Pyridoxine Responsive Anemia

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

With the technical assistance of Katherine Karst

 Naturally occurring vitamin B\(_6\) 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.\(^2\) 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 B\(_6\) requirements.

A general picture of pyridoxine responsive anemia is beginning to emerge which closely resembles that seen in vitamin B\(_6\) deficient animals with nine of the ten reported cases exhibiting hypochromia, microcytosis, and elevated serum iron levels. The patient of Maier's\(^5\) 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 A\(_2\) fraction hemoglobin levels being the chief distinguishing features. In no instance have other signs of vitamin B\(_6\) 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 sternal 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 T¹/₂ 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 T¹/₂ 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 hematinc 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 leptocytes 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 0.20 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](image-url)  
Fig. 1.—Relationship between total estimated vitamin B₆ intake and hemoglobin level (HGB), reticulocyte count, and mean corpuscular hemoglobin concentration (MCHC).
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 Fe59 T½ min.</td>
<td>37</td>
<td>29</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 T½ days</td>
<td>14, 17</td>
<td>18.5, 19</td>
</tr>
</tbody>
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*Normally less than 1 mg. per day 100 ml. of whole blood.11

Pyridoxine responsive anemia (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>
This provides a logical explanation for the marked hemoglobin deficiency seen in vitamin B₆ deficient animals and in humans with pyridoxine responsive anemia. Biochemical evidence of vitamin B₆ 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-nicotinamide.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 A₂ 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 Falls¹⁷ and more recently reviewed by Byrd and Cooper.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 iron¹⁹ as well as to the shortened red cell survival time. The additional defect imposed by vitamin B₆ 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 B₆ 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 B₆ 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.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 B₆. Estimates of vitamin B₆ requirements for the adult range from 3.0 µg to 1.0 mg per day, amounts which are readily attainable. Attempts at producing vitamin B₆ deficiency in adults by dietary restriction alone have been uniformly unsuccessful although it has been produced experimentally and seen clinically in infants.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 Rademaker⁶ who spent three
years in a concentration camp over ten years prior to the known onset of ane-
mia. 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,²¹ and in patients
such as ours and the one reported by Erslev et al.,⁸ 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,³ 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.²² In addition, one must account for the fact that only a small per-
centage of individuals with equivalent degrees of iron excess develop pyridoxine
responsive anemia.

It is probable that pyridoxine responsive anemia has a congenital basis al-
though the same defect may not be common to all patients. Anemia was
present from birth in at least two patients and presumable the present case.
Medal et al.⁹ reported pyridoxine responsive anemia in siblings, and the patient
described by Bishop and Bethell³ 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 B₆ 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.

Summario 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 B₆ demonstrava un augmento del requiriment pro iste vitamina. Approximativemente 2.5 mg per die esseva requirite pro le remission hematologic. Splenectomia esseva sequite per alte concentrationes de siderocytos in le circulation e sever problemas thromboembolic. Es discutite brevemente le mechanismos possiblemente responsabile pro le disveloppamento de anemia responsive a pyridoxina.
PYRIDOXINE RESPONSIVE ANEMIA

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REFERENCES

John N. Bickers, M.D., Chief, Pulmonary Disease Section, Veterans Administration Hospital, and Instructor in Medicine, Tulane University School of Medicine, New Orleans, La.

Charles L. Brown, M.D., Instructor in Medicine, Tulane University School of Medicine, New Orleans, La.

Charles C. Sprague, M.D., Associate Professor in Medicine, Tulane University School of Medicine, and Consultant in Hematology, Veterans Administration Hospital, New Orleans, La.

Katherine Karst, B.S., A.S.C.P., Medical Technologist, Veterans Administration Hospital, New Orleans, La.
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JOHN N. BICKERS, CHARLES L. BROWN, CHARLES C. SPRAGUE and Katherine Karst