STUDIES ON FREE ERYTHROCYTE PROTOPORPHYRIN, PLASMA COPPER, AND PLASMA IRON IN NORMAL AND IN PYRIDOXINE-DEFICIENT SWINE*

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Previous communications from this laboratory (1–7) have demonstrated that swine maintained on a highly purified diet deficient in pyridoxine develop a severe anemia which is characterized by microcytosis, slight hypochromia, and an increase in polychromatophilia, reticulocytes, and nucleated red cells in the blood. There is a marked hyperferremia, normoblastic bone marrow hyperplasia, myelin degeneration in the peripheral nerves, and hemosiderosis in the liver, spleen, and bone marrow. Certain alterations in tryptophan metabolism, namely an increased excretion of xanthurenic acid, kynurenine, and urorosein, occur. The administration of pyridoxine is followed by a sharp reticulocyte response, mobilization of iron from the tissues, and rapid regeneration of blood with restoration of the normal size of erythrocytes. The anemia fails to respond to the administration of either iron or purified liver extract. Appropriate studies have failed to reveal evidence that the anemia is due to increased blood destruction and it has been concluded that it is due to a disturbance in erythropoiesis. The nature of this disturbance has not, however, been discovered.

The purpose of this report is to present data concerning certain aspects of the metabolism of porphyrin, copper, and iron in pyridoxine-deficient swine, which were gathered in an attempt to elucidate the mechanism by which a deficiency of pyridoxine produces anemia. The literature dealing with the relation of pyridoxine to erythropoiesis has recently been reviewed (8).

Materials and Methods

Full details of the experimental methods have been given elsewhere (9). For this study, which forms part of a larger one, forty-five weanling pigs, approximately 21 days of age, were used. Thirty-one animals were placed on the control diet and fourteen animals were fed the same diet as the

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controls except that pyridoxine was omitted. The results of observations on pigs with other types of deficiency will be reported later.

All animals from the day they were received were fed the basal diet consisting of Sheffield "new process" casein 26.1 per cent, sucrose 57.7 per cent, lard 11.0 per cent, salt mixture (swine Salt Mixture 3 (9)) 5.2 per cent. In addition they were given cod liver oil (Mead Johnson, 1800 units of vitamin A, 175 units of vitamin D per gm.), 0.5 gm. per kilo of body weight daily or Natola (Parke, Davis, 55,000 units of vitamin A, 11,000 units of vitamin D per gm.), 0.056 gm. per kilo of body weight per week. Vitamins were supplied in crystalline form in capsules and were administered orally three times a week. The quantities of crystalline vitamins were as follows (mg. per kilo of body weight daily): thiamine hydrochloride 0.25, riboflavin 0.12, nicotinic acid 1.20, pyridoxine hydrochloride 0.20, pantothenic acid 0.50, *p*-aminobenzoic acid 0.10, inositol 0.10, choline chloride 10.0.

Determinations of erythrocyte protoporphyrin were made by the method of Grinstein and Watson (10). Plasma copper was determined by the method of Cartwright, Jones, and Wintrobe (11). For the determinations of plasma iron the method of Kitzes, Elvehjem, and Schuette (12) as well as the method of Barkan and Walker (13) was used. Urinary coproporphyrin determinations were made by the method of Cartwright, Lauritsen, Jones, Merrill, and Wintrobe (14).

Results

Detailed hematologic and chemical data for each of the fourteen pyridoxine-deficient pigs are presented in Table I. In Table II the results of the chemical data are summarized. The amount of free protoporphyrin in the erythrocytes of the normal animals was 118 ± 43.4 γ per 100 ml. of red cells. In the pyridoxine-deficient group this was reduced to an average of 47 ± 13.6 γ. The coproporphyrin excretion in the urine in pyridoxine deficiency was not altered significantly from the normal of about 104 γ per 24 hours. The amount of copper in the plasma of the normal pigs was 206 ± 26.3 γ per cent. In the pyridoxine-deficient group the plasma copper was reduced to 160 ± 38.8 γ per cent. The plasma iron, on the other hand, was markedly increased, being on the average 468 ± 166.6 γ per cent as compared with the normal of 169 ± 38.8 γ per cent.

In Fig. 1 the data for erythrocyte protoporphyrin, plasma iron, and volume of packed red cells are presented as determined in a control animal and in a pyridoxine-deficient pig throughout the course of a 121 day experiment. The significant fact to be noted is that the amount of protoporphyrin in the erythrocytes dropped to the low value of 55 γ per 100 ml. of red cells on the 27th day of the experiment and thereafter remained con-

stantly low. This change took place long before the development of significant anemia (90 days) and differed from the rise in plasma iron in that the latter increased gradually throughout the experiment. Similar

TABLE I

Hematologic and Chemical Data for Fourteen Pyridoxine-Deficient Pigs

Pig No.	Red blood cells	Hb	Volume of packed red blood cells	Mean corpuscular volume	Mean corpuscular Hb	Mean corpuscular Hb concentration	Erythrocyte protoporphyrin	Plasma copper	Plasma iron
	millions per c.mm.	*gm. per cent*	*ml. per 100 ml.*	*cu. micra*	*micro-micrograms*	*per cent*	*γ per 100 ml. red blood cells*	*γ per cent*	*γ per cent*
Normal	8.86	14.7	47.0	53	17	32	118	206	169
9-05	9.70	11.5	44.0	45	12	26	23	183	295
9-06	6.02	5.8	22.0	37	10	26	48	137	578
9-07	4.95	3.7	15.0	31	8	25	47	117	440
9-08	7.00	8.4	32.0	45	10	26	45	102	490
9-20	2.60	3.1	11.5	44	12	27	41	127	392
9-21	6.48	7.6	27.0	42	12	28	79	113	436
9-22	6.50	5.9	20.5	32	9	29	56	201	730
9-23	9.21	9.3	37.0	40	10	25	27	177	376
9-39	7.03	7.3	26.5	38	10	28	63	218	788
9-40	5.85	8.3	28.5	49	14	29	38	143	635
9-41	5.70	7.8	27.0	47	14	29	65		921
9-42	5.35	8.6	28.0	52	16	31	59	237	618
10-20	5.70	8.1	26.0	46	14	31	64		438
10-21	7.40	8.7	29.5	40	12	29	50		466

TABLE II

Summary of Data

Determination	Group	No. of animals	No. of determinations	Mean ± S.D.
Erythrocyte protoporphyrin, γ *per 100 ml. red blood cells*	Control	31	208	118 ± 43.4
	Deficient	14	56	47 ± 13.6
Urinary coporporphyrin, γ *per 24 hrs.*	Control	4	10	104 ± 37.8
	Deficient	3	7	108 ± 36.3
Plasma copper, γ *per cent*	Control	23	79	206 ± 26.3
	Deficient	11	22	160 ± 38.8
Plasma iron, γ *per cent*	Control	30	230	169 ± 38.8
	Deficient	14	67	468 ±166.6

results were obtained in four other animals, followed throughout the course of the deficiency.

In Fig. 2 the results of intravenous therapy in a single animal with small

doses of pyridoxal and pyridoxamine are presented. Within 24 hours after administration of pyridoxal the plasma iron dropped from 720 to 100 γ per cent. A maximum reticulocytosis of 18 per cent was reached on the

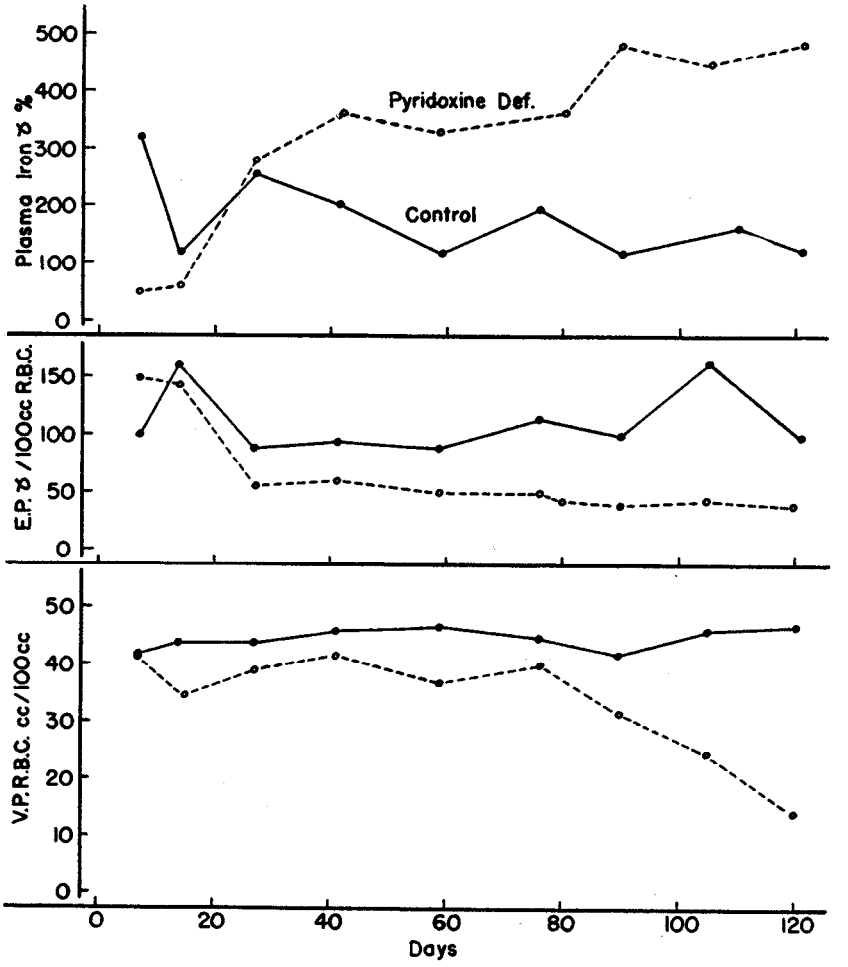

FIG. 1. Rise in plasma iron, reduction of free erythrocyte protoporphyrin, and development of anemia in a pig deficient in pyridoxine in comparison with a control animal.

3rd day of therapy. On the 5th day of treatment the erythrocyte protoporphyrin rose abruptly to 250 γ per 100 ml. of red cells from the previously low level of 55 γ. Following therapy the volume of packed red cells rose rapidly, the size of the erythrocytes increased to normal, the mean cor-

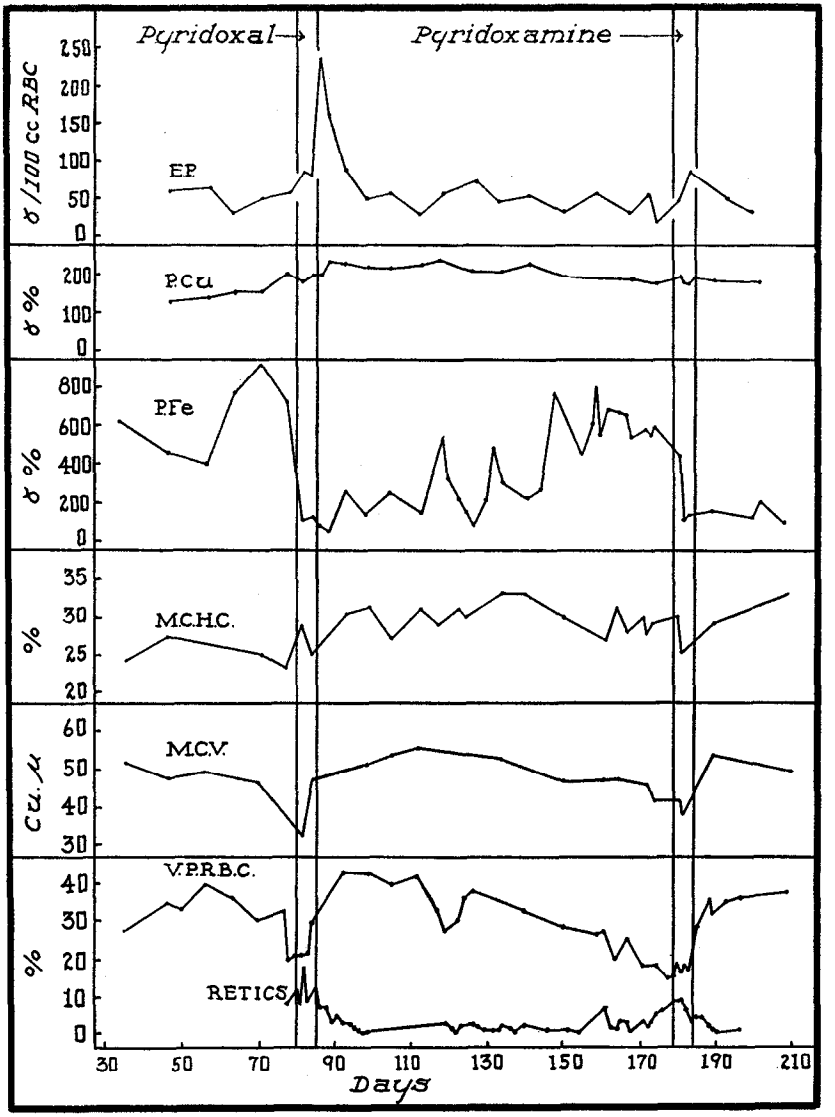

FIG. 2. Chemical and morphological changes in the blood of a pyridoxine-deficient pig following treatment with small doses of pyridoxal and pyridoxamine. The animal received 0.4 mg. per kilo of body weight on the 1st day of therapy and 0.2 mg. per kilo of body weight intravenously for 4 days thereafter.

puscular hemoglobin concentration returned to normal, and there was a slow gradual rise in plasma copper. 15 days after the cessation of therapy the erythrocyte protoporphyrin had returned to its previously low level,

even though the plasma iron and copper levels were normal and anemia was not present. Over the course of the next 80 days a deficiency again developed and the animal was treated a second time with small doses of pyridoxamine. Again there was a reticulocytosis with restoration of the blood to normal, a rapid fall in plasma iron, and a slight rise in erythrocyte protoporphyrin. The experiment was discontinued on the 209th day, when the animal developed a secondary infection.

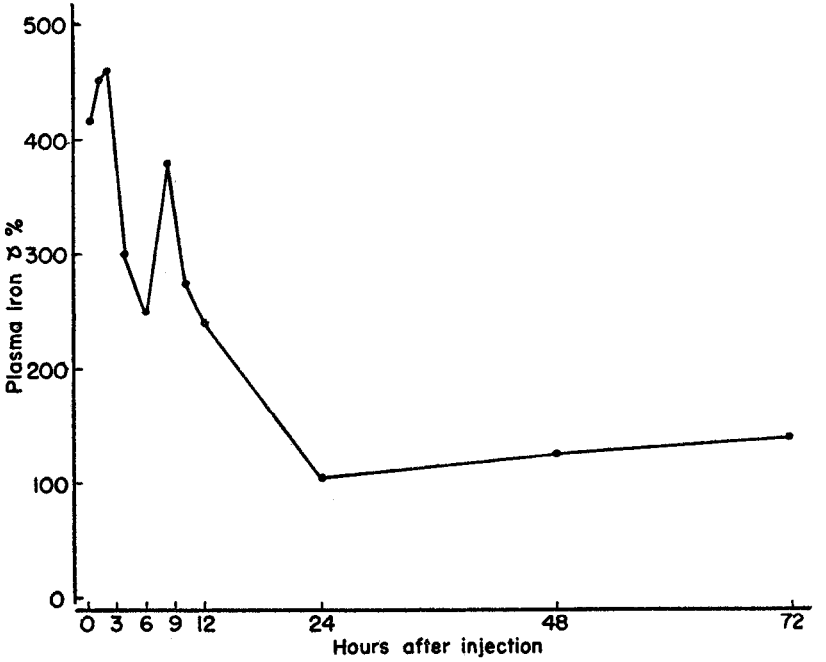

FIG. 3. The rapid fall in plasma iron in a pyridoxine-deficient pig following the intravenous injection of 0.4 mg. of pyridoxamine per kilo of body weight.

In order to determine the rapidity of the fall in plasma iron, determinations were made 1, 2, 3, 4, 6, 8, 10, 12, 24, and 48 hours following therapy. The results are presented in Fig. 3. As can be seen, the plasma iron began to decrease 2 hours after therapy and reached the lowest point within 24 hours.

DISCUSSION

The normal values for free erythrocyte protoporphyrin, plasma iron, and plasma copper in the pig differ significantly from the normal values in human subjects. In the pig the normal mean value for free erythrocyte

protoporphyrin was found to be 118 ± 43.4 γ per 100 ml. of red cells (Table II). In human beings the normal erythrocyte protoporphyrin is less than 50 γ per 100 ml. of red cells (14, 15). The normal plasma copper and plasma iron for the pig have been found to be 206 ± 26.3 γ per cent and 169 ± 38.8 γ per cent, respectively. In human subjects the normal plasma copper is about 125 γ per cent (11) and the normal plasma iron about 126 γ per cent (8).

The reduction in the amount of free protoporphyrin in the erythrocytes of the pyridoxine-deficient animals suggests that the fundamental disturbance in erythropoiesis may be a failure to synthesize protoporphyrin. This is further suggested by the fact that the reduction in the free protoporphyrin appears to take place early in the course of the deficiency and precedes by a long period the development of significant anemia. This hypothesis also explains the hyperferremia and hemosiderosis of the tissues. Since protoporphyrin is not available, iron cannot unite with it to form heme, the plasma iron increases, and iron is stored in the tissues awaiting the time when protoporphyrin becomes available. When pyridoxine is supplied, the synthesis of protoporphyrin is accelerated and as the reaction protoporphyrin + Fe^{++} → heme proceeds, the iron is mobilized from the serum and tissues, and there is an increase in hemoglobin with alleviation of the anemia. It has been demonstrated by Watson, Grinstein, and Hawkinson (15) that the free protoporphyrin in erythrocytes is protoporphyrin type III, No. 9, the protoporphyrin from which hemoglobin is synthesized. Evidence has recently been presented from our laboratory which indicates that the free protoporphyrin in the erythrocytes is a precursor rather than a degradation product of hemoglobin.[1]

At present one can only speculate concerning the manner in which pyridoxine may be related to protoporphyrin synthesis. It is known that the body does not depend upon a dietary source of pyrroles or their derivatives for the production of protoporphyrin. It has long been postulated that the pyrrole rings are synthesized from amino acids. Recently there has been direct evidence for this. Shemin and Rittenberg (16), using glycine labeled with isotopic nitrogen, demonstrated that glycine is a nitrogenous precursor of the protoporphyrin of hemoglobin in both the rat and man. It is now known that pyridoxine and its derivatives are intimately concerned with protein metabolism, especially decarboxylation and transamination of amino acids (17–21). Furthermore pyridoxine is known to be concerned with the metabolism of tryptophan, a pyrrole containing amino acid (8). It does not seem unlikely therefore that pyridoxine may be related to protoporphyrin synthesis through its relation to

[1] Grinstein, M., Silva, J., and Wintrobe, M. M., to be published.

amino acid metabolism. If protoporphyrin synthesis is affected in pyridoxine deficiency, it is necessary to inquire whether other porphyrin-containing compounds such as catalase, the cytochromes, and indophenol oxidase are also affected. A deficiency of pyridoxine in the rat has not been found to cause a significant change in the catalase activity of the liver, kidney, and heart (22). However, the amount of protoporphyrin necessary for the maintenance of these enzyme systems, in comparison with the amount necessary for hemoglobin synthesis, is extremely small and perhaps these compounds would be affected last and not to a significant degree, since the reduction in free erythrocyte protoporphyrin is never complete. It should also be pointed out that in the rat uncomplicated pyridoxine deficiency results in little or no anemia.

The slight reduction in plasma copper in pyridoxine deficiency is difficult to interpret. Very little is known about copper metabolism in various types of anemias. McKibbin *et al.* (23) found total blood copper at a low normal level in anemic pyridoxine-deficient dogs and observed that the level increased to normal during pyridoxine therapy.

We have pointed out previously that several similarities exist between pyridoxine deficiency anemia in swine and pernicious anemia in human beings; namely, hyperferremia, hemosiderosis of the tissues, hyperplasia of the bone marrow, and neurological lesions (2). In respect to the amount of free protoporphyrin in the erythrocytes the conditions are likewise similar, since in pernicious anemia the values tend to be in the low normal range (24).[2]

SUMMARY

1. Determinations of erythrocyte protoporphyrin have been made on thirty-one normal pigs and fourteen pyridoxine-deficient pigs. The mean plus or minus standard deviation for the normal group was 118 ± 43.4 γ per 100 ml. of red cells and for the pyridoxine-deficient group 47 ± 13.6 γ per 100 ml. of red cells.

2. Plasma iron determinations have been made on thirty normal pigs and fourteen pyridoxine-deficient pigs. The mean for the normal group was 169 ± 38.8 γ per cent and for the pyridoxine-deficient group 468 ± 166.6 γ per cent.

3. Plasma copper determinations have been made on twenty-three normal pigs and eleven pyridoxine-deficient pigs. The mean for the normal group was 206 ± 26.3 γ per cent and for the pyridoxine-deficient group 160 ± 38.8 γ per cent.

4. Urinary coproporphyrin excretion was measured in four normal pigs and three pyridoxine-deficient pigs. The mean for the normal group was 104 γ per 24 hours and for the pyridoxine-deficient group 108 γ per 24 hours.

[2] Cartwright, G. E., Huguley, C. M., and Wintrobe, M. M., to be published.

5. It is suggested that the fundamental disturbance in pyridoxine deficiency anemia in swine is a failure to synthesize protoporphyrin.

6. Certain similarities between pyridoxine deficiency in swine and pernicious anemia in human beings are described.

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