The Response of Free Erythrocyte Protoporphyrin to Pyridoxine Therapy in a Patient with Sideroachrestic (Sideroblastic) Anemia

By G. R. Lee, G. E. Cartwright and M. M. Wintrobe

Heilmeyer has applied the term "sideroachrestic anemia" to conditions characterized by the association of hypochromic anemia with excessive iron storage. In referring to the same syndrome, Mollin and others prefer the designation "sideroblastic anemia," a term which emphasizes the importance of abnormal iron accumulation in erythroblasts. Classifications of the reported cases have been proposed.

The patients in whom "sideroachrestic anemia" has been detected differ from one another in several important respects, including the response to pyridoxine therapy, the association with underlying disease, and the evidence of familial transmission of the disorder. It has been suggested that these varied disorders have in common the presence of a metabolic lesion in the hemoglobin synthetic pathways.

We have studied a patient with acquired sideroachrestic (sideroblastic) anemia and carcinoma of the prostate in whom a remarkable amount of protoporphyrin accumulated within his erythrocytes during pyridoxine therapy. Since this observation contributed to the understanding of the biochemical defects present in this patient, the case report is presented. A preliminary report was published elsewhere.

METHODS

The methods employed for the measurement of free erythrocyte protoporphyrin (FEP) and coproporphyrin (FEC), serum iron, total iron-binding capacity, bone marrow iron, tissue iron, iron kinetics, erythrocyte glutathione, and urinary tryptophane metabolites have been published. Routine hematologic procedures were performed as described by Cartwright.

The capacity of erythrocytes to synthesize porphyrins in vitro from delta-aminolevulinic acid (ALA) was measured by incubating one ml. of fresh hemolysate with 10 μM of ALA in 4 ml. of M/15 phosphate buffer, pH 7.2. After incubation for 45 minutes at 37 C., the concentration of porphobilinogen was determined by the method of Granick and Mauzerall, uroporphyrin by the method of Dresel and Falk, and coproporphyrin and proto-
porphyrin by the method of Wranne. This procedure resembles that of Lichtman and Feldman but differs in the concentration of ALA which was used as substrate.

CASE REPORT

J. P., a 79-year-old Caucasian male, was referred to the Salt Lake County General Hospital in March 1962 because of anemia. He had been well until 1950 when, at age 66, he was found to have a duodenal ulcer. He did well on conservative management until 10 years later. when he developed acute gastrointestinal bleeding and was admitted to another hospital for a gastrectomy. At that time the prostate was enlarged but a biopsy was negative for carcinoma. At the end of the hospitalization, the volume of packed red cells (V.P.R.C.) was 37 ml./100 ml and the leukocyte count (W.B.C.) was 3600/mm³.

The anemia was persistent (V.P.R.C. 29–34 ml./100 ml); however, the patient was symptom-free until late in 1961, when weakness and edema developed. In January 1962 the V.P.R.C. was 25 ml./100 ml., the mean corpuscular hemoglobin concentration (M.C.H.C.) 22 per cent, and the mean corpuscular volume (M.C.V.) 77 μm³. Erythrocytes were hypochromic on smear. The patient received blood transfusions and was given oral iron medication for 3 weeks as well as 7 intramuscular injections of iron and copper. When the anemia did not improve, the patient was referred to this hospital for further study.

Past medical history, review of systems and family history were negative.

The patient was an elderly, pale, Caucasian male. The heart was slightly emineralized. The liver was palpable just below the right costal margin and the spleen tip could be felt 4 cm. below the left costal margin. The prostate was enlarged to approximately 3 times its normal size.

Initial hematologic studies were as follows: V.P.R.C. 22 ml./100 ml.; hemoglobin 6.5 Gm./100 ml.; R.B.C. 2.33 × 10⁹/mm³; M.C.V. 95 μm³; M.C.H. 28 μg%; M.C.H.C. 30 per cent; reticulocytes 3.6 per cent; platelets 290,000/mm³; W.B.C. 3320/mm³; differential count: metamyelocytes 2 per cent, segmented neutrophils 43 per cent, lymphocytes 45 per cent, monocytes 10 per cent. Erythrocytes on smear were characterized by anisopoikilocytosis, and there was a population of hypochromic cells (Fig. 1).

The serum iron was 181 μg./100 ml. and the serum iron-binding protein was completely saturated. Stainable iron in the bone marrow reticulum cells was increased markedly, and "ringed" sideroblasts (Fig. 1) and sideroblasts containing excess iron granules were numerous. The myeloid:erythroid cell ratio in the aspirated bone marrow specimen was 1.8:1, and erythrocyte maturation was morphologically normal. Erythroid hyperplasia was observed in fixed sections prepared from a Vim-Silverman needle marrow biopsy.

The total serum bilirubin was 0.8 mg./100 ml.; serum copper, 91 μg./100 ml.; radioactive vitamin B₁₂ excretion, 24 per cent in 24 hours; urinary lead excretion, 0.01 mg./L; hemoglobin A₂, 2.5 per cent of the total hemoglobin; erythrocyte glutathione, 67 mg./100 ml. erythrocytes. Stool and urine analyses were normal.

Two sons were available for examination. They were clinically asymptomatic, and hematologic studies, including examination of the blood smear, were normal.

The anemia and neutropenia persisted throughout the remainder of the patient's life. The anemia was severe and he was given 75 transfusions of whole blood or packed red cells during his 30-month course.

In August 1963 a metastatic survey disclosed evidence of osteoblastic and osteolytic disease in the pelvis, lumbar spine and ribs. A prostatic biopsy disclosed undifferentiated carcinoma. Bilateral orchectomy followed by treatment with diethyl stilbestrol had no appreciable effect on the patient's hematologic status nor on the course of the metastatic disease. The patient expired in September 1964.

Metastatic carcinoma of the prostate was noted at postmortem examination. There was extensive hemosiderosis of the liver, spleen and bone marrow. The hepatic iron was largely parenchymal in distribution. There was little fibrosis. Significant hemosiderosis was not observed in the heart or pancreas. The liver weighed 2700 Gm. and the spleen weighed 520 Gm. The concentration of iron in the liver was 4.9 mg./Gm. of wet liver, and in the spleen it was 1.7 mg./Gm. of wet tissue. The liver contained a total of 13.2 Gm. iron and the spleen, 0.9 Gm.
Fig. 1.—The patient’s blood smear (A) as compared with a smear from a normal subject (B). C, D, E, and F: examples of “ringed” sideroblasts in the patient’s bone marrow aspirate.

Special Studies

Response to Pyridoxine

Initially, the patient was given 100 mg. of pyridoxine hydrochloride daily for 10 days orally, followed by 100 mg. daily for 10 days intramuscularly. The FEP was first measured 2 weeks after the last dose of pyridoxine and was found to be 1976 μg./100 ml. of packed red cells, a value greatly in excess of the upper limit of the normal range (25 to 60 μg./100 ml.). Seven weeks later, this value had decreased to 266 μg./100 ml. The possibility that the increase in FEP was related to pyridoxine therapy was considered; consequently two further trials of pyridoxine were given and the sequential changes in FEP were observed (Fig. 2).

During the second course of pyridoxine therapy (100 mg. daily for 84 days), the FEP increased from a base line value of 300 μg./100 ml. to 2440 μg./100 ml. When the pyridoxine was stopped, the FEP decreased to values of less than 300 μg./100 ml. During the third course of pyridoxine (200 mg. daily for 186 days), the FEP again increased, and declined when the vitamin was withdrawn. During the periods when the FEP values were greatly increased, the erythrocytes were observed to fluoresce when examined with a microscope equipped with an ultra-violet light source.

The concentration of free erythrocyte coproporphyrin (FEC) paralleled that of FEP. During the third course of pyridoxine, for example, the FEC increased
Fig. 2.—Variations in the concentration of free erythrocyte protoporphyrin (FEP) in relation to pyridoxine therapy. The Roman numerals refer to periods during which special studies were performed (see text).

from 0.5 to 8.5 µg./100 ml. while the FEP increased from 160 to 1620 µg./100 ml.

In patients with "pyridoxine-responsive anemia" the FEP is usually in the low normal range and increases to values of 30 to 40 µg./100 ml. following pyridoxine therapy. To determine if the accumulation of large amounts of protoporphyrin observed in our patient was unusual, pyridoxine (100 mg. daily by mouth) was administered to 2 normal subjects and to 8 patients with various disorders in which hemoglobin synthesis might be postulated to be impaired (Table 1). The response of the present patient differed significantly from that of the others, including a second case of acquired sideroachrestic anemia in a 68-year-old male.

During the 3 periods in which our patient received pyridoxine, no significant reticulocytosis was observed, and each time the severity of the anemia tended to increase (Fig. 2). An equivocal reduction in the transfusion requirement was observed during the second of the 3 pyridoxine trial periods but not during the first or the last periods. The mean corpuscular volume ranged from 88 to 107 µm³ and the mean corpuscular hemoglobin concentration from 29 to 32 per cent throughout the course of the study. In general, the highest values for the M.C.V. and the lowest values for the M.C.H.C. were observed when the patient was most anemic. The serum iron failed to decline during the periods of pyridoxine administration, and values of 175 to 260 µg./100 ml. were observed throughout the study. The saturation of the iron-binding protein was always between 80 to 100 per cent. Abnormal sideroblasts and excessive depo-
Table 1.—The Response of Free Erythrocyte Protoporphyrin to Pyridoxine Therapy in Several Disorders

<table>
<thead>
<tr>
<th>Condition</th>
<th>FEP (µg./100 ml.) before Pyridoxine Therapy</th>
<th>FEP (µg./100 ml.) after Pyridoxine Therapy</th>
<th>Days on Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present case—second course</td>
<td>270</td>
<td>1620</td>
<td>42</td>
</tr>
<tr>
<td>Present case—third course</td>
<td>315</td>
<td>1320</td>
<td>42</td>
</tr>
<tr>
<td>Normal male</td>
<td>44</td>
<td>47</td>
<td>46</td>
</tr>
<tr>
<td>Normal female</td>
<td>49</td>
<td>61</td>
<td>46</td>
</tr>
<tr>
<td>Iron-deficiency anemia</td>
<td>89</td>
<td>123</td>
<td>30</td>
</tr>
<tr>
<td>Iron-deficiency anemia*</td>
<td>199</td>
<td>189</td>
<td>10</td>
</tr>
<tr>
<td>Thalassemia minor</td>
<td>38</td>
<td>43</td>
<td>39</td>
</tr>
<tr>
<td>Anemia of chronic infection</td>
<td>231</td>
<td>236</td>
<td>14</td>
</tr>
<tr>
<td>Myelofibrosis</td>
<td>42</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>Acute myeloblastic leukemia</td>
<td>120</td>
<td>149</td>
<td>23</td>
</tr>
<tr>
<td>Acquired sideroblastic anemia</td>
<td>147</td>
<td>194</td>
<td>41</td>
</tr>
</tbody>
</table>

*Complicating paroxysmal nocturnal hemoglobinuria.

sition of iron in the bone marrow reticulum were observed whether or not the patient was receiving pyridoxine.

The 24-hour excretion of tryptophane metabolites was measured before and after a loading dose of 2 Gm. of L-tryptophane by mouth (Table 2). These studies were performed during a period when the patient had not received pyridoxine for 17 weeks (Fig. 2, I) and were then repeated after the patient had received pyridoxine for 18 weeks (Fig. 2, II).

All of the values were within normal limits both before and after the tryptophane load and with and without pyridoxine therapy, except for the excretion of hydroxykynurenine (Table 2). The excretion of this metabolite was increased after the tryptophane load, and this abnormality was corrected by the administration of pyridoxine.

Ferrokinetic studies were performed both before (Fig. 2, I) and during (Fig. 2, II) the administration of pyridoxine. The pyridoxine therapy did not influence the plasma iron turnover rate which was 117 mg./day before pyridoxine and 107 mg./day while the vitamin was being given. Red cell iron utilization was 10 per cent before pyridoxine and 24 per cent during pyridoxine therapy. Surface scanning during both studies indicated an abnormal early uptake of iron by the liver and a subnormal uptake by the sacrum.

In Vitro Synthesis of Intermediates between ALA and Protoporphyrin

When the patient's erythrocytes were incubated with ALA, no impairment in the synthesis of porphobilinogen, uroporphyrin, coproporphyrin, protoporphyrin or total porphyrin was observed (Table 3) either before or during pyridoxine therapy. These studies indicate that the biosynthetic pathway between ALA and protoporphyrin was entirely intact.

*Therapeutic Trial of Desferrioxamine-B*

In May of 1964 (Fig. 2, III) the patient was treated with the iron-chelating agent, desferrioxamine-B, in a dose of 1.0 Gm. per day intramuscularly for 17
Table 2.—Excretion of Tryptophane Metabolites

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Normal</th>
<th>Period I (No Pyridoxine)</th>
<th>Period II (Receiving Pyridoxine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Tryptophane Load After Tryptophane</td>
<td>No Tryptophane After Tryptophane</td>
<td>No Tryptophane After Tryptophane</td>
</tr>
<tr>
<td>Kynurenic acid</td>
<td>2-19</td>
<td>30-99</td>
<td>16 49</td>
</tr>
<tr>
<td>Xanthurenic acid</td>
<td>4-15</td>
<td>18-71</td>
<td>14 45</td>
</tr>
<tr>
<td>Anthranilic acid glucuronide</td>
<td>2-9</td>
<td>2-19</td>
<td>11 20</td>
</tr>
<tr>
<td>Hippuric acid</td>
<td>15-36</td>
<td>23-94</td>
<td>19 31</td>
</tr>
<tr>
<td>0-amino Acetyl-kynurenine</td>
<td>6-22</td>
<td>8-32</td>
<td>13 25</td>
</tr>
<tr>
<td>Kynurenine</td>
<td>6-21</td>
<td>9-83</td>
<td>22 64</td>
</tr>
<tr>
<td>Hydroxykynurenine</td>
<td>11-61</td>
<td>7-76</td>
<td>35 91</td>
</tr>
</tbody>
</table>

These determinations were performed by Dr. J. M. Price. Values are in µ M/24 hours.

days. A total of 180 mg. of iron was excreted into the urine during this period. The reticulocyte count rose from 2.0 per cent before therapy to 7.1 per cent on the twelfth day of therapy; however, the anemia continued to increase in severity (Fig. 3).

DISCUSSION

The features which establish the diagnosis of sideroachrestic (sideroblastic) anemia in the present case are (1) hypersideremia, (2) abnormal "ringed" sideroblasts in the bone marrow, (3) hypochromia of the erythrocytes, (4) erythroid hyperplasia of the bone marrow, (5) markedly reduced incorporation of radioiron into erythrocytes, and (6) prominent siderosis of bone marrow reticulum cells and liver parenchymal cells. The age of onset and the absence of a similar disease in family members suggest that the disorder was acquired rather than inherited. Mollin has noted that in "secondary" sideroblastic anemia the M.C.V. is frequently increased, the M.C.H.C. is normal or low normal, and a population of hypochromic erythrocytes is observed in the blood smear, a "dimorphic" type of anemia. All of these features were observed in our patient. His disorder may be termed either secondary (acquired) sideroblastic anemia or symptomatic sideroachrestic anemia. In 4 of MacGibbon and Mollin's 35 cases of secondary sideroblastic anemia the disease was associated with carcinoma.

It is our interpretation that the remarkable increase of FEP in our patient resulted from a block in the synthesis of heme from protoporphyrin and iron and the stimulation of ALA synthesis by pyridoxine therapy (Fig. 4). Our reasons are as follows.

Despite increasing concentrations of protoporphyrin in the erythrocytes, no increase in the circulating hemoglobin was observed. This indicates failure to incorporate the protoporphyrin into hemoglobin. The reaction by which this incorporation is accomplished requires the enzyme heme synthetase, glutathione, globin and iron. Since erythrocyte glutathione levels were normal, and since iron was present in abundance, it seems likely that the activity of the
enzyme was impaired. However, we cannot exclude the possibility that the iron was present in a form which was unavailable for heme synthesis or that there was a defect in globin synthesis. In any event, the block was not removed by pyridoxine therapy.

As judged by the in vitro studies (Table 3), the biosynthetic pathway between ALA and protoporphyrin was intact. These observations suggest that pyridoxine acted by stimulating the synthesis of ALA. This suggestion fits with previous observations establishing the role of pyridoxal phosphate as a cofactor for the enzyme ALA synthetase as well as with the observed changes in FEP when pyridoxine was given to pyridoxine-deficient swine or to certain patients with pyridoxine-responsive sideroachrestic anemia.

No effect of pyridoxine therapy was noted in control studies (Table 1). These indicated either that the observed stimulation of ALA synthesis is not a normal phenomenon or that it cannot be detected in the absence of block in the heme synthetase reaction. It seems probable that the effect of the vitamin in our patient was similar to that observed in patients with so-called pyridoxine-responsive anemia—namely, reversal of a pathologic block in ALA synthesis rather than correction of a deficiency of the vitamin. The details of the way in which pyridoxine produces this effect are unknown and the reader is referred to the discussion of the subject by Horrigan and Harris.

It is of considerable interest that the enzymes which are involved in the synthesis of coproporphyrinogen from ALA are located in the cytoplasm and that the activities of these enzymes (ALA dehydratase, porphobilinogen deaminase, uroporphyrinogen isomerase, and uroporphyrinogen decarboxylase) were normal (Table 3). On the other hand, the enzymes delta-ALA synthetase...
and heme synthetase are located in mitochondria. The relationship of these enzymes to mitochondria is shown diagrammatically in Figure 4. Both of the mitochondrial steps in heme synthesis seemed to be impaired in our patient, whereas the cytoplasmic steps were normal. Bessis and Breton-Gorius have shown that the iron-laden mitochondria in sideroblasts of patients with sideroachrestic anemia are swollen and that their cristae are indistinct, a possible submicroscopic demonstration of the morbid anatomy in this type of disorder.

It should be emphasized that neither of the two metabolic lesions thought to be present in our patient was confirmed by direct assay of the reactions involved. There are several reasons for this omission, notably the fact that these enzymes are absent from mature erythrocytes. It is necessary to assay reticulocytes or bone marrow in order to detect such activity. Difficulty in obtaining sufficient numbers of reticulocytes and in quantitating the marrow erythroid tissue and the nonheme iron pool at present creates formidable technical problems.

Fig. 4.—Schematic representation of hemoglobin synthesis in relation to mitochondria. The proposed sites of the blocks in hemoglobin synthesis in the patient presented are indicated by the letters A and B. The postulated effect of pyridoxine is the reversal of the block at “A.”

1. Δ-ALA Synthetase
2. ΔALA Dehydratase
3. Porphobilinogen deaminase
4. Uroporphyrinogen isomerase
5. Uroporphyrinogen decarboxylase
6. Coproporphyrinogen oxidase
7. Heme synthetase
Table 3.—Synthesis of Porphobilinogen and Porphyrins from Delta-ALA

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Normal</th>
<th>Receiving B6 (Fig. 2, II)</th>
<th>No B6 (Fig. 2, III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulocytes (per cent)</td>
<td>0.5–2.5</td>
<td>5.1</td>
<td>5.6</td>
</tr>
<tr>
<td>Porphobilinogen</td>
<td>274–1186</td>
<td>730</td>
<td>715</td>
</tr>
<tr>
<td>Uroporphyrin</td>
<td>2.3–8.9</td>
<td>7.5</td>
<td>7.9</td>
</tr>
<tr>
<td>Coproporphyrin</td>
<td>5.3–13.5</td>
<td>9.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Protoporphyrin</td>
<td>0.6–3.2</td>
<td>1.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Total porphyrins</td>
<td>8.9–21.3</td>
<td>18.5</td>
<td>17.7</td>
</tr>
</tbody>
</table>

Values are in μM/hour/ml erythrocytes.

The mild reticulocytosis observed in our patient during desferrioxamine therapy is of interest in that it lends some support to the thesis that iron in excessive amounts or in certain subcellular loci may impair hemoglobin synthesis.\(^{30}\) In the patient reported by Moeschlin\(^{31}\) there was an increase in circulating hemoglobin levels during therapy when desferrioxamine was given over a prolonged period of time. No such increase was observed in our patient during a much shorter observation period. Unfortunately, the patient's clinical condition did not permit further studies on the desferrioxamine effect.

**SUMMARY**

Studies of a patient with acquired sideroachrestic anemia and metastatic carcinoma of the prostate are presented. Although the administration of pyridoxine did not elicit a hematologic response, the administration of this vitamin was associated with a remarkable accumulation of protoporphyrin within the erythrocytes. It is suggested that there were two defects in the hemoglobin biosynthetic pathway: impaired incorporation of protoporphyrin into hemoglobin and defective delta-aminolevulinic acid synthesis. Pyridoxine had no effect on the former but partially corrected the latter.

**SUMMARIO IN INTERLINGUA**

Es presentate studios in un patiente con acquirite anemia sideroachrestic e carcinoma metastatic del prostata. Ben que le administration de pyridoxina non evocava un responsa hematologic, le administration de iste vitamina esseva associate con un remaricable accumulation de protoporphyrina intra le erythrocytos. Es suggestonate le presentia de duo defectos in le circuito biosynthetic de hemoglobina: un defective incorporation de protoporphyrina ad in hemoglobina e un defective synthese de acido delta-aminolevulinic. Pyridoxina habeva nulle effecto super le prime de iste defectos sed corrigeva in parte le secunde.

**ACKNOWLEDGMENT**

The studies on tryptophane metabolites were performed by Dr. J. M. Price of the University of Wisconsin. Dr. Edgar A. Jack of Ciba Pharmaceutical Company supplied the desferrioxamine-B. We are indebted to Miss Jacqueline Thomas for technical assistance and to Dr. Joseph Quagliana for clinical assistance.
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