Pyridoxine-Responsive Anemia Conditioned by Isonicotinic Acid Hydrazide

By Paul R. McCurdy and Robert F. Donohoe

Since the first report in 1956 of a patient with pyridoxine-responsive anemia, a number of other patients have been reported. The red blood cells usually are hypochromic and microcytic but occasionally they may be macrocytic and accompanied by megaloblastic bone marrow maturation. It is unlikely that many of these patients have a simple dietary deficiency of pyridoxine, since large doses of the vitamin are needed to elicit a response. Furthermore, this response is commonly incomplete and the hemoglobin concentration and red cell morphology do not return to normal. Thus, the mechanism may be dysmetabolism or impairment of an enzyme system in some way other than by simple coenzyme deficiency.

Isonicotinic acid hydrazide (INH) is a pyridoxine antagonist, both in vitro and in vivo, and many of its side effects can be attributed to interference with the proper action of this vitamin. It occupies a key position in the therapy of tuberculosis. Although the occurrence of anemia is quite uncommon during INH therapy, two patients have been reported who developed anemia responsive to pyridoxine administration during treatment of tuberculosis with INH. A third patient, whose anemia originally responded to small doses of pyridoxine, suffered a recurrence of anemia during therapy of tuberculosis with INH. When administration of this drug was stopped and relatively small doses of pyridoxine (5 mg./day, i.m.) were given, there was a reticulocytosis followed by marked hematologic improvement.

It is the purpose of this paper to describe three additional patients who had pyridoxine-responsive anemia while being treated for tuberculosis with INH.

Material and Methods

The majority of technics used in this study have been described or referred to elsewhere. The spot test of Fairbanks and Beutler was used to screen for glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. Procedures for the single tube assay of G-6-PD activity and for starch gel electrophoresis of G-6-PD have also been described. Bone marrow aspirates were examined with the Prussian Blue stain.

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The technic of Karmen\textsuperscript{12} was adapted to the determination of erythrocyte glutamic-oxaloacetic transaminase (EGOT). Red cells were washed three times in saline. Lysis was accomplished by the addition of distilled water and by freezing and thawing. A suitable dilution was made (usually 1:20) and the mixture centrifuged at 1000 g for 15 minutes. The decrease in optical density with time at 340 μm was determined in a Beckman model DB spectrophotometer. Generally, first order kinetics were observed. With very low reaction rates, however, the optical density curve was sigmoid and the straight central portion was used to determine EGOT. This sigmoid curve was probably caused by insufficient malic dehydrogenase in the system.\textsuperscript{13} EGOT values in 17 normal individuals (married, young adult males and females) were 1200 ± 250 units/ml of packed red cells.

**Case Reports**

**Case 1.**\textsuperscript{14} A 68-year-old Negro male was hospitalized for pulmonary tuberculosis and treated with INH (300 mg./day or 5 mg./Kg./day) and p-amino-salicylic acid (PAS). His course is depicted in Figure 1. The hematocrit value gradually fell from 33 per cent at the time of admission to 25 per cent 4 months later. He lost 30 pounds and appeared cachectic. A bone marrow aspirate was hypocellular. A biopsy was done and the marrow found to be cellular with partially megaloblastic red cell maturation. After 15 days of therapy with pharmacologic doses of vitamin B\textsubscript{12} (100 μg./day, i.m.) he was clinically unimproved despite a reticulocytosis and slight increase of hematocrit value. Accordingly, the daily injection of 0.5 mg. folic acid was begun. There was prompt clinical improvement, but incomplete laboratory follow-up precluded detection of a possible secondary reticulocyte peak. After 6 weeks the hematocrit reading was 40 per cent. A modified Schilling test was normal.\textsuperscript{15} Thereafter, he was treated nearly continuously with both vitamin B\textsubscript{12} and folic acid in the above-stated doses. Nevertheless, after another 6 weeks the hematocrit value had again fallen to 25 per cent. On the blood smear peripheral red cells were now hypochromic. Some erythrocytes contained Howell-Jolly bodies. There was moderate variation in their size and shape and target cells were seen (Fig. 2A). Erythrocyte precursors in the bone marrow had reduced hemoglobin formation, but bone marrow iron was increased. A few typical ringed sideroblasts were seen. Many other probable ringed sideroblasts were present, but the pronounced cytoplasmic accumulation of iron tended to obscure the fine perinuclear ring. Serum iron was 150 μg./100 ml., and the iron binding protein was completely saturated. He suffered from severe anorexia, especially in the morning, and breakfast was frequently vomited.

Six days after commencing parenteral injections of 100 mg. pyridoxine daily the reticulocytes had risen from 1.9 per cent to 16.6 per cent. The hematocrit value rose to 37 per cent within 2 weeks. Six to 8 hours after the first injection of pyridoxine the patient became semicomatose and appeared critically ill. By the next morning, after nonspecific therapy with intravenous fluids, he was alert and his appetite had returned with gusto. Pyridoxine administration was stopped after 6 days, but treatment with INH and PAS was continued. Six weeks after the last vitamin B\textsubscript{6} injection the hematocrit value had again fallen to 31 per cent, and his appetite and feeling of well-being were gone again. The ECOT was 273 units/ml red cells. At this point INH therapy was discontinued and 11 days later a mild persistent reticulocytosis developed. Anorexia and malaise gradually disappeared. The hematocrit value fell further to 27 per cent, then returned to 30 per cent. One month after INH therapy had been withdrawn, PAS administration was also stopped. A further improvement in well-being occurred and the hematocrit reading rose progressively to 38 per cent in 4 weeks. The ECOT had gradually risen after INH therapy was stopped and by now was 934 units/ml red cells.

At this time parenteral pyridoxine administration (10 mg./day for 4 days, and 100 mg./day for 2 days) resulted in no further clinical or laboratory improvement except for a further increase in the EGOT value. INH was then given in double the previous dose for 10 weeks. During this time the hematocrit value fell to 34 per cent, and mild paresthesias
Fig. 1—Hospital course of Case 1. See text for discussion. Note gaps in time scale.
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Fig. 2.—A: Peripheral blood, Case 1, day 221, before initial treatment with pyridoxine. Note target cells, hypochromia, and Howell-Jolly body (arrow). B: Peripheral blood, Case 1, day 636. Although the patient was taking INH and PAS but not pyridoxine, his hematocrit value had been 34–38 per cent for at least 5 months before and 6 months after this smear was made. C: Peripheral blood, Case 2, just prior to therapy with pyridoxine. It was the same four months previously when the bone marrow was done. D: Peripheral blood, Case 2, at the time of readmission to the hospital with a normal hematocrit reading. Note Howell-Jolly body (arrow). E: Peripheral blood, Case 3, at the time of admission to the hospital. F: Peripheral blood, Case 3, 9 months later.

developed. Red cell maturation in the bone marrow was normoblastic. This time no ringed sideroblasts were seen. Again the ECOT had dropped below 300 units/ml. Thirteen days after the last INH and 10 days after the start of a 4-day course of pyridoxine, (100 mg./day, i.m.) a minimal increase of reticulocyte count occurred (from 1.7 per cent to 3.2 per cent). The ECOT promptly increased. Cessation of INH therapy and administration of pyridoxine had again been followed by marked improvement in well-being and appetite with a gain in weight. The paresthesias rapidly disappeared.

After 3 months without any therapy his red blood cells were tagged with Cr 51 to measure the autologous red cell survival. When this study had been underway for 7 days, PAS was administered (3 Gm./day for 3 days; 6 Gm./day for 5 days; and then 9 Gm./day). On the
Fig. 3.—Survival of Cr$^{51}$ tagged autologous red cells in Case 1. The departure from linearity when INH was added is probably inherent in the technic rather than significant of accelerated hemolysis.$^{16}$

On the twenty-eighth day of the survival study the daily administration of 300 mg. of INH was added to his regimen. There was no significant change in the survival curve (Fig. 3) and no change in hematocrit value or reticulocyte count to suggest hemolysis. The T 1/2 was 24 days. He was known to have erythrocyte G-6-PD deficiency. The hemolysate assay and enzyme-electrophoresis were consistent with the primaquine-sensitive type of abnormality (G-6-PD-A$^c$).

The patient was then discharged from the hospital and continued on antituberculosis therapy. During the ensuing months his hematocrit value remained at or just below normal, and he had essentially no symptoms. Approximately 10 days after antituberculosis therapy was stopped, he had hemoptysis and was rehospitalized. Treatment with INH and PAS was reinstituted without concomitant administration of pyridoxine. Over the next month he again became anorexic, lost weight, and suffered a fall in hematocrit value from 40 to 30 per cent. There then was no clinical or laboratory change during 2 weeks of therapy with 10 mg. of pyridoxine daily, intramuscularly. Within 10 days, after the dose was increased to 50 mg./day, his appetite and sense of well-being had improved and by the fourteenth day the reticulocytes had reached 14 per cent. The hematocrit value slowly rose to 35 per cent where it remained. Intramuscular administration of 650 mg. of sorbitol$^*$ resulted in no additional hematologic response, nor did increasing the pyridoxine dose to 100 mg./day. Thereafter, the patient gradually slipped clinically and 3 months later quietly expired. Despite administration of up to 200 mg./day pyridoxine and the substitution of o-hydroxybenzal isonicotinyl hydrazine (Nupa-sal)$^f$ for INH, the hematocrit reading remained between 31 and 34 per cent during this period. The appearance of his peripheral red cells remained abnormal throughout (Fig. 2B). Postmortem examination was not done and cause of death is not accurately known.

By means of the spot test the patient's brother was also found to have G-6-PD deficiency.

Administration of INH for 10 weeks did not produce any hematologic change, nor was there hematocrit or reticulocyte response to pyridoxine therapy at the end of this time. During treatment with INH his ECOT fluctuated between 568 and 962 units/ml red cells. After 6 days of pyridoxine therapy (100 mg./day, orally) his ECOT was 1305.

Case 2. This 35-year-old Negro female was admitted for therapy of a diffuse pulmonary infiltrate believed to be tuberculous. She had been treated for anemia 6 years previously with blood transfusions. No further information about this episode is available. Her course is depicted in Figure 4A. At the time of admission to D. C. General Hospital her hematocrit value was 20 per cent and reticulocytes were 1.8 per cent. Her red cells were hypochromic and there were numerous target cells. There was normoblastic red cell hyperplasia in the bone marrow. Bone marrow iron was increased and typical ringed sideroblasts were present. After 6 days of hospital care, including a blood transfusion given on day 4, the hematocrit reading was 32 per cent and reticulocytes were 10 per cent. There was no hyperbilirubinemia and the Coombs test was negative. No evidence for bleeding was found. She was given a short course of intramuscular penicillin and daily medications consisting of 900 mg. ferrous sulphate for 6 weeks, INH (300 mg.), PAS (12 Gm.), one hexavitamin tablet (N.F.) (contains no folic acid, vitamin B12, or pyridoxine). She became afebrile and the hematocrit value rose slowly to about 35 per cent. After 6 weeks, Nupa-sal (750 mg./day) was substituted for INH because of a psychosis believed related to INH therapy. During the next 2 1/2 months the hematocrit value gradually fell to 29 per cent but the reticulocyte count remained between 1 and 2.5 per cent. The peripheral red cell morphology was unchanged (Fig. 2C) but now Howell-Jolly bodies were readily found. The bone marrow was not examined at this time. Because the ECOT was 280 units/ml, a very low value, pyridoxine was administered intramuscularly. 100 mg./day, for 10 days and there was an excellent hematologic response. Nupa-sal and PAS therapy were continued. The hematocrit value reached 47 per cent on one occasion, but stabilized at about 43 per cent. Her red cell G-6-PD was normal by assay, but on starch gel electrophoresis showed a double band consistent with heterozygosity for the normal enzyme and the G-6-PD-A or the G-6-PD-A- variant. After an additional 11 months she was readmitted with anasarca. The hematocrit value was 40 per cent and red cell morphology abnormal as before (Fig. 2D). By cellulose acetate electrophoresis of her hemolysate, hemoglobin A2 was found to be 2.4 per cent (normal range 2.0 to 3.8 per cent). Alkali resistant hemoglobin was 1.5 per cent and the remainder was the normal adult pigment. In the clinic she had been given Nupa-sal and PAS but no further pyridoxine.

Case 3. This 103-year-old Negro female was admitted to the hospital because of pulmonary tuberculosis and treated with INH and PAS. Her course is depicted in Figure 4B. During the first 2 months in the hospital the hematocrit reading was between 35 and 39 per cent. During the third month, however, it fell to 27 per cent. Reticulocytes were nearly absent from the peripheral blood. Her red cells appeared normal in size but there was some hypochromia. A few target cells were seen, but no Howell-Jolly bodies were found (Fig. 2E). A bone marrow aspirate was interpreted as partially megaloblastic and therapy was begun with the daily injection of 200 μg. folic acid after a serum test for vitamin B12 absorption was normal.15 The marrow specimen was inadequate for examination with the Prussian Blue stain. On the fourth day of this therapy the serum iron concentration was 114 μg./100 ml. and the serum iron binding protein was 76 per cent saturated. On the fifth day, the hematocrit value was 23 per cent and the reticulocyte count was 0.5 per cent. On the eighth day, the hematocrit value was 22 per cent and the daily intramuscular injection of 100 mg. of pyridoxine was begun. That evening, 6 to 8 hours after the first dose of vitamin B6, the patient became semicomatose and appeared critically ill. By the next morning, however, after nonspecific therapy with intravenous fluids, she was alert and hungry. There was a good hematologic response to this therapy and after 2 months the hematocrit value was stabilized at about 36 per cent despite a trial of larger doses of pyridoxine. After an additional 7 months the hematocrit reading was unchanged, but red cell morphology was still not normal (Fig. 2F). Her red cell G-6-PD was normal by assay and by electrophoresis.
Discussion

In each of these cases there was hypochromic anemia which responded to the parenteral administration of pyridoxine. Target cells were observed in the peripheral blood of all three cases and Howell-Jolly bodies in the erythrocytes of two. With treatment, the red cell morphology improved but did not become normal. In Case 1, a response also followed cessation of INH therapy. During this period the patient was hospitalized and there was no change in diet. He relapsed with further INH administration and had another response to vitamin B₁₂. After a later relapse during INH therapy, he showed no improvement with the injection of 10 mg./day of pyridoxine but had a clinical and reticulocyte response to the injection of 50 mg./day. This sequence implicates pyridoxine antagonism by INH in the pathogenesis of his anemia and suggests that the patient needed between 10 and 50 mg. of pyridoxine to balance the daily ingestion of 300 mg. INH.

For Cases 2 and 3, the relationship to INH or Nupa-sal is less clear. INH was discontinued after 6 weeks in the second patient because of a psychotic episode and Nupa-sal therapy was substituted. The latter drug has not been shown to be a pyridoxine antagonist although it is apparently metabolized to INH. No anemic relapse occurred with continued Nupa-sal therapy, even though pyridoxine administration was not continued. The third patient was initially given folic acid because the bone marrow maturation was partially megaloblastic and B-12 absorption was normal. Inadvertently, pyridoxine therapy was added before the therapeutic trial with folic acid was completed. Nevertheless, the timing of the reticulocytosis suggests that the patient responded to the addition of pyridoxine rather than to the administration of folic acid alone. The first increase of reticulocyte count was noted 3 days after the initial dose of pyridoxine, or a full 11 days after the initial dose of folic acid. The peak reticulocyte count was 5 days after pyridoxine therapy was started,
or 13 days after folic acid administration was begun. Nevertheless, it is possible that this patient had a defect involving both vitamins. A small group of individuals with pyridoxine-responsive anemia have megaloblastic erythroid maturation in the bone marrow. A few of these appear to respond to folic acid with a mild reticulocytosis, a moderate increase of hematocrit value, and a reversion of the marrow maturation to normoblastic. They then have a more striking response to pyridoxine.

The somnolent reaction observed in Cases 1 and 3 after the administration of large doses of pyridoxine deserves comment. During experimental treatment of tuberculosis with large doses of INH (15 to 20 mg./Kg./day), central nervous system stimulation and excitement were often noted. When these large doses of INH were discontinued, a temporary depressive reaction sometimes ensued. Certain of the essential brain enzymes are pyridoxine-dependent. Although the mechanism is not known, it seems reasonable that the somnolence was a reaction to sudden flooding of the body with pyridoxine.

The mechanism for the induction of pyridoxine-responsive anemia by INH is unknown. The drug probably exerts its anti-pyridoxine effect by forming a hydrazone compound with pyridoxal-5-phosphate (PLP), the active coenzyme. This complex is inactive enzymatically and is excreted in the urine. In vitro INH is able slowly to remove PLP from the holoenzyme, leaving an inactive apoenzyme. Enzyme inhibition by removal of the coenzyme differs from the usual mechanism of enzyme inhibition. Pyridoxine has been implicated as a coenzyme in a wide variety of biochemical reactions involving amino acids. Studies in cell-free systems have shown that it is a necessary cofactor to ALA-synthetase in the synthesis of delta-aminolevulinic acid from glycine and succinyl-CoA. Although in some animals pyridoxine deficiency results in a hypochromic microcytic anemia, in others it may impair the erythropoietic response to blood loss instead of producing anemia. Furthermore, stressing different metabolic pathways by varying the amino acid content of the diet may alter the nonhematologic manifestations of pyridoxine deficiency in animals. Hence, pyridoxine-responsive anemia in the first two cases could have been conditioned by the episode of increased erythropoiesis that occurred in each during INH therapy 4 months previously. In the first patient this was a response to the administration of vitamin B12 and folic acid. He was also G-6-PD deficient and might have had continued, slowly accelerated hemolysis from his drugs or infection. Jewish G-6-PD deficient subjects may have hemolytic episodes from PAS. Primaquine-sensitive Negro males have also been observed to have mild brief accelerated hemolysis from INH, PAS, or both together. There was no detectable hemolysis from these drugs during the Cr-51 red cell survival study, but the PAS dosage was inadvertently held at 9 Gm./day instead of being increased to a more usual 12 Gm./day. The reason for the reticulocytosis in the second case is not certain. Although the patient described by Erslev et al. had a hematologic response to the transfusion of whole blood as well as to treatment with pyridoxine, our patient's reticulocytosis was but 2 days after a transfusion. Consequently, it may have had
another cause such as an undetected episode of bleeding several days previously or an improved diet after hospitalization.

Nevertheless, rather than the induction of pyridoxine-responsive anemia by accelerated erythropoiesis and INH, it would appear more likely that each patient has an enzymatic defect rendering his bone marrow more susceptible to pyridoxine deprivation by INH. None became hematologically normal. Patients 1 and 3 remained anemic and all three patients retained a considerable abnormality of red cell morphology. Furthermore, Case 3 had no recognized episode of increased erythropoiesis prior to the development of pyridoxine-responsive anemia.

Glutamic-oxaloacetic transaminase like other transaminases is a pyridoxine-dependent enzyme. In a few reported instances, the concentration of this enzyme in the serum has been normal in patients with pyridoxine-responsive anemia. It has been suggested that the EGOT might be of greater worth, but no values for the EGOT have been reported in this type of patient. The red cell concentration of this enzyme may fall during INH therapy, but rarely to very low levels. In Case 1 the EGOT level was very low before administration of pyridoxine and rose with treatment. It roughly paralleled the need for vitamin B₆ as determined by other parameters. In Case 2 the very low EGOT level led to the administration of pyridoxine. Sass and Spear found EGOT concentrations to be normal or elevated in their patients with anemia from a variety of causes. The measurement of EGOT deserves further investigation for the evaluation of patients with pyridoxine-responsive anemia.

Summary

Three patients are described who had a pyridoxine-responsive anemia while under treatment for pulmonary tuberculosis with INH and PAS. The red cells were hypochromic and target cells were common. Two cases also had microcytosis and one had ringed sideroblasts in the bone marrow. None became hematologically normal with therapy. A somnolent reaction occurred in two of the patients following the parenteral administration of large amounts of pyridoxine, and seemed related to it. The concentration of erythrocyte glutamic oxaloacetic transaminase was less than 25 per cent of the normal mean in two of the patients at the time of relapse.

Summario in Interlingua

Es describite 3 patientes qui manifestava un anemia responsive a pyridoxina durante que illes esseva sub tractamento pro tuberculosis pulmonar con hydrazida de acido isonicotinic e acido p-amino-salicylic. Le erythrocytos esseva hypochromic, e cellulas in forma de cappello mexican esseva commun. In 2 casos, unassociate microcytosis esseva constatate, e in un sideroblastos annulate esseva observate in le medulla ossee. Le condition non se renormalisa vento hematologicamente durante le therapia. Un reaction somnolente occurreva in 2 del patientes post le administration parenteral de grande quantitates de pyridoxina e pareva esser relationate con illo. Le concentration de
transaminase glutamic-oxaloacetic in le erythrocytos esseva minus que 25 pro cento del valor medie normal in 2 del pacientes al tempore de un recidiva.

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