Pyridoxine-Responsive Anemia

By Spencer O. Raab, Arthur Haut, George E. Cartwright and Maxwell M. Wintrobe

Since the original report by Harris et al. in 1956, six additional patients with anemia responsive to the administration of pyridoxine have been described. With the exception of the case reported by Maier, the features which have been common to this group of patients have been microcytic, hypochromic anemia, hyperferremia, hemosiderosis and a partial or complete response of the anemia to the continued administration of relatively large amounts of pyridoxine. The condition has been termed "pyridoxine-responsive anemia" since in none of the subjects reported has there been evidence suggestive of a dietary deficiency of pyridoxine. Furthermore, the amounts of pyridoxine required to elicit a response have been in excess of the ordinary dietary requirement and, when the therapy has been discontinued, relapses have been observed in spite of the ingestion of a normal diet.

The purpose of this paper is to report studies on two patients with microcytic, hypochromic anemia, hyperferremia, hemosiderosis, and a partial hematologic response to pyridoxine.

A preliminary report of one of these patients (M. Mc.) has been published.

Methods

The routine hematologic methods used, including Heinz body stain, examination of urine and bone marrow for hemosiderin, osmotic fragility, alkali resistant hemoglobin, paper electrophoresis, serum bilirubin, urobilinogen estimation and urinary porphobilinogen have been described elsewhere.

To identify reticulocytes in the presence of iron-containing Pappenheimer bodies, fresh smears were stained with brilliant cresyl blue followed by Prussian blue, and were counterstained with a mixture of basic fuchsin and neutral red. The details of this method are to be published.

The method employed for the measurement of total serum copper was described by Gubler et al.; for serum iron by Hamilton et al.; for the total iron-binding capacity of the serum by Cartwright and Wintrobe; for xanthurenic acid by Glazer et al.; for starch block electrophoresis of hemoglobin by Kunkel et al.; for free erythrocyte protoporphyrin and coproporphyrin by Schwartz and Wikoff; or urinary coproporphyrin by Schwartz et al.

Case Presentations

Case 1 (M. Mc.)

M. Mc., an adopted white boy of eight years of age was first seen in our clinic in March 1957. He lived in a state distant from Utah and was seen and studied again in our clinic on two subsequent occasions, the summer of 1958 and the summer of 1960.
History: The child was born four weeks prematurely on October 13, 1948. His parents were of Irish, Dutch and English descent. At the age of one month he was noted to be anemic and the liver and spleen were palpable. His hemoglobin at two months of age was 6.4 Gm./100 ml. and there was marked anisocytosis, poikilocytosis and hypochromia of the erythrocytes. He was given multiple transfusions and started on iron therapy. The latter was continued until he was 21 months of age. Between the ages of 4 and 21 months, transfusions were not given and the hemoglobin concentration ranged between 8 and 10 Gm./100 ml. The platelet count was reported to vary between 26,000 and 610,000/mm.$^3$ At seven months of age the child was adopted and nothing more is known concerning his true parents or siblings.

Between the ages of 2 and 5 years, the hemoglobin concentration repeatedly declined to 4 Gm./100 ml. and he was given 1000 ml. of whole blood at four to six week intervals. The liver and spleen continued to enlarge and epistaxes became frequent. The total and differential leukocyte counts were found repeatedly to be within normal limits. Several bone marrow aspirates were interpreted as showing hypoplasia of all elements. No benefit resulted from the administration of cortisone, iron, parenteral liver extract, folic acid or Vitamin $B_{12}$.

At the age of 5 years the patient was admitted to Stanford University Hospital. The platelet count at this time was 35,000/mm.$^3$. The leukocyte count was 4800/mm.$^3$. A liver biopsy obtained at abdominal laparotomy was interpreted as indicative of "pigmentary cirrhosis of the liver." (fig. 1). The spleen was removed. There was "hyperplasia of the spleen" and no myeloid metaplasia was seen in the histologic sections, according to the examining pathologist.

The patient recovered uneventfully from the splenectomy; the platelet count increased after splenectomy to 94,000/mm.$^3$. The leukocyte count increased to 9,600/mm.$^3$. The patient continued to require 1000 ml. of whole blood every four to six weeks.

First visit (March 1957): This alert, somewhat tanned red-haired and freckled-faced boy was slightly small for his age of eight years, being 45.5 inches tall and 46 pounds in weight. He was not jaundiced. Koilonychia was not observed. There was an alternating exotropia. The heart was diffusely enlarged. A thrill was palpable over the pulmonic area and a grade II systolic, ejection murmur was audible over this area and along the left sternal border. The liver was palpable nine cm. below the right costal margin and filled most of the abdomen. The testes were undescended. The remainder of the physical examination was normal.

The heart appeared diffusely enlarged by x-ray. After intravenous injection of dye and oximeter measurements it was determined that the circulation was rapid, the cardiac output was increased and there was no evidence of a left to right shunt.

The initial hematologic findings are summarized in table 1. There was considerable variation in the size and shape of the erythrocytes. There were a moderate number of hypochromic microcytes and a few macrocytes (fig. 1). Only an occasional Pappenheimer body, stippled cell or siderocyte was seen. Many target cells were present. The platelets were reduced. The differential leukocyte count was as follows: neutrophils, 12 per cent; eosinophils, 5 per cent; basophils, 1 per cent; lymphocytes, 72 per cent; and monocytes, 10 per cent.

Bone marrow aspiration revealed a reduced myeloid-erythroid ratio of 2:1. Fifty-four per cent of the cells seen were lymphocytes. The normoblasts were immature and showed little hemoglobinization. Surgical biopsy revealed a moderately hypercellular marrow with an increased deposition of pigment (fig. 1).

Heinz bodies were not present. Paper electrophoresis of serum and of hemoglobin revealed no abnormalities. Alkali resistant hemoglobin was absent. The osmotic fragility of the erythrocytes was decreased. The Coombs' test was negative and the excretion of urobilinogen in both stool and urine was normal. Serum albumin was 4.1 Gm./100 ml.; serum globulin, 4.2 Gm./100 ml. Liver function studies were normal except for an alkaline phosphatase of 12 King-Armstrong units.
Fig. 1.—Case 1 (M. Mc.). (Upper left) Blood smear taken in March 1957, prior to pyridoxine therapy. The patient had received 1000 ml. of whole blood two weeks before. Wright's stain (X 1220). (Upper right) Blood smear taken in June 1958. The patient had been receiving pyridoxine for 11 months, and had not received transfusions during this time. Note the numerous Pappenheimer bodies. Wright's stain (X 1220). (Lower left) Bone marrow biopsy performed on April 8, 1957, and stained with hematoxylin-eosin. Specimen is cellular and there is no significant fibrosis. (X 120). (Lower right) Biopsy of liver (July 1, 1954) stained with Giemsa. Dark area represent hemosiderin deposition in both parenchymal and Kupffer cells (X 120).
Table 1.—Hematologic Values in Case 1 (M. Mc.)

<table>
<thead>
<tr>
<th>Determination</th>
<th>March 1957*</th>
<th>June 1958†</th>
<th>June 1960‡</th>
</tr>
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<tbody>
<tr>
<td>Red blood cells, X 10^6/mm.³</td>
<td>3.04</td>
<td>5.43</td>
<td>5.08</td>
</tr>
<tr>
<td>Hemoglobin, Gm. 100 ml.</td>
<td>8.1</td>
<td>7.7</td>
<td>8.7</td>
</tr>
<tr>
<td>Volume packed red cells, ml. 100 ml.</td>
<td>26</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Mean corpuscular volume, cµ</td>
<td>87</td>
<td>63</td>
<td>67</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin, µg/µl.</td>
<td>27</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin conc., %</td>
<td>31</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>Reticulocytes, %</td>
<td>0.3</td>
<td>4.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Platelets, X 10^9/mm.³</td>
<td>90</td>
<td>485</td>
<td>500</td>
</tr>
<tr>
<td>Leukocyte count, X 10^3/mm.³</td>
<td>27.7</td>
<td>27.0</td>
<td>12.7</td>
</tr>
<tr>
<td>Nucleated red blood cells, mm.³</td>
<td>52</td>
<td>2800</td>
<td>2800</td>
</tr>
<tr>
<td>Pappenheimer bodies</td>
<td>Rare</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Siderocytes</td>
<td>Rare</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Serum iron, µg./100 ml.</td>
<td>234</td>
<td>274</td>
<td>300</td>
</tr>
<tr>
<td>Unsaturated iron binding capacity, µg./100 ml.</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*The patient had received 1000 ml. of whole blood two weeks prior to these determinations.
†The patient was receiving 25 mg. of pyridoxine daily.
‡The patient was receiving 50 mg. of pyridoxine daily.

The excretion of xanthurenic acid in the urine was 31 mg./day before tryptophane and increased to 55 mg./day after the ingestion of one Gm. of L-tryptophane.

A definitive diagnosis was not made and the child was returned to the care of his physician with the recommendation that he be given a trial of brewer's yeast and that, if this was ineffective, he should be given a therapeutic trial of pyridoxine.

Interval history: Treatment with brewer's yeast, 30 Gm. daily for one month, was ineffective. Administration of pyridoxine, 25 mg. daily by mouth, was associated with reticulocytosis and the appearance of Pappenheimer bodies in most of the erythrocytes. The exact degree of reticulocytosis could not be determined because the presence of the Pappenheimer bodies made difficult the identification of reticulocytes. The hemoglobin concentration stabilized at 7 to 8 Gm./100 ml. The child was maintained on this therapy and has required no further transfusions to the present time. Prior to this time he had been receiving 1000 ml. of whole blood every 4 to 6 weeks and had received a total of more than 120 whole blood transfusions of 500 ml. each.

Second visit (June 1958): Because of the apparent response to pyridoxine the patient returned to our clinic for more definitive studies. He was taking 25 mg. of pyridoxine daily at this time.

The physical examination was unchanged from the examination in 1957 except that both testes had descended into the scrotum. He was 46.5 inches tall and weighed 47 pounds. The initial blood studies are presented in Table 1.

The blood smear was quite unusual. At least 90 per cent of the red cells contained one or more Pappenheimer bodies (fig. 1). These granules stained blue with PRussian blue. There was a marked degree of anisocytosis, poikilocytosis, polychromatophilia, hypochromia and microcytosis. Many target cells and nucleated red cells were present. The platelets appeared adequate. The differential leukocyte count was as follows: neutrophils, 24 per cent; eosinophils, 10 per cent; basophils, 1 per cent; lymphocytes, 57 per cent; and monocytes, 8 per cent.

Bone marrow aspiration revealed a myeloid-erythroid ratio of 1.7:1. Fifty per cent of the cells were lymphocytes. Polychromatophilic and orthochromic normoblasts were present. Hemoglobin electrophoresis on starch block was normal. The A₂ component represented 2.4 per cent of the total hemoglobin. The urine was not remarkable except for the presence...
of large amounts of hemosiderin. A qualitative test for urine porphobilinogen was negative. The 24-hour excretion of coproporphyrin was 344 \( \mu g \).

The patient was observed for a period of 29 days while receiving 25 mg. of pyridoxine daily (fig. 2). During this period, a 24-hour urine was collected for the study of tryptophane metabolites in the urine. He was then given 2 Gm. of L-tryptophane and the studies were repeated. The results, which were entirely within normal limits, are presented in table 2.

Pyridoxine medication was then withdrawn. There was a prompt decline in the reticulocyte values from the range of 2 to 5 per cent to essentially zero values on the sixth day without therapy. The hemoglobin decreased from 8.4 Gm./100 ml. to 5.8 Gm. in 10 days. The free erythrocyte protoporphyrin (FEP), which had been 38 \( \mu g./100 \) ml. of packed cells, was 18 \( \mu g./100 \) ml. The free erythrocyte coproporphyrin (FEC) decreased from 3.4 to 1.5 \( \mu g./100 \) ml. The patient became weak, listless and tired easily; the mother became quite apprehensive. A second tryptophane load test was performed with 2 Gm. of L-tryptophane. The metabolism of tryptophane was quite abnormal as compared with the previous study (table 2). The excretion of kynurenic acid and kynurenine was increased, but again the excretion of xanthurenic acid was within the expected values for normal adult subjects.

Starting on the 10th day after withdrawing therapy, 100 mg. of pyridoxine was given intravenously daily (fig. 2).

The reticulocyte count increased promptly and reached a maximum of 8.2 per cent on the fifth day of therapy. The FEC increased and reached a maximum of 12.7 \( \mu g./100 \) ml. on the eighth day. The FEP reached a maximum of 73 \( \mu g. \) on the 10th day of therapy, by which time the hemoglobin had increased from 5.6 to 7.8 Gm./100 ml. The patient's vigor increased; the mother's anxiety decreased. Hyperferremia persisted. The appearance of the erythrocytes in the blood smear remained unchanged throughout the study period.

Calcium pantothenate, 100 mg. daily by mouth, was given for a 10-day period while the patient was receiving 100 Gm. of pyridoxine daily by the intravenous route. A second

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Table 2.—Excretion of Tryptophane Metabolites in the Urine of Patient M. Mc. Before and After Administration of Tryptophane

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Patient M. Mc.</th>
<th>Normal Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>While Receiving</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pyridoxine</td>
<td>No Pyridoxine</td>
</tr>
<tr>
<td></td>
<td>before tryptophane</td>
<td>after tryptophane</td>
</tr>
<tr>
<td>Kynurenic acid</td>
<td>8</td>
<td>173</td>
</tr>
<tr>
<td>Xanthurenic acid</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Anthranilic glucuronide</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>O-Aminohippuric acid</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Acetyl-kynurenine</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Kynurenine</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Hydroxy-kynurenine</td>
<td>4</td>
<td>28</td>
</tr>
</tbody>
</table>

We are indebted to Dr. J. M. Price, University of Wisconsin, for performing these determinations. The values are expressed in \( \mu M \) of metabolite excreted in the urine in a 24-hour period. The normal values (adult subjects) were supplied by Dr. Price. The figures in parentheses refer to range.
Fig. 2.—Case 1 (M. Mc.). Response to pyridoxine. The patient was studied for 28 days while receiving 25 mg. of pyridoxime hydrochloride daily by mouth. The pyridoxine medication was then withheld. The reticulocytes and the hemoglobin concentration decreased. Following this, pyridoxine hydrochloride was given daily by the intravenous route. This was followed by reticulocytosis, an increase in hemoglobin concentration, an increase in free erythrocyte protoporphyrin (FEP µg. 100 ml. of packed cells) and an increase in free erythrocyte coproporphyrin (FEC µg. 100 ml. packed cells). The serum iron concentration (S.Fe), the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) did not change appreciably.

The reticulocyte peak was not observed and there was no further increase in hemoglobin concentration. Pyridoxine and pantothenate were discontinued and he was given 100 mg. of pyridoxal daily by the oral route. There was no change in reticulocytes or hemoglobin. After 10 days, the pyridoxal was discontinued, and he was discharged on 100 mg. of pyridoxine daily by mouth.

Interval history: The patient continued to take 100 mg. pyridoxine daily by mouth. The hemoglobin was maintained at about 8 Gm./100 ml. without blood transfusions. While being maintained on this quantity of pyridoxine, he was given consecutive 30-day trial periods of pyridoxal (100 mg. daily, orally), ascorbic acid (100 mg. daily, orally), Valentine’s crude liver extract (45 ml. daily), and brewer’s yeast (45 Gm. daily) with no apparent benefit. Glutamic acid, niacin, and riboflavin were likewise ineffective.

The oral dose of pyridoxine was decreased to 50 mg. daily but the hemoglobin remained at the previous level of about 8 Gm./100 ml.

Third visit (June 1960): The physical examination and the blood value (table 1) were unchanged from the previous examination in 1958.

Liver function studies were as follows: total serum bilirubin, 1.6 mg. per cent; direct-reacting serum bilirubin, 0.1 mg. per cent; thymol turbidity, 2 units; alkaline phosphatase, 11 King-Armstrong units; serum albumin, 4.6 Gm. per cent; and serum globulin, 3.9 Gm.
PYRIDOXINE-RESPONSIVE ANEMIA

per cent. FEP, measured on several occasions, was 43 to 49 μg./100 ml of packed cells. FEC ranged from 1.2 to 1.8 μg./100 ml.

After an appropriate base line period of 10 days, during which he continued to receive 50 mg of pyridoxine daily, the patient was given daily for 10 days a special concentrate (No. 11–10) of Valentine’s liver extract (Lot No. 21038) prepared by Dr. D. L. Horrigan of Western Reserve University, in an amount equivalent to 144 ml of crude extract. This material did not elicit an hematologic response but unfortunately there was no way of knowing if this particular batch of extract contained the hemopoietic factor reported by Dr. Horrigan.

Pyridoxamine, 100 mg daily for 10 days, and L-tryptophane, one Gm. daily for 30 days, were not associated with further hematologic improvement.

Case 2 (E.O.R.)

E.O.R., a 44 year old white Chilean male of Irish descent, was first seen in our clinic on March 16, 1960, complaining of mild diarrhea and lassitude of 21 years’ duration.

History: The patient was born in 1916 and he was in excellent health until 1939, at which time he developed a severe watery diarrhea. He was given a course of anti-amebic therapy with improvement but mild bouts of intermittent diarrhea continued. In 1943 he was again treated for amebiasis but his symptoms persisted and he developed generalized weakness.

In 1944 he was admitted to a Naval Hospital in South America. Stool cultures for amebiasis, x-rays of the gastro-intestinal tract and sigmoidoscopic examinations were normal repeatedly. He was discovered to have a microcytic hypochromic anemia with a hemoglobin of 7.0 Gm. per cent. The leucocyte count was 3,800/mm.³ and the platelets were normal. Normoblastic hyperplasia was observed on a marrow aspirate smear. Because it was noted that his skin had become pigmented, a potassium ferrocyanide skin test was performed and interpreted as showing an increased iron content. Liver biopsy revealed “pigmentary cirrhosis of the liver.” A glucose tolerance was normal. He was treated with anti-amebic agents, anti-diarrhea mixtures, vitamins, oral iron, sulfaguanidine and 42 whole blood transfusions.

In December 1945 he was admitted to the Navy Medical Center at Bethesda, Md., complaining of weakness, mild diarrhea and occasional bouts of mild abdominal pain. Bronzing of the skin, hepatomegaly and splenomegaly were observed. The red cell count was 3.5 X 10⁶/mm.³; hemoglobin, 5.7 Gm./100 ml.; volume of packed red cells, 26 ml./100 ml.; reticulocytes, 1.7 per cent; leucocyte count, 5600/mm.³; and platelets, 250,000/mm.³. Hypochromia, microcytosis, anisocytosis, and poikilocytosis were noted. Aspiration of the bone marrow again revealed normoblastic hyperplasia. Erythrocyte osmotic fragility was decreased. Needle biopsy of the liver was interpreted as compatible with hemochromatosis. Liver function tests were normal. X-ray studies of the gastro-intestinal tract and stool examinations for ova, parasites and enteric pathogens were repeatedly negative. He was treated with whole blood transfusions and was discharged April 16, 1946.

He continued to fatigue easily and to have loose bowel movements. He was treated with oral and parenteral iron, oral and parenteral folic acid and vitamin B₁₂, as well as whole blood transfusions.

In October 1956 he was readmitted to the Naval Medical Center in Bethesda, Md. with essentially the same physical and laboratory findings as on the first admission. The serum iron concentration was 230 μg./100 ml. Bone marrow examination revealed “erythroid hyperplasia with severe hemosiderosis and poor hemoglobinization of the normoblasts.” An hematologic consultant suggested the possibility of a “pyridoxine-responsive anemia” and on November 17, 1956, pyridoxine therapy, 50 mg./day intramuscularly, was instituted. The patient experienced subjective improvement in strength and the hemoglobin concentration increased from 8.3 to 10.8 Gm./100 ml. A reticulocytosis was looked for but was not observed. He was discharged on February 26, 1957.

In the period from 1957 to 1960 he continued to receive pyridoxine parenterally. He continued to have poorly formed stools and occasional bouts of right upper quadrant pain but he felt considerably stronger, his appetite was excellent and he was able to perform his duties as a Commander in the Chilean Navy.
At the request of one of the authors (M. M. W.) who saw the patient in Chile in the fall of 1959, the patient discontinued the pyridoxine therapy on February 24, 1960, three weeks prior to coming to our clinic.

**Family history:** There was no known family history of anemia, splenomegaly or hemochromatosis. The blood of his mother, one brother, four sisters, two children, one uncle and one aunt had been examined elsewhere and "found to be normal."

**Physical examination:** The patient was tall and thin although the abdomen was enlarged and protuberant. The liver extended 20 cm., and the spleen 12 cm., below the right and left costal margins, respectively. The skin was diffusely tanned. The testes were not atrophic. The remainder of the physical examination was normal except for the presence of a soft systolic murmur over the apex of the heart.

**Hematologic studies:** The initial hematologic studies are presented in table 3. The erythrocytes were hypochromic and microcytic (fig. 3). There was considerable variation in size and shape and target cells were prominent. Nucleated red blood cells, Pappenheimer bodies, basophilic stippling and siderocytes were not observed. There was a moderate degree of polychromatophilia. The platelets were reduced. The leukocyte differential count was as follows: neutrophils, 65 per cent; eosinophils, 4 per cent; lymphocytes, 21 per cent; and monocytes, 10 per cent.

Needle aspiration of the bone marrow revealed marked deposition of hemosiderin. The myeloid-erythroid ratio was 1:1. The erythroid cells consisted entirely of basophilic normoblasts. There was little evidence of hemoglobinization.

Serum proteins and hemoglobin, as determined by electrophoresis on paper, were normal. The Coombs' test was negative. Bile pigment excretion was not increased. The erythrocyte osmotic fragility was decreased.

**Other laboratory studies:** The results of liver function studies were as follows: total serum bilirubin, 1.2 mg. per cent; direct reacting serum bilirubin, 0.15 mg. per cent; cephalin flocculation, 3 plus; alkaline phosphatase, 5 King-Armstrong units; serum albumin, 5.9 Gm. per cent; serum globulin, 1.5 Gm. per cent; thymol turbidity, 1 unit; serum glutamic transaminase, 34 units; bromsulphthalein retention, 1 per cent in 45 min. An electrocardiogram was within normal limits. A fasting blood sugar was 103 mg. per cent and the two hour postprandial value was 108 mg. per cent.

### Table 3.—Hematologic Values in Case 2 (E. O'R.)

<table>
<thead>
<tr>
<th>Determination</th>
<th>March 16, 1960</th>
<th>March 29, 1960</th>
<th>April 28, 1960</th>
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<tbody>
<tr>
<td>Red blood cells, X 10⁶/mm³.</td>
<td>4.22</td>
<td>3.88</td>
<td>4.77</td>
</tr>
<tr>
<td>Hemoglobin, Gm./100 ml.</td>
<td>8.4</td>
<td>7.7</td>
<td>9.8</td>
</tr>
<tr>
<td>Volume packed red cells, ml./100 ml.</td>
<td>30.5</td>
<td>27.0</td>
<td>38.0</td>
</tr>
<tr>
<td>Mean corpuscular volume, cμ.</td>
<td>72</td>
<td>70</td>
<td>80</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin, μg.</td>
<td>20</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin conc., %</td>
<td>27</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>Reticulocytes, %</td>
<td>0.9</td>
<td>0.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Platelets, X 10³/mm³.</td>
<td>78</td>
<td>146</td>
<td>140</td>
</tr>
<tr>
<td>Leukocyte count, X 10³/mm³.</td>
<td>5.9</td>
<td>4.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Serum iron, μg./100 ml.</td>
<td>281</td>
<td>229</td>
<td>215</td>
</tr>
<tr>
<td>Unsaturated iron binding capacity, μg./100 ml.</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Free erythrocyte protoporphyrin, μg./100 ml.</td>
<td>20</td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td>Free erythrocyte coproporphyrin, μg./100 ml.</td>
<td>0.4</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Serum copper, μg./100 ml.</td>
<td>104</td>
<td>119</td>
<td>95</td>
</tr>
</tbody>
</table>

*Pyridoxine was discontinued on February 24, 1960.*
Response to pyridoxine. Pyridoxine had last been given 21 days before the beginning of our observations. He was observed for an additional 14 days without pyridoxine. During this period, the reticulocytes varied between 0.1 and 2 per cent. The hemoglobin concentration remained relatively constant, between 8.4 and 7.8 Gm. per cent (fig. 4). A 24-hour urine was collected for the study of tryptophane metabolites in the urine after which the patient was given 2 Gm. of L-tryptophane by mouth and the studies were repeated. The results are presented in table 4. All of the values were within the limits of normal, both before and after the administration of tryptophane.

Pyridoxine, 100 mg. daily, was then administered intravenously. The patient felt subjectively better within 24 hours after the first injection. The reticulocytes reached a maximum of 7.7 per cent on the eighth day of therapy. The FEC increased from a value of 0.40 µg./100 ml. of packed cells to 0.88 µg. on the eighth day of therapy. The hemoglobin concentration reached a maximum of 10 Gm. per cent on the 22nd day of treatment. During this period, the patient continued to feel stronger, his appetite and exercise tolerance increased, and there was lessening of the diarrhea. The hematologic values, after 30 days of therapy, are shown in table 3. The excretion of tryptophane metabolites in the urine was again studied, both before and after the administration of 2 Gm. of L-tryptophane. The results were within the limits of normal both before and after tryptophane (table 4).

While the patient was receiving 100 mg. of pyridoxine intravenously daily, he was given six infusions of 3.0 Gm. of calcium disodium ethylene-diamine tetra-acetic acid (EDTA) over a period of seven days. No further hematologic response was observed.

The patient was discharged to the care of his private physician in Chile. Since discharge he has been maintained on the following consecutive regimens of pyridoxine; 200 mg. daily by the oral route for 70 days; 1000 mg. daily by the oral route for 30 days; 200 mg. daily by the oral route, plus 0.05 mg. of ethinyl estradiol (Estinyl) daily by the oral route for 30 days; and 100 mg. of pyridoxine twice weekly intramuscularly. During this time, his volume of packed red cells has remained between 34 and 40 ml./100 ml. and hemoglobin between 8.7 and 12.2 Gm./100 ml. No clear relationship between these variations and any of the above regimens could be made out.
Fig. 4.—Case 2 (E. O'R.). Response to Pyridoxine. Pyridoxine therapy had been discontinued 21 days before day "O." The patient was observed for an additional 14 days without pyridoxine therapy. Pyridoxine hydrochloride, 100 mg. daily, was then administered by the intravenous route. This therapy was followed by reticulocytosis, an increase in hemoglobin concentration, a slight transient decrease in serum iron concentration (S.Fe), a slight but equivocal increase in free erythrocyte protoporphyrin (FEP) and an increase in the mean corpuscular volume (MCV). The mean corpuscular hemoglobin concentration (MCHC) did not change significantly.

**DISCUSSION**

**Comparison with Reported Cases**

Certain pertinent data concerning the seven reported cases of pyridoxine-responsive anemia and the two patients reported by the authors, are summarized in table 5. Unfortunately, several of the cases have been reported rather sketchily and some critical data are lacking.

The patient reported by Maier is unusual in several respects. His patient is the only female in the group and the anemia was "hyperchromic" and associated with a megaloblastic bone marrow. The anemia presumably failed to respond to vitamin B₁₂, folic acid or liver extract. Information concerning the corpuscular constants, serum iron and metabolism of tryptophane is not supplied so that it is difficult to be certain that this patient appropriately belongs to the group under discussion. The only feature in common with the others is the reported response to the administration of pyridoxine.

With the exception of the patient described by Maier, the other eight cases
We are indebted to Dr. J. M. Price, University of Wisconsin, for performing these determinations. The values are expressed in μM of metabolite excreted in the urine in a 24-hour period. The normal values (adult subjects) have been supplied by Dr. Price. The figures in parentheses refer to range.

have a number of features in common, in addition to a response to pyridoxine: all were males with a microcytic, hypochromic anemia, normoblastic hyperplasia of the bone marrow, hyperferremia, and some degree of hemosiderosis of the tissues. It is possible that additional characteristics in common are an incomplete response to pyridoxine and relapse of the anemia on cessation of therapy (table 5). It is not possible to be certain that these were features in all nine cases since inadequate information is given for three of them (table 5, Cases 2, 3, 5). In six of the patients (Cases 2, 4, 6–9), the hemoglobin response was incomplete; in all seven cases in which mean corpuscular hemoglobin concentration after therapy is reported, hypochromia persisted. Hyperferremia is known to have persisted after pyridoxine therapy in three cases. In the three patients (Cases 1, 4, 7) in whom a decrease in the serum iron was reported following therapy, it is not clear whether this decreased during active blood regeneration and remained low or normal or whether it returned shortly to hyperferremic levels. In all seven patients in whom therapy was discontinued, the anemia relapsed. A statement in this regard is not included in the other two case reports (Cases 2, 5).

In two patients (table 5, Cases 4, 8), the anemia was present from birth. In one (Case 4), the anemia was probably hereditary. In the remaining patients, anemia was first detected later in life.

Abnormalities in the metabolism of tryptophane have not been consistent findings in this group of patients. The excretion of xanthurenic acid after administration of tryptophane was normal in four patients (table 5, Cases 4, 6, 8, 9), possibly increased in one (Case 3) and definitely increased in another
Table 5.—Comparison of Reported Cases of Pyridoxine-Responsive Anemia

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Patient</th>
<th>Age at onset in years</th>
<th>Before Pyridoxine</th>
<th>After Pyridoxine</th>
<th>Relapse off therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lowest Hb. Gm./100 ml.</td>
<td>MCV c. u.</td>
<td>MCHC %</td>
<td>Serum iron µg./100 ml</td>
</tr>
<tr>
<td>1</td>
<td>Harris (1)</td>
<td>27</td>
<td>5.9</td>
<td>66</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>Maier (2)</td>
<td>61</td>
<td>9.0</td>
<td>66</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>Gehrmann (3)</td>
<td>43</td>
<td>5.7</td>
<td>Low</td>
<td>237</td>
</tr>
<tr>
<td>4</td>
<td>Bishop (4)</td>
<td>cong.</td>
<td>6.0</td>
<td>75</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>Leeming (5)</td>
<td>40</td>
<td>5.9</td>
<td>22</td>
<td>Inc</td>
</tr>
<tr>
<td>6</td>
<td>Verloop (6)</td>
<td>33</td>
<td>8.4</td>
<td>75</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>Erslev (7)</td>
<td>25</td>
<td>3.8</td>
<td>63</td>
<td>22</td>
</tr>
<tr>
<td>8</td>
<td>Authors’ Case 1</td>
<td>cong.</td>
<td>5.8</td>
<td>58</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td>Authors’ Case 2</td>
<td>28</td>
<td>7.7</td>
<td>70</td>
<td>27</td>
</tr>
</tbody>
</table>

Figures in parentheses refer to bibliographic reference. Cong. = congenital; Inc. = increased; Hb. = hemoglobin; MCV = mean corpuscular volume; MCHC = mean corpuscular hemoglobin concentration.
(Case 7). Harris et al.\(^1\) state that abnormalities in the excretion of tryptophane metabolites were observed in their patient, but they give no details. In one of our patients, the excretion of kynurenic acid and kynurenine was increased even though the excretion of xanthurenic acid was within normal limits (Case 8). In the other (Case 9), no abnormality in the excretion of any of the tryptophane metabolites was observed.

Whether the cases of "pyridoxine-responsive anemia" represent a single disease entity cannot be determined until a greater number of patients has been described in detail. There are sufficient dissimilarities between the few reported cases to raise the possibility that the reported cases represent the result of several different pathogenetic mechanisms.

**Comparison of Pyridoxine-Responsive Anemia in Human Subjects and Pyridoxine-Deficiency in Experimental Animals**

Dogs\(^{22,23}\) and swine\(^{24}\) made deficient in pyridoxine by the exclusion of pyridoxine from the diet develop microcytic hypochromic anemia, normoblastic hyperplasia of the bone marrow, hyperferremia and hemosiderosis of the liver, spleen and bone marrow.

In all of the above respects the patients with "pyridoxine-responsive anemia" resemble the experimental dietary deficiency. The degree of hemosiderosis in the reported cases had no doubt been enhanced by the multiple transfusions and the oral and parenteral iron therapy which a number of the patients had received over the course of many years. In addition, it is quite likely that these patients, like pyridoxine-deficient rats\(^{23}\), absorb iron more readily than do normal subjects.

In swine deficient in pyridoxine,\(^{24}\) the free erythrocyte protoporphyrin (FEP) decreases to low levels early in the course of the deficiency, preceding the development of anemia. In both of our patients, the FEP values were in the low normal range\(^{27}\) when pyridoxine was withheld (18, 20, 20, 36 \(\mu g./100\) ml.), and were somewhat higher (33, 38, 43, 49 \(\mu g./100\) ml.) after prolonged administration of pyridoxine. Verloop and Rademaker\(^8\) likewise found a low normal concentration of FEP (25 \(\mu g./100\) ml.) in their patient. It should be pointed out that the mean value for FEP in normal pigs is higher (208 \(\mu g./100\) ml.) than the mean value for FEP in normal human subjects (32 \(\mu g./100\) ml.) and it is, therefore, considerably easier to demonstrate lower than normal values in the pig.

In the pig, the anemia and the associated metabolic abnormalities revert rapidly and completely to normal after the administration of pyridoxine. The plasma iron, after the administration of pyridoxine, decreases from values of 400 to 900 \(\mu g./100\) ml. to levels of about 100 \(\mu g./100\) ml. within 24 hours after the administration of a single dose of pyridoxine.\(^{26}\) In either experiments,\(^{24}\) when the animals were not always on a complete diet, the anemia was frequently only partially alleviated. In more recent experiments,\(^{28}\) when the basal diet of casein, sucrose and lard was supplemented with niacin, riboflavin, nicotinic acid, pantothenic acid, biotin, inositol, para-aminobenzoic acid, choline, folic acid and cobalamin, the anemia was completely alleviated by the administration of pyridoxine.
Swine deficient in pyridoxine excrete large quantities of xanthurenic acid.\textsuperscript{29} The quantity is so great that the addition of a drop of dilute ferric ammonium sulfate to alkaline urine causes it to turn deep green or black. However, it is not surprising that “pyridoxine-responsive” human subjects do not excrete large quantities of xanthurenic acid since species differences in the excretion of tryptophane metabolites have been observed.\textsuperscript{19} Furthermore, in human subjects the administration of deoxypyridoxine, a pyridoxine antagonist, is associated with a pattern of excretion of tryptophane metabolites which differs from that which follows the administration of another pyridoxine antagonist, isoniazid.\textsuperscript{20}

Swine deficient in pyridoxine develop epileptiform convulsions early in the course of the deficiency and later ataxia is observed in association with degenerative changes in the sensory neuron.\textsuperscript{24,30} Neither convulsions nor peripheral neuritis have been observed in patients with pyridoxine responsive anemia. Convulsions without anemia have been observed in infants fed a milk diet low in pyridoxine.\textsuperscript{31} Neurologic abnormalities in the absence of convulsions and anemia have been observed in patients receiving isoniazid.\textsuperscript{32} Patients given deoxypyridoxine develop seborrheic dermatitis and peripheral neuritis but not anemia or convulsions.\textsuperscript{33} Thus, different clinical syndromes have been observed in accordance with the manner in which the alterations in pyridoxine metabolism were induced. This is not surprising since pyridoxal phosphate functions as a coenzyme in a great many different enzyme systems.\textsuperscript{34} It seems possible that these enzymes may be influenced selectively.

Pathogenesis of Pyridoxine Responsive Anemia

The precise biochemical role of pyridoxine in porphyrin synthesis has been defined by Kikuchi et al.\textsuperscript{35,36} A pyridoxal phosphate derivative of glycine condenses with active succinate to form $\Delta$-aminolevulinic acid. The fact that low normal FEP, hyperferremia and hypochromic anemia occur in pyridoxine responsive anemia suggests that a defect in the synthesis of $\Delta$-aminolevulinic acid may be present in such patients. All of the available information indicates that this defect in not due to a dietary deficiency of pyridoxine. In general, the reported patients ingested a normal diet, the amounts of pyridoxine required to elicit a response were in excess of the amounts needed for simple replacement and, finally, relapse of all of the manifestations occurred promptly after withdrawal of pyridoxine therapy. It would be expected from our experience in growing pigs that if a simple dietary deficiency of pyridoxine existed, the anemia would not return within a period of two months or less after the parenteral administration of such large amounts of the vitamin, particularly in subjects ingesting a normal diet.

The possibility that the absorption of pyridoxine from the gastro-intestinal tract is impaired in these patients must be considered. Indeed, the long history of diarrhea in our patient E. O’R. suggests that he did have a chronic gastrointestinal disorder. However, failure to absorb pyridoxine seems like an unreasonable explanation since it would be expected, in adults at least, that the parenteral administration of massive doses of pyridoxine would replenish the stores of pyridoxine for a long period, certainly greater than two
months. The prompt return of the manifestations after the withdrawal of "massive" pyridoxine therapy suggests that some type of inhibition of the pyridoxal phosphate enzyme exists. It was suggested by Bishop and Bethell\(^4\) that the excessive iron stores and the increased iron concentration at the site of hemoglobin synthesis cause the formation of an inactive iron-pyridoxal complex. Although inhibition of \(\Delta\)-aminolevulinic acid synthesis by excessive iron can be demonstrated in in vitro systems,\(^{37,38}\) this explanation seems inadequate since a defect in hemoglobin synthesis is not usually observed in patients with hemochromatosis. Furthermore, in the experimental animal the hemosiderosis is secondary to a deficiency of pyridoxine.

In our two patients and, as discussed above, in at least several of the other reported cases, pyridoxine did not restore the hematologic abnormalities to normal. This suggests that at least one other defect is present in these patients. This defect would appear to involve heme synthesis since the hypochromic anemia persists, albeit in lesser degree than before pyridoxine therapy. The nature of this defect is not known. In our own experiments the abnormality was not corrected by the administration of brewer's yeast, pantothenic acid, pyridoxal, pyridoxamine, ascorbic acid, niacin, riboflavin, Valentine's liver extract, L-tryptophane, glutamic acid, calcium disodium ethylene diamine tetra-acetic acid, or ethinyl estradiol.

**Summary**

1. Two patients with microcytic hypochromic anemia, hyperferremia, and hemosiderosis have been described. A partial hematologic remission was observed in both patients following the administration of pyridoxine.

2. Interruption of pyridoxine therapy resulted in reticulocytopenia, a decline in the concentration of hemoglobin and a decrease in free erythrocyte protoporphyrin. Increased excretion of kynurenine and kynurenic acid, but not xanthurenic acid, was observed in the urine of one of the patients following administration of tryptophane. The excretion of tryptophane metabolites was within normal limits in the second patient.

3. Reinstition of pyridoxine therapy was followed by reticulocytosis, an increase in free erythrocyte protoporphyrin and an increase in the concentration of hemoglobin. The excretion of tryptophane metabolites in the urine following the administration of tryptophane was within normal limits in both patients. However, microcytosis, hypochromia, anemia and hyperferremia persisted.

4. The administration of brewer's yeast, Valentine's liver extract, calcium disodium ethylenediamine tetra-acetic acid, ethinyl estradiol, pantothenic acid, pyridoxal, pyridoxamine, ascorbic acid, niacin, riboflavin, glutamic acid and tryptophane failed to elicit a further hematologic response.

5. These patients have been compared with the seven other reported cases of "pyridoxine-responsive" anemia.

**Summario in Interlingua**

1. Es describite duo patientes con anemia hypochromic microcytic, hyperferremia, e hemosiderosis. Un partial remission hematologic esseva observate in ambe le patientes post le administration de pyridoxina.
2. Le interruption del therapia a pyridoxina resultava in reticulocytopenia, un declino del concentration de hemoglobina, e un reduction del liber proteoporphyrina erythrocytic. Un augmento del excretion de kynurenina e de acido kynurenic (sed non de acido xanthurenic) esseva observe in le urina de un del patientes post le administration de tryptophano. Le excretion de metabolitos de tryptophano esseva intra limites normal in le secunde paciente.

3. Le re-institution del therapia a pyridoxina esseva sequite per reticuloctyosis, un augmento del liber proteoporphyrina erythrocytic, e un augmento in le concentration de hemoglobina. Le excretion de metabolitos de tryptophano in le urina post le administration de tryptophano esseva intra limites normal in ambi patients. Tamen, microcytosis, hypochromia, anemia, e hyperferremia persisteva.

4. Le administration de levatura de birero, de extracto Valentine de hepate, de acido ethylenodiamino-tetra-acetic dimatric calcic, de estradiol ethinylic, de acido pantothenic, de pyridoxal, de pyridoxamina, de acido ascorbic, de niacina, de riboflavina, de acido glutamic, e de tryptophano non succedeva a evocar un responsa hematologic additional.

5. Iste patientes es comparete con le septe alteres in qui anemia "responsive a pyridoxina" ha essite reportate.

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PYRIDOXINE-RESPONSIVE ANEMIA


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Pyridoxine-Responsive Anemia

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