Pyridoxine Responsive Anemia

By John N. Bickers, Charles L. Brown and Charles C. Sprague

With the technical assistance of Katherine Karst

Naturally occurring vitamin B₆ deficiency in adult man was unknown until 1956 when Harris et al.¹ described an individual with a hypochromic microcytic anemia which responded to the administration of pyridoxine. Since that time nine additional cases have been reported in the literature.²⁻⁹ The information available suggests that these patients do not have a simple dietary deficiency in the ordinary sense, but some metabolic disturbance which results in abnormal vitamin B₆ requirements.

A general picture of pyridoxine responsive anemia is beginning to emerge which closely resembles that seen in vitamin B₆ deficient animals with nine of the ten reported cases exhibiting hypochromia, microcytosis, and elevated serum iron levels. The patient of Maier's⁵ with macrocytosis and red cell maturation arrest is the single exception. There is uniform failure of response to iron and other common hematinsics. In two instances, anemia was known to have been present since birth; whereas in the remainder it was discovered at ages ranging from 26 to 54 years. In one patient, normal blood values were obtained three months prior to the appearance of illness,⁴ but in the other patients, the onset of anemia could not be documented with certainty. All the reported instances have occurred in males. Splenomegaly and shortening of red cell survival appear to be common findings. Erythroid hyperplasia of the bone marrow is an almost constant feature, with only one patient showing hypoplasia.⁷ In all patients so examined, there has been extensive hepatic hemosiderosis. Response to treatment with pyridoxine has been incomplete in four patients with varying degrees of anemia and red cell morphologic abnormalities persisting, the two patients with congenital anemia showing the poorest responses. In general, pyridoxine responsive anemia closely resembles thalassemia with the absence of elevated fetal and A₂ fraction hemoglobin levels being the chief distinguishing features. In no instance have other signs of vitamin B₆ deficiency appeared, such as neurologic and dermatologic abnormalities.

The present report describes a patient with a chronic, hypochromic, microcytic anemia who exhibited a partial response to the administration of pyridoxine. In addition to representing another instance of a rare condition, the patient presents some features which we believe to be of particular interest. The patient was originally reported by Stiles et al.¹⁰ in 1946 as a case of Mediterranean anemia, and hematologic data spanning 22 years are available. This is the first reported patient in whom splenectomy was carried out and it was at-
tended by an unusually severe series of thrombotic episodes. In addition, an attempt was made to estimate the patient's vitamin B₆ requirements.

**CASE REPORT**

W. H. (N.O. V.A.H. A-1003), a Negro male, was born in 1910 and his significant medical history relates entirely to the illness to be described.

From 1939, when it was first discovered, until 1955, the patient had a stable and asymptomatic hypochromic anemia which was unresponsive to iron. Slight hepatosplenomegaly was first noted in 1944. Aside from a value of 5 Gm. per 100 ml. at the time of an acute infection, his hemoglobin values ranged from 9 to 11 Gm. per 100 ml., red cell counts averaged 5 million per cu. mm., and hematocrits averaged 35 to 37 per cent.

In the summer of 1955, a rather sharp deterioration in his health occurred, and in September he was admitted to the New Orleans Veterans Administration Hospital. He complained of marked weakness, dyspnea and abdominal pain. Pallor was apparent as were hepatosplenomegaly, cardiomegaly, and clinical signs of congestive heart failure.

On admission his red cell count was 4.3 million per cu. mm., hemoglobin content 6.1 Gm. per 100 ml., and hematocrit 26 per cent. The white cell count was 12,500 per cu. mm. with a normal differential. Reticulocytes averaged 3–5 per cent and occasional normoblasts were seen. The red cells were intensely hypochromic with marked variations in size and shape. Many bizarre forms were apparent and target cells were common. The normal marrow exhibited extreme erythroid hyperplasia with no evidence of maturation arrest. A few random urine urobilinogen determinations were normal. A Cr₅¹ red cell survival determination revealed a T₅¹ value of 17 days.

The hemoglobin level did not rise, and occasional transfusions were required to maintain cardiac compensation. In January 1956, splenectomy was carried out. The spleen which weighed 935 Gm. exhibited slight extramedullary hematopoiesis as well as moderate hemosiderosis. Postoperative platelet counts were four to six times the upper limits of normal and the white cells increased to 20,000 per cu. mm. Inclusion bodies appeared in 70 to 90 per cent of the red cells. These bodies gave a positive Prussian blue reaction for iron and were peroxidase negative. His hemoglobin level failed to rise following splenectomy, and a follow-up red cell survival determination revealed a T₅¹ value of 14 days. Numerous normoblasts appeared in the peripheral blood. Thrombophlebitis was troublesome but not severe at this time.

With the aid of occasional transfusions, his hemoglobin level averaged 7.5 Gm. per 100 ml. until the sixth postoperative month when there was a sudden and unexpected rise to 12.8 Gm. per 100 ml. Questioning disclosed that on the advice of a friend, he had begun taking an oral hematonic containing vitamin B₁₂, ferrous sulfate, liver fraction II, folic acid, thiamine, riboflavin, nicotinamide, pyridoxine and calcium pantothenate (Iberol). The remission was well maintained with two tablets of the preparation daily, and hemoglobin levels fell to 6–7 Gm. per 100 ml. when it was stopped. All components of the preparation were administered individually, and it was shown that response occurred only to pyridoxine and that this was as effective in maintaining a remission as the whole product. A specific response to pyridoxine was demonstrated on two occasions, the average hemoglobin rise being 5 Gm. per 100 ml. Administration of the drug produced a striking subjective improvement in strength and sense of well-being that preceded the hemoglobin rise.

Each remission induced by pyridoxine was associated with severe venous thrombosis. At times, virtually all palpable veins were thrombosed. Showers of pulmonary emboli occurred with resulting cor pulmonale. Priapism was a particularly distressing event. Anticoagulation and a femoral vein ligation were utilized during this period. Episodes of thrombophlebitis recurred until the spring of 1959.

Maintenance therapy with oral pyridoxine hydrochloride, 25 mg. per day, maintained his hemoglobin level at 11 to 12 Gm. per 100 ml., and there was a slow, gradual fall in his platelet count until it averaged 600,000 per cu. mm. in the summer of 1959.

On September 1, 1959 pyridoxine was discontinued, and on September 10 the patient
was admitted to the hospital for the purpose of studying him in greater detail. He was placed on a diet consisting of foods about which there was general agreement regarding vitamin B₆ content. It was rather monotonous and ultimately depressed his food intake somewhat, but good nutrition was maintained. Hemograms and glutamic-oxalacetic transaminase (SGOT) levels were obtained twice weekly. Serum iron determinations were carried out weekly as were the administration of tryptophane loads (10 Gm. d, 1, tryptophane). Ferrokinetic studies and bone marrow aspirations were performed when full relapse occurred and were repeated when maximal hemoglobin values were obtained. When the hemoglobin level fell to 7 Gm. per 100 ml., about eleven weeks after stopping pyridoxine, small incremental supplements of pyridoxine hydrochloride were given intramuscularly.

The data obtained during this period are summarized in figure 1 and table 1. On paper electrophoresis only hemoglobin A was demonstrated. Fetal hemoglobin content was less than 1.5 per cent and the A₂ hemoglobin fraction was 2.6 per cent. White cell alkaline phosphatase was normal. The red cells showed extreme hypochromia with many leptoctyes and target cells. Microcytes were common as were a variety of pleomorphic forms. Normoblasts were common in the peripheral blood during relapse, but there was no detectable difference in red cell morphology or in the percentage of siderocytes. Red cell fragility was not affected by pyridoxine (hemolysis complete at .20 per cent NaCl in both instances). Withdrawal of pyridoxine was associated with definite marrow changes. In contrast to the typical normoblastic hyperplasia seen while he was on supplemental pyridoxine, there was considerable immaturity of the red cell elements. Large basophilic normoblasts were numerous and the nuclear chromatin showed delayed maturation. The other marrow elements exhibited no changes. No increase in xanthurenic acid or indolacetic acid excretion was noted during relapse (xanthurenic acid excretion did not exceed 23 mg. per 24 hours after

![Graph](https://via.placeholder.com/150)

**Fig. 1.**—Relationship between total estimated vitamin B₆ intake and hemoglobin level (HGB), reticulocyte count, and mean corpuscular hemoglobin concentration (MCHC).
PYRIDOXINE RESPONSIVE ANEMIA

Table 1.—Comparative Data Obtained Before and After Supplemental Pyridoxine

<table>
<thead>
<tr>
<th></th>
<th>Off Pyridoxine</th>
<th>On Pyridoxine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cell count (X 10^6/mm^3)</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Hematocrit (per cent)</td>
<td>30</td>
<td>38</td>
</tr>
<tr>
<td>Mean corpuscular volume (cu. µ)</td>
<td>75</td>
<td>70</td>
</tr>
<tr>
<td>White cell count (X 10^3/mm^3)</td>
<td>38</td>
<td>34</td>
</tr>
<tr>
<td>Platelets (X 10^4/mm^3)</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>Serum iron (µg. per cent)</td>
<td>400</td>
<td>360</td>
</tr>
<tr>
<td>Plasma iron turnover (mg./day/100 ml. blood)</td>
<td>7.3</td>
<td>7.7</td>
</tr>
<tr>
<td>Cr^{51} RBC T ½ days</td>
<td>14, 17</td>
<td>18.5, 19</td>
</tr>
</tbody>
</table>

*Normally less than 1 mg. per day 100 ml. of whole blood.

In November 1960 the patient was re-admitted with typical, uncontrolled diabetes mellitus which ultimately required 30 to 35 units of long acting insulin for control. There is no family history of diabetes and he is not obese. A glucose tolerance test in 1944 was normal. A needle biopsy of the liver in August 1961 disclosed marked hemosiderosis with most of the pigment in the parenchymal cells. The degree of iron deposition was as great as is seen in typical idiopathic hemochromatosis. This iron excess cannot be accounted to transfusions as he has received less than 5,000 ml. of whole blood. Hepatic morphology was well preserved.

The patient is maintained on pyridoxine 25 mg. daily with hemoglobin levels averaging 11 Gm. per 100 ml. Phlebotomies have been started in an effort to reduce his iron stores.

Family studies are summarized in table 2. Stiles et al. reported his mother to be anemic, but she is no longer living. A sister and his five children were available for limited study. Hypochromia was apparent in all, but only in Y.H. and B.H. was it more than minimal. Occasional microcytes were noted in B.H., E.N., and Y.H. A few target cells were present in U.C. All smears exhibited slightly more than normal variation in red cell size and shape. The minimal nature of the over-all changes is emphasized by the blood indices.

**DISCUSSION**

Vitamin B_6_ is composed of three compounds: pyridoxine, pyridoxal, and pyridoxamine; each of which may be metabolized to the active form, pyridoxal-5-phosphate. The latter compound serves as a coenzyme for a variety of reactions involving decarboxylation and transamination and is essential in heme formation, being a participant in the formation of delta-aminolevulenic acid

Table 2.—Family Data (V. C. is a Sister—The Remainder Are Children of the Patient)

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Serum Fe (µg. %)</th>
<th>Hgb. (Gm./100 ml.)</th>
<th>Hemat. (%)</th>
<th>RBC (X 10^6/mm^3)</th>
<th>MCV</th>
<th>MCHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. C.</td>
<td>40</td>
<td>F</td>
<td>100</td>
<td>13.0</td>
<td>40.5</td>
<td>4.7</td>
<td>89</td>
</tr>
<tr>
<td>W. H.</td>
<td>8</td>
<td>F</td>
<td>200</td>
<td>12.0</td>
<td>36.0</td>
<td>4.5</td>
<td>80</td>
</tr>
<tr>
<td>A. H.</td>
<td>7</td>
<td>M</td>
<td>140</td>
<td>13.7</td>
<td>38.5</td>
<td>4.7</td>
<td>84</td>
</tr>
<tr>
<td>Y. H.</td>
<td>6</td>
<td>F</td>
<td>165</td>
<td>12.8</td>
<td>37.0</td>
<td>4.3</td>
<td>88</td>
</tr>
<tr>
<td>E. H.</td>
<td>4</td>
<td>M</td>
<td>70</td>
<td>11.5</td>
<td>35.5</td>
<td>4.4</td>
<td>81</td>
</tr>
<tr>
<td>B. H.</td>
<td>1.5</td>
<td>F</td>
<td>Smear only available</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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from glycine and succinic acid.\textsuperscript{13,14} This provides a logical explanation for the marked hemoglobin deficiency seen in vitamin B\textsubscript{6} deficient animals and in humans with pyridoxine responsive anemia. Biochemical evidence of vitamin B\textsubscript{6} deficiency is generally manifested by increased urinary excretion of xanthurenic acid, kynurenin, and kynurenic acid, as well as a failure to convert tryptophane to N-methyl-nicotinamid.\textsuperscript{15,16}

Two phases of the patient’s illness are apparent, the first presumably a congenital hypochromic anemia and the second, appearing late in life, related to pyridoxal need. His basic anemia closely simulates thalassemia. However, the normal fetal and A\textsubscript{2} hemoglobin levels together with the paucity of abnormal findings in his children makes this unlikely. His primary anemia appears to be of the congenital, hypochromic, iron-loading type which was first well described by Rundles and Falls\textsuperscript{17} and more recently reviewed by Byrd and Cooper.\textsuperscript{18}

The hematologic data reflect multiple abnormalities in erythrokinetics. Hemoglobin synthesis or the incorporation of hemoglobin into the erythron is markedly depressed. An intracorpuscular red cell defect is apparent from the shortened survival time and the abnormal morphologic changes observed. There is gross collection of non-heme iron in the red cells, and plasma iron turnover is increased about sevenfold. The extreme elevation of the latter is probably related to the recirculation of siderocyte iron\textsuperscript{19} as well as to the shortened red cell survival time. The additional defect imposed by vitamin B\textsubscript{6} need manifests itself largely by impairment of red cell production as is evident from the “left-shift” seen in the red cell series of both the marrow and the peripheral blood. The previously existing defect in red cell hemoglobin content is further intensified by vitamin B\textsubscript{6} need. The known actions of pyridoxal as a coenzyme lead us to the interpretation that its deficiency in this patient depresses heme synthesis and interferes with protein metabolism essential to normal cell maturation.

No other metabolic defects attributable to vitamin B\textsubscript{6} deficiency were detected although this may be due, in part, to the selection of xanthurenic acid as the measured by-product of tryptophane metabolism. Abnormal excretion of xanthurenic acid is common, but by no means constant, in these cases, and it is likely that kynurenine and kynurenic acid are more sensitive indicators of pyridoxine deficiency as noted by Raab et al.\textsuperscript{7} The slight fall in serum SGOT observed during relapse is probably only a reflection of decreased total red cell destruction. Whole blood transaminase levels would have been more indicative of true transaminase activity.

The major question raised by these cases is: Why do they exist at all? One cannot easily explain them on the basis of a simple dietary deficiency of vitamin B\textsubscript{6}. Estimates of vitamin B\textsubscript{6} requirements for the adult range from 3.0 \(\mu\text{g}\)\textsuperscript{15} to 1.0 \(\text{mg}\)\textsuperscript{20} per day, amounts which are readily attainable. Attempts at producing vitamin B\textsubscript{6} deficiency in adults by dietary restriction alone have been uniformly unsuccessful although it has been produced experimentally and seen clinically in infants.\textsuperscript{16} The only other instances of pyridoxine deficiency in adults have occurred following the use of drugs such as desoxypyridoxine.
and isonicotinic acid hydrazide which compete biochemically with vitamin B₆. There is little to suggest dietary inadequacies in the cases of pyridoxine anemia with the exception of the patient of Verloop and Rademaker who spent three years in a concentration camp over ten years prior to the known onset of anemia. Careful questioning failed to reveal any gross dietary inadequacies in our patient. Estimating vitamin B₆ intake, like its requirements, is at best inexact. Our studies indicate that his daily requirement for vitamin B₆ is approximately 2.5 mg., but we feel this estimate serves only to indicate that his needs are in excess of any estimates of normal requirements. His requirements are close enough to estimated physiologic requirements, however, to indicate that the effect of pyridoxine in this patient is specific and not "vicarious."

One is forced to resort largely to speculation to explain an increased need for the vitamin. There is little evidence to support or deny the existence of a decreased absorption rate. It has been shown that accelerated erythropoiesis in thalassemia results in increased requirements for folic acid, and in patients such as ours and the one reported by Erslev et al., it is conceivable that a similar mechanism may be operative. Most of the clinical data tend to favor the hypothesis put forth by Bishop and Bethell that increased iron stores in some way bind pyridoxal and render it ineffective. Certainly, iron excess is a common denominator in all cases of pyridoxine responsive anemia and is well established by the time the anemia is discovered. Such a mechanism would readily explain the superimposition of a relative vitamin B₆ deficiency upon a case of chronic anemia of another etiology. At least a partial answer to this part of the problem should be forthcoming soon as Harris and Verloop and Rademaker are performing venesections on their respective patients in an effort to reduce iron stores. An alternate experimental approach to this would be to observe the effect of a pyridoxine deficient diet upon an individual with typical idiopathic hemochromatosis.

The cases of pyridoxine responsive anemia reported to date appear to fall into two groups. In approximately half of the cases it occurs as a secondary feature in the course of a chronic, hypochromic anemia with hyperferricemia and iron-loading. In the remainder it is seen in association with iron-storage excess without other obvious hematologic defects. This suggests an etiologic relationship between the two groups, as in each group iron excess is present and apparently precedes the pyridoxal defect. However, as logical as it may appear to explain increased pyridoxine need on the basis of binding by excess tissue iron, it must be remembered that the actions of vitamin B₆ are complex and its deficiency will itself result in increased iron absorption in experimental animals. In addition, one must account for the fact that only a small percentage of individuals with equivalent degrees of iron excess develop pyridoxine responsive anemia.

It is probable that pyridoxine responsive anemia has a congenital basis although the same defect may not be common to all patients. Anemia was present from birth in at least two patients and presumably the present case. Medal et al. reported pyridoxine responsive anemia in siblings, and the patient described by Bishop and Bethell was associated with other instances of
hypochromic anemia in the family. Verloop and Rademaker found elevated serum iron levels in their patient's three children in the absence of other hematologic abnormalities. We believe that iron metabolism is abnormal in some of our patient's children but do not believe the available data warrant any firm conclusions. The apparent relationship between pyridoxine responsive anemia, congenital hypochromic iron-loading anemia, and hemochromatosis appears to be an important one which may be clarified by careful family studies.

The severe post-splenectomy thrombotic complications were a singular feature of the patient's illness. Such complications in the face of high siderocyte levels have been reported by other authors. Six of eight reported adults with hypochromic iron-loading anemia who have undergone splenectomy have suffered fatal thrombotic episodes. In addition, vascular thrombosis appears to be common in these patients without splenectomy. In our patient, pyridoxine undoubtedly played a role as immediate postoperative thromboembolic problems were not as great with maximal platelet levels and normal blood values maintained by transfusions as they proved to be later following the administration of pyridoxine. Once platelet levels had fallen to about twice the normal level, the administration of pyridoxine produced only minor venous thrombosis. Platelet counts were not affected by pyridoxine. It appears that an extreme hypercoagulable state immediately after splenectomy was prevented by the associated pyridoxine deficiency. We believe the pyridoxine corrected some platelet defect although changes in any of the clotting factors or the vascular endothelium could have been responsible.

SUMMARY
A patient is reported with pyridoxine responsive anemia superimposed upon a chronic hypochromic anemia with excess iron storage. A specific response to pyridoxine was demonstrated on several occasions with restoration of the blood picture to its original state. Dietary estimates of vitamin B intake demonstrated an increased requirement for the vitamin, approximately 2.5 mg per day being required for hematologic remission. Splenectomy was followed by high circulating siderocyte levels and severe thromboembolic problems. The possible mechanisms for the development of pyridoxine responsive anemia are discussed briefly.

SUMMARY IN INTERLINGUA
Es reportate le caso de un patiente con anemia responsive a pyridoxina e imponite super chronic anemia hypochromic con excessive magasinage de ferro. Un responsa specific a pyridoxina esseva demonstrate in plure occasiones, con restauration del hemogramma a su stato original. Estimationes dietari del ingestion de vitamina B esseva demonstrata un augmento del requirimento pro iste vitamina. Approximativemente 2,5 mg per die esseva requisite pro le remission hematologic. Splenectomia esseva sequite per alte concentrationes de siderocytos in le circulation e sever problemas thromboembolique. Es discuimite brevemente le mechanismos possiblemente responsabile pro le disveloppamento de anemia responsive a pyridoxina.
PYRIDOXINE RESPONSIVE ANEMIA

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