92.5	420	1.3	505	3.2	10.5

* These values were calculated as percentage of total nucleated (myeloid-erythroid) bone marrow cells.

† These values were calculated as percentage of total nucleated and non-nucleated (myeloid erythroid) bone marrow cells.

‡ These rabbits differed from the others in being unusually large and old.

§ Frequent normoblasts in peripheral blood.

rabbits with post hemorrhagic anemia and low oxygen tension are similar though at a lower level of porphyrin concentration.

In the rabbits receiving phenylhydrazine, the findings are of unusual interest because of the marked elevations of ECP and, to a relatively lesser extent, of the EP in two of the three animals. As noted in Table II, many normoblasts were seen in the stained blood smears of these rabbits. The high ratio of ECP to EP agrees with previously reported data(1,2).

As shown in Table II, it is clear that a variable number of mature erythrocytes are present in the bone marrow. While no attempt has been made to apply a correction factor for these cells, it is obvious that their presence does not affect the general conclusions to be drawn.

Discussion. The present findings offer further evidence pointing to a close relationship of coproporphyrin to hemoglobin synthesis. Two divergent concepts regarding this relationship have recently been advanced. One is that coproporphyrin III is a normal intermediary in the formation of the hemoglobin protoporphyrin(7,8). The other hypothesis (9) emphasizes oxidative decarboxylation of a dipyrrylmethene precursor of coproporphyrin to one of protoporphyrin. According to this, coproporphyrin is formed as a side product and is not a precursor of the hemoglobin protoporphyrin. It is believed that the present data are compatible with either hypothesis. There is, however, one consideration which is more readily explained by the assumption that coproporphyrin is a direct precursor of hemoglobin protoporphyrin. This involves the correlation of previous data obtained for urinary and fecal coproporphyrin(1,10,11) with those now obtained for bone marrow and blood. In instances of heightened erythro-

7. Grinstein, M., Kamen, M. D., Wikoff, H. H., and Moore, C. V., *J. Biol. Chem.*, 1950, v182, 715.

8. Neuberger, A., Muir, H. M., and Gray, C. H., *Nature*, 1950, v165, 948.

9. Lemberg, R., and Legge, J. W., Hematin Compounds and Bile Pigments, Interscience Publishers, Inc., New York, 1949.

10. Dobriner, K., and Rhoads, C. P., *Physiol. Rev.*, 1940, v20, 416.

11. Watson, C. J., and Larson, E. A., *Physiol. Rev.*, 1947, v27, 478.

poiesis as seen in acute blood-loss anemia or in hemolytic (phenylhydrazine) anemia there is a considerable increase of total coproporphyrin in both bone marrow and circulating erythrocytes. Studies in this laboratory(2) revealed 90% of the total erythrocyte coproporphyrin to be the type III isomer in animals subjected to repeated bleedings, while approximately 60% was type III in animals with phenylhydrazine anemia. The major fraction of the red cell coproporphyrin thus has the same configuration as hemoglobin protoporphyrin. On the other hand, the increase in urinary and fecal coproporphyrin with increased erythropoiesis is due to the type I isomer, with little or no change in the type III coproporphyrin(1,10,11). This is consistent with the concept that there is efficient utilization of coproporphyrin III for protoporphyrin synthesis, with a liberation from the cells of increased amounts of coproporphyrin I formed as a side product. However, if coproporphyrin III also were only a side product, one might expect that it, too, would be excreted in increased amount. As noted, this is not the case though it can be argued that other unknown factors may operate to influence the excretion or further metabolism of coproporphyrin III.

The data obtained in the lead poisoned animals agree equally well with either hypothesis. Thus, Lemberg and Legge point out that their scheme would predict the increase in type III isomer which is known to exist. According to the precursor concept, too, it would be logical to assume that lead interferes with the utilization of coproporphyrin III; hence its increased excretion in the urine. The evidence also suggests interference by lead in other stages of hemoglobin synthesis, *i.e.* in the utilization of protoporphyrin as indicated by its accumulation in marrow and circulating cells, and perhaps of porphyrin precursors. In this connection it is of interest to point out that the porphyrinuria of lead poisoning is readily harmonized with the present bone marrow findings while previous studies of the circulating erythrocytes(2,3) failed to reveal any correlation of the coproporphyrin concentration in urine and erythrocytes. The data suggest that the coproporphyrin is liberated from intact young erythrocytes in the bone marrow. Actual destruction of these cells appears unlikely since the excesses observed in the rabbits urine would require the destruction of 100 ml or more of such cells per day.

Despite the evident relationship of coproporphyrin to erythropoiesis, it may be pointed out that the present investigation has not revealed any strict correlation between the degree of erythropoietic activity (percentage of normoblasts) in the bone marrow, and its coproporphyrin concentration nor between the percentage of circulating normoblasts and the coproporphyrin concentration of the circulating erythrocytes.

An ether-insoluble uro-type porphyrin has also been demonstrated in the bone marrow of lead poisoned rabbits. Its absorption and fluorescence spectra are identical with those of uroporphyrin I. Further studies of this porphyrin are in process.

Summary and conclusions. 1. The copro- and protoporphyrins of bone marrow and circulating erythrocytes in the rabbit have been found to exhibit dynamic changes under a variety of stimuli to erythropoiesis, including lead poisoning, phenylhydrazine, hemorrhage, and reduced oxygen tension. These consist of (1) a striking increase of coproporphyrin in the developing erythrocytes of the bone marrow, with a marked relative reduction of its concentration in circulating red blood cells, (2) an inconstant but generally increased level of protoporphyrin in the circulating as compared with the marrow erythrocytes. 2. These observations point to a close relationship between coproporphyrin and hemoglobin synthesis, and appear to be consistent with concepts which consider coproporphyrin either as a direct precursor of hemoglobin protoporphyrin or as a by-product of the synthesis. The former concept, however, seems to permit a more reasonable correlation of the porphyrin findings in bone marrow and blood with those in urine and feces.

Received October 30, 1950. P.S.E.B.M., v75.