Pyridoxine-Responsive Anemia: Report of 2 Cases in Brothers and a Review of the Literature

By Luis Sanchez Medal, Jorge Elizondo, Jesus Torres Gallardo and Carlos Gittler

In 1953 Synderman et al. demonstrated that vitamin B₆ has a role in human hematopoiesis by producing anemia in an infant fed with a vitamin B₆-free synthetic diet. In 1956 Harris et al. reported the first patient with pyridoxine-responsive anemia (PRA). Since this report appeared, further similar cases have been published.

In the present article, two brothers with PRA are reported. The pyridoxine responsiveness of the anemia was ascertained in one of them and seemed likely in the other, due to the marked clinical and laboratory resemblances with his brother and to his recovery while receiving a parenteral vitamin B complex-containing pyridoxine.

Case 1

A butcher, 54 years old, was first seen on August 4, 1958. He had been in good health except for a moderate polyuria and polydipsia during the previous year. Two weeks before consultation he had noticed weight loss (6 Kg.) and symptoms of anemia. There was no known bleeding.

Obesity had been present since he was 25 years old. His daily diet during the month period previous to his hospitalization included 2027 calories, with 250 Gm. of carbohydrates, 96 Gm. of proteins, 67 Gm. of fats, and about 2.5 mg. of vitamin B₆.

Physical examination.—Weight 109 Kg. (ideal weight 70 Kg.); pulse 120; B. P. 130/60. Important findings were: very marked pallor, subicterus, hepatomegaly (liver edge at 9 cm. below the costal margin in the midclavicular line), splenomegaly (the tip of the spleen was palpable on normal breathing), slight ankle edema, and presence of a presistolic gallop rhythm. There were acanthosis nigricans-like lesions in the neck, the axillae, the groins, and the pubis, probably related to the obesity.

Laboratory data.—Venous blood pressure: 20 cm. of water. Six stool examinations were negative for parasites and blood. Urine: normal, except for the presence of glucose. Blood chemistry: glucose 225 mg.; urea 43 mg.; creatinine 1.55 mg.; uric acid 4.4 mg. Serologic tests for syphilis: negative. Liver function tests: direct bilirubin 0.50 mg.; indirect bilirubin 0.56 mg.; bromosulphalein retention at 30 minutes (dose used 2 mg. per Kg.) 8 per cent; serum proteins 6.35 (albumin 4.20, globulins 2.15); total cholesterol 108 mg., cholesterol esters 70 per cent; cephalin flocculation ++; alkaline phosphatase 2.6 B. U.

Blood count.—Hemoglobin 4.5 Gm., hematocrit 15 per cent, MCHC 30 per cent, reticulocytes 0 per cent, WBC 19,500, lymphocytes 20 per cent, monocytes 4 per cent, neutrophils 76 per cent, with 23 per cent stabs and 3 per cent metamyelocytes. Platelets moderately increased (528,000 per cu.mm.). Slight anisocytosis, poikilocytosis, and hypochromia were observed in the blood smear.

The MCV was determined accurately on two occasions. Two flask dilutions were made and the red cell count of 16 chambers were averaged. The results ranged between 77 and 81 cu.micra.

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The direct Coombs test was negative. The serum iron was 260 μg./100 ml. and the unsaturated iron-binding capacity was 0.

Bone marrow: hypercellular with normoblastic reaction and maturation arrest of the normoblasts at the basophilic stage. No abnormality was observed in megakaryocytes and leukocytes (table 1). Hemosiderin stain: greatly increased; both fine and coarse iron granules were present.

The L-tryptophan loading test gave abnormal results (table 2).


Evolution.—During the first two weeks of hospitalization the patient received 2500 ml. of blood and a 1500-calorie sodium-free diabetic control diet. Fifteen units of protamine zinc insulin and 1 Gm. of chlorothiazide were given daily, with great clinical improvement manifested by disappearance of the gallop rhythm, diminution of dyspnea, edema, and hepatomegaly, and a 10-Kg. weight loss. The hemoglobin rose to 8 Gm. and the blood glucose diminished to 112 to 130 mg. The glycosuria decreased to a ± reaction.

Beginning on the fourteenth day of hospitalization, the patient received 200 mg. I.M. of pyridoxine daily for 6 days. Two days after starting therapy the reticulocytes rose from 0 to 4.6 per cent, reaching a peak of 23 per cent on the fourth day. On this day, a bone marrow examination showed that the maturation arrest had disappeared, since there were numerous poly- and orthochromatic normoblasts (table 1). Improvement of the anemia followed the reticulocyte rise (fig. 1).

One month after discontinuing pyridoxine therapy, the patient relapsed. Reticulocytes fell again to 0 per cent and the hemoglobin dropped from 10.8 to 7.1 Gm. A three-day oral treatment period of pyridoxine was followed by a reticulocyte response equal in promptness and magnitude to that seen after the intramuscular therapy (see figure 1). Although the total second dose was low (112.5 mg.), the patient recovered and has maintained a normal level of hemoglobin (13.85 to 14.5 Gm.) for the past 18 months without additional therapy.

The effect of therapy on the leukocytes is shown in fig. 1. The WBC and neutrophils became normal in number and the young cells disappeared with therapy. The platelets, although not counted, appeared normal on all posttherapy smears.

Case 2

A 44-year-old administrative employee, brother of the patient of Case 1, was hospitalized on January 19, 1954.

Fig. 1.—Case 1: effect of intramuscular and oral pyridoxine therapy on hemoglobin, reticulocytes, serum iron, fecal urobilinogen, and leukocytic picture in the peripheral blood. Notice the poor effect of blood transfusions and the striking reticulocyte response to therapy.
Table 1.—Bone Marrow Cell Distribution in Both Cases of Vitamin B₁₂ Deficiency Anemia, Before (B) and After (A) Pyridoxine Administration

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>8-11-58</td>
<td>1-21-54</td>
</tr>
<tr>
<td></td>
<td>8-21-54</td>
<td>7-21-54</td>
</tr>
<tr>
<td>Date</td>
<td>B</td>
<td>A*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A†</td>
</tr>
<tr>
<td>Cellularity</td>
<td>Increased +</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased +</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Megakaryocytes</td>
<td>Increased +</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased +</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>E-L ratio</td>
<td>2.5-1</td>
<td>3.5-1</td>
</tr>
<tr>
<td></td>
<td>1.8-1</td>
<td>3.5-1</td>
</tr>
<tr>
<td>Pronormoblasts</td>
<td>3%</td>
<td>1%</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td>1%</td>
</tr>
<tr>
<td>Normoblasts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basophilic</td>
<td>56%</td>
<td>56%</td>
</tr>
<tr>
<td></td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>Polychromatic</td>
<td>8%</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>58%</td>
<td></td>
</tr>
<tr>
<td>Orthochromatic</td>
<td>0.2%</td>
<td>0.2%</td>
</tr>
<tr>
<td></td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>Immature granulocytes</td>
<td>7%</td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Adult neutrophils</td>
<td>15%</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td>1%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>3%</td>
<td>0%</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>5%</td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td>5%</td>
</tr>
</tbody>
</table>

* Four days after starting to receive 200 mg. of pyridoxine I.M. daily.
† After one month of receiving 2 mg. of pyridoxine I.M. daily.
‡ Myeloblasts through metamyelocytes are included in this group.

Table 2.—Urinary Excretions of Xanthurenic Acid* Observed in Patient No. 1 Before and After the Oral Administration of 5 Gm. of L-Tryptophan

<table>
<thead>
<tr>
<th>Period of study†</th>
<th>Before tryptophan</th>
<th>First day</th>
<th>After tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[Mg./24 hours]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>43</td>
<td>106</td>
<td>96</td>
</tr>
<tr>
<td>B</td>
<td>—</td>
<td>46.8</td>
<td>51.8</td>
</tr>
<tr>
<td>C</td>
<td>—</td>
<td>88.2</td>
<td>36.6</td>
</tr>
</tbody>
</table>

* The Rosen-Lowy-Sprince method,²₉ was used.
† The tryptophan-load tests were done before (A), during (B), and 10 days after (C) a six-day period of intramuscular administration of 200 mg. of pyridoxine per day.
‡ Refers to the values obtained during the 24- to 48-hour period following the administration of the L-tryptophan.

In November, 1953, he had presented symptoms and laboratory data of diabetes. At that time he was 18 Kg. overweight. He consulted a private physician and the diabetic condition was satisfactorily controlled with a 1400-calorie diet with 122 Gm. of carbohydrates, 61 Gm. of proteins, 75 Gm. of fats, and about two mg. of vitamin B₁₂.

One month before being hospitalized, he had fever, which appeared every other day and continued until hospitalization. At the same time he noticed the presence of pallor, and the existence of anemia was confirmed by the laboratory (hemoglobin of 75 and 50 per cent, on January 8 and 18, respectively).

He was aware of having had splenomegaly for several years as well as repeated malarial attacks. He had received vitamin B₁₂ injections without any effect on the anemia.

Physical examination.—Weight 80 Kg. (ideal weight 68 Kg.), pulse 135, temperature 38.6° C., B.P. 130/50. There was marked pallor. Hepatomegaly, grade II. The spleen was felt at 2 cm. below the costal margin on deep inspiration. No other abnormalities were found at physical examination.

Laboratory data.—The Kahn and Mazzini tests were negative, and urine normal. Six stool examinations were negative for parasites and blood. Liver function tests: direct bilirubin 0.56 mg.; indirect bilirubin 1.0 mg.; bromosulphalein retention 5 per cent; serum proteins 6.35 (albumin 4.00, globulins 2.35); total cholesterol 84 mg., cholesterol esters 65 per...
This could be explained by his past malarial history.29

Fig. 2.—Hematological course of case 2. Notice the large requirements of blood transfusions, the ineffectiveness of several therapeutic agents, and the long-lasting remission consecutive to vitamin B complex administration.

cent; cephalin flocculation 4+;* serum fats 300 mg. (normal values: 670 to 1025 mg.)

Blood count: hemoglobin 4.1 Gm. per cent; hematocrit 14 per cent, MCHC 29 per cent, MCV 81 cu.micra, reticulocytes 0 per cent, anisocytosis +, poikilocytosis +, hypochromia +; WBC 10,650, lymphocytes 12 per cent, monocytes 9 per cent, eosinophils 2 per cent, and neutrophils 77 per cent with 7 per cent stabs and 2 per cent metamyelocytes. Platelets moderately increased. The direct and indirect Coombs tests were negative, as well as the saline and trypsin search for anti-RBC antibodies, at 4°C and 37°C.

Three bone marrow studies were performed, on Jan. 19, Feb. 22, and June 18, 1954, with normoblastic hyperplasia and striking maturation arrest at the level of basophilic normoblasts as the only relevant finding.

The investigation of malarial parasites in the blood was negative on two occasions. Six blood, two stool, and two urine cultures were negative for pathogenic microorganisms. Intradermal reactions to Koch bacilli and brucella were negative.

X-ray studies.—Thorax: normal. Esophageal varices: absent. Barium enema: some diverticula were observed in the descending colon and sigmoid.

On March 27, a peritoneoscopy was performed. Liver was moderately enlarged, slightly reddish in color, and of granular appearance. The opinion of the endoscopist was “hepatomegaly and splenomegaly. Liver cirrhosis?” Liver biopsy showed microscopically a slight increase of connective tissue in the periportal areas, with occasional formation of septae among the trabeculae of hepatic cells. The cytoplasm of the parenchymal cells showed abundant deposits of hemosiderin and a slight amount of hemofuchsin; these pigments were also found in Kupffer cells and in connective tissue cells; there was a concentration of hemosiderin around the portal spaces. The pathological diagnosis was of early pigmentary cirrhosis (figs. 3 and 4).

Evolution.—During the first week, the patient received 2900 ml. of whole blood (fig. 2), with an unsatisfactory rise in the hemoglobin from 4.1 to 8.65 Gm./100 ml. The anemia continued for the next five months and required 18 further blood transfusions of 400 to 800 ml. each (a total of 10 L.) in order to maintain the hemoglobin at a 7.5- to 9-Gm. level.

Several therapeutic agents were tried: vitamin B12 (30 mg. daily for 15 days); folinic acid (3 mg. daily for 15 days); copper gluconate (2 mg. daily for 12 days); ascorbic acid (500 mg. daily for 24 days); and cortisone on several occasions, once for 22 days at decreasing doses from 500 mg. Copper and cortisone were given by mouth; the other therapeutic agents were parenterally administered. None of them improved the anemia and, al-

*This could be explained by his past malarial history.29
though reticulocytes appeared during the six-month period of study on three different occasions, their number was very low (below 3 per cent), without any appreciable relationship to therapy, and without effect on the anemia.

Continuous fever with a maximum of 38.5 to 40° C. was present during the first six
days of hospitalization; it disappeared 48 hours after starting chloroquine administration. No fever recurrence was observed.

The diabetes was easily controlled with diet. Insulin was required occasionally, particularly when receiving cortisone.

During the first 20 days of hospitalization the patient lost 12 Kg. of body weight. In June he noted an additional loss of 10 Kg. Besides weight losses and symptoms of anemia, the patient’s only complaint was weakness of the legs. He had a good appetite.

On June 21 the patient started to receive I.M. 1 ml. daily of a vitamin B complex, which contained 2 mg. of pyridoxine per ml. This therapy was continued during the following two months. In July the patient was transfused with 1500 ml. of whole blood and, in clear contrast with what had occurred before, the hemoglobin rose from 7.0 to 11.0 Gm. In addition, 2.5 per cent reticulocytes were found in the peripheral blood, and a new bone marrow study showed (table 1) that most nucleated erythrocytes were polychromatophilic. Since then, and up to the present time, the patient has not required any blood transfusions and the hemoglobin level has ranged from 15 to 17 Gm. per 100 ml. during the past six years. He did not receive the vitamin $B_6$ preparation after the one-month trial mentioned above.

From January to June, 1954, the WBC ranged from 8,000 to 11,000 per cu.mm. There was moderate neutrophilia (75 to 90 per cent) with a shift to the left (1 to 5 per cent metamyelocytes), and platelets were always reported as increased on the smears. After remission of the anemia the WBC ranged from 4,500 to 7,000, neutrophilia and metamyelocytes were not observed, and in all the blood smears the platelets have been considered as normal in number.

**DISCUSSION**

(1) **Diagnosis.**—There is adequate evidence that patient 1 is a case of PRA. The nature of the anemia in case 2 was not proven, but the same diagnosis seems probable in view of the similar laboratory and clinical data. Both had hypochromic anemia, iron overload, striking erythroblastic maturation arrest, and no leukocytic or thrombocytic abnormalities. In addition, case 2 recovered while receiving a vitamin B complex that contained pyridoxine.
Table 3.—Study of the Intestinal Absorption of Different Forms of Vitamin B₆ as Measured by the Increase in the Urinary Excretion of Pyridoxic Acid

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
<th>Normal *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridoxal-5-phosphate</td>
<td>2.0</td>
<td>2.82</td>
<td>3.9</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>1.53</td>
<td>—</td>
<td>1.75</td>
</tr>
<tr>
<td>Pyridoxamine</td>
<td>0.75</td>
<td>0.74</td>
<td>—</td>
</tr>
</tbody>
</table>

*These figures represent the increments over the basal values observed in the urine voided during the four hours following ingestion of 10 mg. of the substance tested.
†Mean normal values according to Sarett.31

(2) Erythrocytic characteristics in vitamin B₆ deficiency.—Hypochromia has been constantly found in the reported cases with PRA. The anemia of experimentally induced pyridoxine deficiency has been hypochromic in most animals, but this latter has not been observed in human beings.30,40 Probably because the time-lapse has been insufficient for its development.*

Microcytosis has been as constant as hypochromia, although in our cases it was not as marked as that reported by others (table 3).

Anisocytosis and poikilocytosis were only slight in our cases and in Gehrmann’s case,11 but they have been very marked in others.

Other noteworthy factors in the peripheral blood are the absence of reticulocytes (0 to 0.2 per cent); the absence of normoblasts in spite of the presence of young granulocytic cells, and moderate leukocytosis with neutrophilia and thrombocytosis.

Bone marrow studies4,11,15,19,38 have shown definite normoblastic hyperplasia with maturation arrest of red cells, and normal megakaryocytes and leukocytes. In our cases, maturation arrest was striking, i.e., most of the erythroblasts were basophilic; the polychromatophilic cells were scarce and showed only traces of hemoglobin (table 1); practically no pyknotic normoblasts were seen. The distribution of erythroblasts in small nests around a reticulum cell, as described by Bessis,1 was conspicuously and constantly present even at the beginning of remission. This arrangement of cells has been a common finding in all types of hypochromic hypersideremic anemias.

The patient reported by Maier20,21 clearly differs from all the others in that the RBC were not microcytic-hypochromic but macrocytic “hyperchromic,” and erythropoiesis in the bone marrow was interpreted as megaloblastic; this megaloblastosis was not corrected by pyridoxine therapy and could be attributed to his “anemia refracta sideroblastica” since megaloblastosis has been observed in the latter condition.9 However, the possibility of megaloblastosis in vitamin B₆ deficiency should be kept in mind, since it has been demonstrated that absorption of vitamin B₆ is impaired in pyridoxine-deficient rats,18 and that pyridoxine-deficient dogs may require liver in addition to vita-
min B₆ to fully correct the anemia. Furthermore, intravenous injection of pyridoxine in some patients with macrocytic anemia due to pellagra and to pernicious anemia has induced striking reticulocytosis. It seems possible that erythropoiesis in pyridoxine deficiency may be megaloblastic as a result of an associated deficiency.

(3) Leukocytes and thrombocytes.—In human beings and in most animals, vitamin B₆ deficiency is usually accompanied by peripheral leukocytosis and neutrophilia. Our patients, in addition, had metamyelocytes and thrombocytosis.

In bone marrow, no abnormality in megakaryocytes or leukocytes has been observed.

Thus, vitamin B₆ does not appear to be necessary for the development, maturation, and release of granulocytes and thrombocytes.

(4) The nature of the anemia.—The absence of reticulocytes in peripheral blood and the erythroblastic maturation arrest observed in bone marrow are strong evidence that diminished RBC production is the main cause of the PRA. Another suggestive evidence of this is that utilization of Fe for hemoglobin synthesis is greatly diminished in human beings and in pyridoxine-deficient pigs.

In human beings estimation of the RBC survival has given variable results: normal and shortened (Gri T½ of 20 and 18 days). In our case 2 (fig. 5), an Ashby study showed a RBC survival of 50 days; a second study initially had an even deeper slope and, coinciding with the remission, the RBC destruction rate became normal. Thus, at least in some instances, an abnormal extracorporeal hemolytic component probably exists that may explain the poor effect of blood transfusions; i.e., patient 2 received 2,370 ml. of blood per month in order to maintain hemoglobin concentration at 7.5 to 9 Gm./100 ml., and the patient of Harris et al. required 113 units of blood (about 56 L.) over a two-year lapse to hold a hemoglobin level of about 7 Gm.

(5) Iron abnormalities.—Serum iron is constantly high with 92 to 100 per cent saturation of the siderophilin. These parameters rapidly revert to normality after pyridoxine therapy in some patients with PRA and in experimental animals. This reversion does not occur in others even after large doses of pyridoxine. In our case 1, the serum iron and the saturation index of siderophilin decreased from 260 μg. and 100 per cent to 120 μg. and 43 per cent, respectively (fig. 1).

A marked overload of iron in bone marrow and liver also has been a constant feature in both types. In some cases, this overload might be explained by the primary disorder or by previous therapy, but this is not the explanation in our 2 cases. Case 1 had not received blood transfusions when the first bone marrow and serum iron studies were done. Case 2 had been transfused with only 6 L. of blood at the time liver biopsy was performed.

*Maier's patient had hemochromatosis, and the one studied by Harris et al. (1958) had received large amounts of iron (30 Gm.) by means of blood transfusions and ferrotherapy. This was also true of most of the other patients.
Evidence that vitamin B6 deficiency enhances the intestinal absorption of iron has been reported.14,38

The iron kinetic pattern is also abnormal: the plasma turnover rate is markedly increased in spite of poor utilization for hemoglobin synthesis.5,39

6 The status of different organs and systems.—Sebrell and Harris34 list many alterations in animals with vitamin B6 deficiency; dermatologic, neurologic, hematologic, muscular, renal, and adrenal, and modifications in the blood urea values and in antibody production. Yet the reported patients with the PRA syndrome apparently do not present any other abnormality besides anemia, in spite of the fact that human beings with the experimentally induced deficiency have shown neurologic abnormalities and skin and mucous alterations, such as seborrheic dermatitis, glossitis, conjunctivitis, and intertrigo.4

The possible occurrence of muscular, renal, and adrenal changes were investigated in our case 1. The heart was studied clinically, radiologically, and electrocardiographically. The adrenal and renal status was determined by repeated 17-keto- and 17-OH-steroid urinary excretion estimations, blood urea and creatinine determinations, and urine analysis. Results of all studies were normal and did not vary with vitamin B6 administration.

Liver function apparently remains normal or nearly normal in spite of the iron overload; i.e., liver function tests repeatedly carried out in our cases showed only slight direct bilirubinemia, hypocholesteremia, and positive cephalin flocculation. The hypocholesteremia was corrected by the pyridoxine therapy.

7 Metabolic abnormalities.—A participation in the metabolism of tryptophan is one of the roles of vitamin B6 in the body. Its deficiency promptly results in an abnormally high conversion of tryptophan to xanthurenic acid. Greenberg et al.13 observed it as early as 14 days after starting administration of a vitamin B6-free diet to human subjects. Therefore, the tryptophan loading test "may be used as a biochemical sign of a latent vitamin B6 deficiency or at least of an increased requirement for vitamin B6."14 Abnormal results with this test have been obtained in some patients,11,15,48 but not in others.4,25,39 In our case 1 the test was abnormal before therapy, normal during vitamin B6 administration, and abnormal again 10 days after the first therapeutic trial, at the time the anemia was in remission (table 2).

Serum transaminases have been normal in the only two cases in whom they have been reported.4,39 Both had a normal response to the tryptophan loading test.

In our case 1, the serum glutamic transaminases were determined during recovery and in relapse, 7 and 32 days after stopping I.M. therapy. The results were 30 and 40 units for the pyruvic, and 50 and 25 for the oxalacetic, respectively.

* A different iron kinetic pattern was observed in Erslev’s48 patient, but their studies were done during an anemic period probably due to pulmonary infection, since the patient recovered from the anemia, apparently without pyridoxine therapy, when the infection receded.
The fact that hemopoiesis becomes affected later than l-tryptophan metabolism (Greenberg et al.,13 Vilter et al.,16 and our case 1) and earlier than the activity of serum transaminases,* may be the result of different affinities for the pyridoxal-5-phosphate coenzyme of the various enzymes involved.

Vitamin B₆ is required for the synthesis of porphyrins, and Schulman and Richert31 have shown that “pyridoxal-5-phosphate acts specifically in the utilization of glycine and succinate for delta aminolevulinic acid synthesis.” No increased excretion of porphyrins has been observed in some cases,11,16 and in animals,6 but abnormally high urinary excretion of porphyrins has been reported in others.30,43

Similarly, a normal or subnormal production of biliary pigment could be expected to accompany vitamin B₆ deficiency. This has been observed in pigs6 and in our two patients. During relapse, our patients both excreted low amounts of fecal urobilinogen in spite of the increased rate of RBC destruction.* A remarkable and unexpected fact was the early and great increase in the excretion of urobilinogen observed when vitamin B₆ therapy was instituted. In case 1, it rose from 49 mg. to 237 to 310 mg. per day; in the nine-day period immediately following the initiation of therapy, our patient excreted a total of 2451 mg. of urobilinogen, an amount equivalent to more than 60 Gm. of hemoglobin, or about 200 ml. of packed RBC.

The same two patients30,41 that showed a high urinary excretion of porphyrins also had an increased excretion of fecal urobilinogen. Therefore, their pigment pattern resembled that seen in the sideroblastic or refractory normoblastic anemias,16,17 rather than that observed in animals with experimentally induced pyridoxine deficiency.6

Vitamin B₆ also has some role in fat metabolism.35 This problem apparently has not been studied in human beings with PRA. The low cholesterol and serum fat values observed in our patients may indicate that fat metabolism was altered in them.

(8) Pathogenesis of pyridoxine-responsive anemia.—It is possible that patients with PRA constitute not a single but a miscellaneous group. Data suggestive that some of them are actually deficient in pyridoxine (References 11, 15, 19, 43 and our cases), follows: (a) similarity between their abnormalities and those presented by the animals with experimentally induced vitamin B₆ deficiency; (b) increased excretion of xanthurenic acid after the l-tryptophan loading test; (c) full regression of all abnormalities after therapy, particularly anemia, serum iron parameters, and abnormal degradation of l-tryptophan; (d) effectiveness of small doses of pyridoxine (5 mg. orally per day16 and lower doses parenterally (Gehrmann11 and our case 2); and (e) long-lasting remissions. In one case,11 hemoglobin remained above 12 Gm. four months after

*Sass and Murphy32 observed an early significant drop in the oxalacetic transaminating activity in human beings given isoniazide nicotinic acid. This discrepancy with our findings might be due to the fact that these workers assayed whole blood.

*Case 1 had a hemolytic index of 13, and case 2, had 4 and 14.8, which are lower than the normal average of 17 and still lower than the 22 average value obtained in patients with iron-deficiency anemia.30 In case 2, higher hemolytic indexes were observed later on, but blood was being transfused on such occasions.
discontinuing therapy; in another, a spontaneous remission lasting five years occurred. Our 2 cases have been in remission 1.5 and 6 years respectively, without maintenance therapy. Therefore, the terms “true” or “actual” vitamin B₆ deficiency seem adequate for their condition. In the other cases, except for a partial response to pyridoxine, no other evidence of deficiency in this element has been present. The reason for this partial hemopoietic affect might be, as Bishop and Bethell suggested, that large doses of vitamin B₆ break the inhibition in heme synthesis produced by the excessive iron stores present in these patients as a result of several conditions: primary hemochromatosis, iatrogenic hemochromatosis, and sideroblastic anemia. A mass action effect of pyridoxine is also a possibility, in a manner similar to that of folic acid in pernicious anemia. In the group with the apparently true deficiency, metabolic aberration and intestinal malabsorption have been suggested as causes of the disorder. However, spontaneous and long-lasting remissions and the full effect of small doses of pyridoxine make the first alternative very unlikely. Malabsorption of vitamin B₆ can also be ruled out in most cases. Harris' case showed a good hematological response to the daily oral ingestion of 5 mg. of pyridoxine; Leeming and Wilkinson found no anatomical or physiological intestinal alterations in their case. Our two cases showed significant increases in the excretion of pyridoxic acid after ingestion of pyridoxine, pyridoxamine, and pyridoxal phosphates (table 3).

Since a dietary defect has also been excluded in these patients, an increased requirement of the vitamin seems to be the only remaining etiologic possibility. Conditions in which a deficiency is induced by increased consumption of an element are not rare; thus it has been shown that in most types of hemolytic anemias the turnover of folic acid is markedly increased and that, as a consequence of it, folic acid deficiency with megaloblastic erythropoiesis may occur.

In our patients, several possible explanations for the increased requirements of vitamin B₆ were present. One of them had had fever, which has been considered as a causative factor; both developed anemia shortly after a diabetic condition became evident. Stutinsky has shown that vitamin B₆ has an insulin-saving effect of the order of 25 per cent and Rosen et al. found some evidence of pyridoxine deficiency in diabetic patients.

A genetic determination of the increased requirements also may be considered in our patients since, in addition to their own relation as brothers, they stated that their sister had died some years earlier with severe anemia not due to bleeding or to any other obvious cause. In three out of four cases with roentgenological studies of the digestive tract (Leeming and Wilkinson and the present report), diverticula of the duodenum or colon have been observed. This could be a mere coincidence, but it may also support the genetic influence hypothesis.

Summary

The clinical and laboratory findings in two brothers with severe anemia are presented. These findings were very similar in both cases. Evidence that at least one of them suffered from a pyridoxine-responsive anemia is presented.
It was assumed that the other had the same disorder since, in addition to the striking similarity in the clinical and laboratory abnormalities, the latter's anemia disappeared completely with the parenteral administration of vitamin B complex, which provided him with 2 mg. of pyridoxine daily. These patients have not relapsed 1.5 and 6 years after stopping the therapy.

A review of the reported cases with anemia responding to vitamin B₆ administration is presented. Common factors observed in some of these cases are: (1) severe anemia, microcytic and hypochromic in type, chiefly due to a striking maturation erythroblastic arrest at the basophilic stage with no defect in leukocytes or thrombocytes; (2) hyperferremia and hemosiderosis; (3) an abnormal tryptophan-loading test; and (4) complete or almost complete correction of all abnormalities with administration of vitamin B₆, even at small doses. These data were considered to suggest that the patients had a true deficiency in pyridoxine.

In other patients, no increased excretion of xanthurenic acid has been observed after a tryptophan-loading dose, and pyridoxine administration has improved the anemia only partially and has not reversed the serum iron parameters to normality. All of these other patients suffered from a familial hypochromic anemia not due to iron deficiency or from a "sidero-achrestic" or "refractory normoblastic" anemia.

The cause of the disorder in patients in the first group is unknown, but, by exclusion, increased requirement of vitamin B₆ seems to be the most likely possibility.

**Summario in Interlingua**

Es presentate le constatationes clinic e laboratorial ab le casos de duo fratres con sever anemia. Le constatationes esseva multo simile in le duo casos. Es presentate evidentia que indica que al minus un del fratres suiffreva de un anemia responsive a pyridoxina. Il es supponite que le altere fratre habeva le mesme morbo, proque—a parte le frappante similaritate in lor anormalitates clinic e laboratorial—le anemia del secunde fratre disparsa completamente post le administration parenteral de complexo a vitamina B, le qual provideva 2 mg de pyridoxina diamente pro ille. Iste patientes non ha suffritle un recidiva in le curso de 1, 5 e 6 annos post le cessation del therapia.

Es presentate un reporte del reportate casos de anemia responsive a vitamina B₆. Le factores commun observate in alicunes de iste casos es le sequentes: (1) sever anemia, de typo microcytic e hypochromic, debite principalmente a un marcate arresto de maturation erythrocytic in le stadio basophilic, sin defecto de leucocytos o thrombocytes; (2) hyperferremia e hemosiderosis; (3) reacções anormal al test de administration de tryptophano; e (4) correction complete o quasi complete de omne anormalitates post administrationes de vitamina B₆, mesmo in debile dosages. Iste datos esseva interpretate como indicationes que le patientes habeva un ver deficientia in pyridoxina.

In altere patientes nulle augmento in le excretion de acido xanthurenic ha essite observate post le administration de tryptophano. Hic le administration de pyridoxina ha meliorate le anemia solo partialmente e illo non ha revertite le parametos del ferro seral verso le norma. Omne iste altere patientes ha-
beva suifrite (1) de un hypochromic anemia familial non debite a deficientia de ferro o (2) de un anemia “sidero-achrestic” o “refractori normoblastic.”

Le causa del disordine in patientes del prime gruppo non es cognoscite, sed, per exclusion, le plus probable possibilitate es un augmentate requirimento de vitamina B₆.

**ADDENDUM**

In February 1961, patient 1 complained again of weakness and dyspnea. Laboratory studies gave results similar to those observed in 1958: RBC 3,160,000 per cu.mm.; Hb. 6.4 Gm.; Ht. 22 per cent; MCV 69 cubic micra; MCHC 29 per cent; reticulocytes 0.4 per cent; WBC 13,600, with metamyelocytes 4, stabs 7 and PMN 50 per cent; Serum iron 280 µg.; UIBC 0.

He was given 5 mg. of pyridoxine daily per os. This therapy was followed by reticulocytosis and improvement of the anemia. On the 22nd day of treatment, hemoglobin had risen to 12.1 Gm.

The patient had not had any concurrent illness that could explain his relapse. He had been eating his usual diet, but in December 1960, he had stopped drinking beer, of which he used to take at least one liter daily. This diminished his daily intake of vitamin B₆ by at least 0.5 mg.

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Pyridoxine-Responsive Anemia: Report of 2 Cases in Brothers and a Review of the Literature

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