inhibits hemolytic activity. The rate of hemolysis is influenced by temperature(11) as well as concentration of the mercurial reagent. Benesch *et al.*(5) found a striking variation in the reactivity of the –SH groups of crystalline hemoglobin among different species. Those of dogs and man were less reactive than those of sheep. Racker(6) suggested that the difference in rate of reactivity between sulfhydryl groups and reagents may be influenced by properties of adjacent groups on the protein molecule.

That –SH groups are necessary for the integrity of the erythrocyte is a fact. The thiols responsible for this effect are not known nor is the mechanism known. A recent report (5) indicates there are 8 moles of –SH per mole of hemoglobin in man. Previous studies have demonstrated the presence of ergothioneine(7-9) and glutathione(10) within the erythrocyte. The glutathione is in a dynamic state(3) and may traverse the cell envelope. Less is known of the role of ergothioneine.

The necessity of –SH groups for the integrity of the erythrocyte suggests that control of the thiol system may be advantageous in the preservation of whole blood stored for transfusion.

Conclusions. 1. The sulfhydryl reagent, p-chloromercuribenzoic acid, causes hemolysis of human erythrocytes. This action can be prevented by prior reaction with sulfhydryl groups. 2. The hemolytic action of PCMB is reversible by washing cells for as long as 30 minutes after contact with this agent. Reversibility of hemolysis lasts up to 60 minutes after addition of free sulfhydryl group. 3. No protection from PCMB hemolysis was noted with stroma whereas hemoglobin solution was effective. 4. The reaction between PCMB and the –SH group of erythrocytes is relatively slow. 5. A physiologic concentration of glucose offered no protection from hemolysis in this system.

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Pyridoxine Responsive Anemia in the Human Adult.* (22284)

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Although pyridoxine is necessary for normal hematopoiesis in dogs(1,2), swine(3-5) and monkeys(6), no evidence is available that abnormal hematopoiesis occurs in humans secondary to a naturally occurring deficiency of pyridoxine or alteration in its metabolism(7,8). In general, the hematologic alterations resulting from pyridoxine deprivation in animals have been characterized by a hypochromic, microcytic anemia with

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high plasma iron; although a moderate leukopenia is usually observed, neutrophilic leukocytosis has also been described. After pyridoxine deprivation abnormal metabolites of tryptophan are excreted following a loading test dose of this amino acid(9,10). The administration of pyridoxine is followed by a well-defined reticulocyte response, rapid fall of plasma iron and restoration of normal leukocyte, erythrocyte and hemoglobin values; concomitantly, the abnormalities of tryptophan metabolism are corrected.

The following observations concern a 35-year-old white male who had a severe hypochromic anemia unresponsive to the usual hematopoietic agents and associated with abnormalities in tryptophan and iron metabolism; these abnormalities were promptly reverted to normal following the intramuscular administration of pyridoxine hydrochloride.

Results. Patient H.S. was first admitted to the hospital in 1947 at 27 years of age, because of weakness and pallor. Except for nocturia, tibial pain and pedal edema that became very bothersome in 1955, the patient's signs and symptoms during entire subsequent course could be directly attributed to decreased hemoglobin level and were abolished by transfusions. No signs or symptoms of glossal, neural, or dermal involvement occurred. His appetite and food intake always remained excellent and he maintained a constant weight. Detailed dietary history showed that the daily caloric intake averaged 2900 and was comprised of 122 g protein, 284 g carbohydrate and 143 g fat; the daily intake of pyridoxine was estimated at approximately 3 mg.† The spleen never became palpable. Admission blood studies showed a hypochromic anemia (4.5 g hemoglobin/100 ml) with normal leukocyte and platelet counts; the leukocyte differential count was within normal limits. Gastric analysis demonstrated 47 units free acid following histamine. A bone marrow biopsy was interpreted as showing some degree of erythrocyte maturation arrest but not consistent with that seen in classic Addisonian pernicious anemia. The anemia was unresponsive to parenteral liver injections (both purified and crude), pteroylglutamic acid, Brewer's yeast, iron and ascorbic acid in the dosages, routes and time duration of treatment indicated in Table I. Transfusions were given as necessary to maintain his hemoglobin at approximately 8 g/100 ml. Continuing studies (roentgenographic studies of the chest, upper gastrointestinal tract, small and large bowel, intravenous pyelograms; basal metabolic rate; stool for ova, parasites, blood excess fat or nitrogen; lymph node biopsy; blood chemistries, electrocardiogram) were done with normal or negative results.

In May, 1948 a spontaneous remission apparently occurred and repeated observations showed red count of over $4x10^6/mm^3$ and hemoglobin of 12 to 13 g/100 ml. He was thereafter asymptomatic and received no therapy until November, 1953 at which time he again noticed onset of weakness and pallor.

Administration of cyanocobalamin and intravenous iron by private physician apparently did not alter progression of symptoms. In December, 1953 the hemoglobin was 8.5 g/100 ml; erythrocyte count $3x10^6/mm^3$, hematocrit, 26%; reticulocyte count, 0.2% and the leukocyte count, $13.2x10^3/mm^3$. The leukocyte differential showed 81% polys, 2% bands, 17% small lymphocytes; the platelet count was $420x10^3/mm^3$; and blood urea nitrogen was 9.9 mg/100 ml. Tests for abnormalities found in various(11) hemolytic states were within normal limits including direct Coombs' test, electrophoresis of hemoglobin, alkaline denaturation for estimation of fetal hemoglobin, Donath-Landsteiner test, cold agglutination test, Ham test, bilirubin partition and total amount: no hemolysins or agglutinins could be demonstrated in the patient's serum in the pH range of 6.5 to 7.5. The indirect Coombs' test was positive at pH 7.5 but consistent with a transfusion subgroup incompatibility. The erythrocyte osmotic fragility was within normal limits; after 24 hours sterile incubation the osmotic fragility had shifted into the range indicating *increased resistance* to osmotic stress com-

† We are indebted to Miss H. Ochi of the Dietetic Service of Crile Veterans' Administration Hospital for these calculations.

TABLE I. Agents Ineffective in Producing a Hematopoietic Response in Patient H.S.

Medication	Route admin.	Amt admin./day	Dates of treatment
Purified liver extract (15 U.S.P. units/ml)	I.M.	1 ml	1/28/47– 2/ 6
Crude liver extract	I.M.	2	4/21 – 4/30
(2 U.S.P. units/ml)	I.M.	5	1/ 8/54– 1/14
Bio-hepulin*	I.M.	2	5/14 – 5/28
Valentine's liquid extract of liver, U.S.P.	P.O.	30	4/21/47– 4/30
	P.O.	90	4/14/54– 4/28
	P.O.	120	4/29 – 5/10
Brewer's yeast tablets	P.O.	20 tablets	2/20/47– 4/30
Ferrous sulfate	P.O.	1 g	2/ 7 – 4/30
Cobaltous chloride	P.O.	180 mg	12/19 –12/24
Pteroylglutamic acid	P.O.	45	3/ 7 – 3/13
	P.O.	100	3/14 – 4/30
	I.M.	15	12/22/53–12/29
	P.O.	30	3/30/54– 4/12
Ascorbic acid	P.O.	150	3/ 6/47– 3/12
	P.O.	200	4/ 4 – 4/30
	P.O.	100	3/30/54– 4/12
Leukovorin	P.O.	30	1/ 2 – 1/ 8
	I.M.	6	12/30/53– 1/ 8/54
Cyanocobalamin	I.M.	100 μg	3/30/54– 4/12
	I.M.	1000	1/ 8/55– 1/30
Pantothenic acid	P.O.	10 mg	12/ 1/47
Cortisone	P.O.	100	1/15/54– 1/20
	P.O.	200	1/21 – 2/ 2
	P.O.	150	1/ 8/55– 1/28
Fresh human plasma	I.V.	1400 ml	6/ 8/54– 6/16
Vit. A	P.O.	75000 units	7/ 8/55– 8/28
D		3000	
Ascorbic acid		450 mg	
Thiamine		15	
Riboflavin		15	
Niacin		750	

* Extract prepared from pregnant mare's liver.

pletely outside the normal range. The erythrocyte mechanical fragilities determined before and after 24 hours of sterile incubation were only slightly increased above normal. An erythrocyte autosurvival time determined by Cr51 demonstrated a half life of 20 days; all the labelled cells had been removed from the circulation by 90 days. These values are significantly below the normal for the method (19). In addition to several agents which had been tried previously, the patient was treated with citrovorum factor, crude liver extract and 1400 ml of fresh plasma intravenously as indicated in Table I. To none of these therapeutic trials did he make any subjective, clinical or hematologic response. His hemoglobin level was maintained at approximately 7 g/100 ml by repeated transfusions of whole blood. In January, 1955 he was readmitted for a trial of cyanocobalamin and cortisone(12): there was no response to this combination of drugs. Transfusions were continued periodically until August, 1955 by which time he had received a total of 113 units of blood. He was then readmitted with an erythrocyte count of $2.86 \times 10^6/mm^3$, 5.9 g hemoglobin/100 ml, hematocrit 22%, leukocyte count $25.5 \times 10^3/mm^3$; the leukocyte differential showed the following percentage distribution: 76 polymorphonuclears, 16 small lymphocytes, 4 monocytes and 4 eosinophils. The mean corpuscular volume was 66 μ^3 and the mean corpuscular hemoglobin concentration, 27%. The erythrocytes on smear showed definitely abnormal variation in size and shape with frequent bizarre forms and

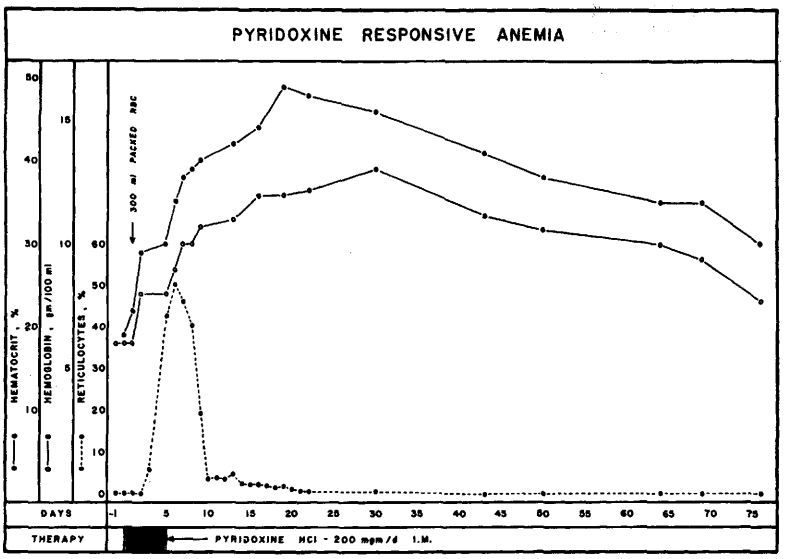

FIG. 1. Initial hematologic response of patient H.S. to intramuscular administration of pyridoxine and subsequent relapse following cessation of therapy.

target cells; the cells appeared hypochromic with decrease in hemoglobin content. During the previous 2 months the patient had been taking one tablet of a vitamin preparation 3 times a day. Each tablet contained: vit. A, 25,000 units; vit. C, 150 mg; vit. D, 1,000 units, thiamine chloride, 5 mg; riboflavin, 5 mg; and niacin, 250 mg. He was also taking quinine, grains 5 p.r.n. usually once every other week for night leg cramps; no other medicines were taken. A transfusion of 300 ml packed red cells was given on the same day pyridoxine hydrochloride, 200 mg intramuscularly daily was started. After 4 doses of this vitamin he noted a subjective improvement in general well-being and on the seventh day after institution of therapy, the reticulocytes had risen to 50.8%. Following this there was a rapid rise in the hematocrit and hemoglobin to maximum values of 49% and 13 g/100 ml respectively, on Sept. 12, 1955. The leukocyte count decreased to 9.2×10^3/mm. As soon as the reticulocytes were found to be elevated significantly, the pyridoxine administration was stopped, 5 doses having been given. By Nov. 14, 1955 the hematocrit had fallen to 27% and the hemoglobin to 7.8 g/100 ml. This response and subsequent relapse are outlined in Fig. 1. Before the response to pyridoxine the patient had complained of very troublesome nocturia, tibial pain and swelling of the feet constant throughout the day. Coincident with the reticulocyte rise the tibial pain disappeared and the edema subsided; shortly thereafter, the nocturia disappeared. After hematologic relapse when the hemoglobin had fallen to 7.9 g/100 ml and the hematocrit to 30%, the patient was started on pyridoxine, 1 mg intramuscularly daily. Except for the l-tryptophan loading tests as indicated below, no medicines or transfusions were given at this time. No significant reticulocyte response occurred following the 1 mg pyridoxine dose and as indicated in Fig. 2, after 8 days it was increased to 10 mg intramuscularly daily. A reticulocyte peak of 17.7% on the seventh day of this therapy, was followed by a rapid rise in hemoglobin and hematocrit. Again, there had been subjective improvement coincident with the reticulocyte response. On Dec. 14, 1955 the hemoglobin reached 14.1 g/100 ml and the hematocrit 51.9%. The patient received 3 oral loading tests of 4 g l-tryptophan, the first before pyridoxine therapy was started, the second during the 1 mg dose, and the final one shortly following the reticulocyte response during the 10 mg dose. Measurements of the urinary excretion of kynurenin, kynurenic acid, acetylkynurenin, xanthurenic

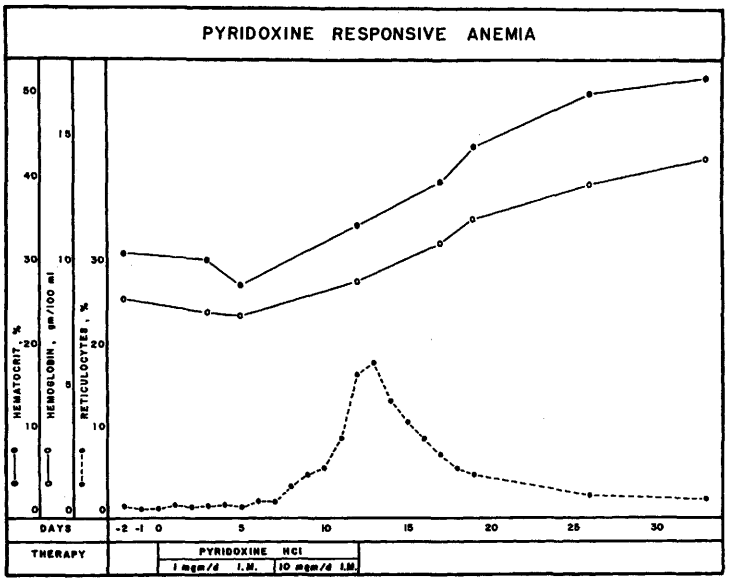

FIG. 2. Lack of hematologic response of patient H.S. to intramuscular administration of 1 mg pyridoxine daily with subsequent response to 10 mg pyridoxine daily. The l-tryptophan loading tests were done on days −2, 5 and 17.

acid, anthranilic acid glycuronide, ortho-aminohippuric acid, and n-methyl-pyridone-5-carboxymid indicated abnormalities of tryptophan metabolism(13). These abnormalities were partly reverted toward normal on the 1 mg pyridoxine dose and completely normalized at the 10 mg level(14). Before therapy the serum iron level was 170 μg/100 ml with a total iron binding capacity of 184 μg/100 ml and an iron binding protein saturation of 92%. Following therapy the serum iron was 67 μg/100 ml; the total iron binding capacity was 214 μg/100 ml and the saturation, 31%.‡

Discussion. Studies concerning pyridoxine have been accomplished in humans by eliminating the vitamin from the diet, counteracting the effect of the vitamin with an antagonist (4-desoxypyridoxine) and administering the vitamin as a therapeutic test(8). Although abnormalities in tryptophan metabolism have been readily produced(10), in only one instance has it been possible to show that pyridoxine deprivation in the human has resulted in altered hematopoiesis manifested by a severe microcytic hypochromic anemia, remediable by pyridoxine alone. Snyderman and associates(15) studied 2 hydrocephalic infants maintained on a synthetic diet devoid of pyridoxine; after 130 days anemia developed in one infant and abnormalities in tryptophan metabolism were demonstrated. These abnormalities disappeared after pyridoxine administration. No instances of naturally occurring abnormalities of hematopoiesis have been reported in humans that were responsive to the administration of pyridoxine. Hunt and associates(16) reported an infant who suffered from intractable convulsions which were controlled by pyridoxine. This infant was also described as having had a moderate anemia during the neonatal period; however, the only values reported for the first 23 days of life are: hemoglobin 13.1 g/100 ml and an erythrocyte count of 4.2×10^6/mm^3.

Employing the metabolic antagonist 4-desoxypyridoxine(17,18) dermatitis, glossitis, neuritis, lymphopenia and alterations in the handling of tryptophan and alanin were

‡ We are indebted to James A. Bowerfind, M.D., of University Hospitals, Cleveland, for these determinations.

produced. However, the anemia that sometimes occurred could not be related to pyridoxine metabolism.

Despite extensive empirical use of pyridoxine as a therapeutic test agent for many different conditions, its essential role in human hematopoiesis has been demonstrated only in the infant maintained on a synthetic diet lacking pyridoxine. The present observations indicate that abnormal hematopoiesis (associated with abnormalities in the metabolism of tryptophan and iron) can occur in the adult human secondary to a naturally occurring alteration in pyridoxine metabolism. Since the dietary intake of pyridoxine was estimated to be normal, the possibilities exist at present that either an absorptive defect or metabolic aberration accounts for the findings in this patient. The nature of the alteration and its role in the steps involved in hematopoiesis are unknown.

Summary. An adult patient is reported with hematologic abnormalities that were unresponsive to the usual hematopoietic agents, and characterized by a hypochromic anemia, leukocytosis, high serum iron and high per cent iron binding protein saturation. Measurements of the abnormal urinary excretion of the metabolites of l-tryptophan following oral loading tests demonstrated an alteration in the metabolism of this amino acid. The clinical and laboratory abnormalities were promptly reverted to normal, and hematological remission followed intramuscular administration of pyridoxine hydrochloride.

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Acceptance of Tumor Homografts by Mice Injected with Antiserum. II. Effect of Time of Injection.* (22285)

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With certain combinations of transplantable tumors and inbred strains of mice, a normally resistant host will accept a tumor homograft if the animal is injected with preparations of mouse tissues prior to tumor grafting(1-4). The altered response of the host may persist for many months after the

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