Congenital Erythropoietic Porphyria, Diminished Activity of Uroporphyrinogen Decarboxylase and Dyserthropoiesis

By James P. Kushner, Neville A. Pimstone, Carl R. Kjeldsberg, Marsha A. Pryor, and Arthur Huntley

A 51-yr-old white male with congenital erythropoietic porphyria (Günther’s Disease) is described. The disease was first manifested in infancy and mutilating cutaneous photosensitivity eventually developed. The source of excess porphyrin production was a markedly dyserthropoietic bone marrow. The dyserthropoietic changes were most marked in the orthochromic and polychromatophilic normoblasts, and these cells demonstrated a red-orange nuclear fluorescence. The morphological features of the dyserthropoietic cells, studied with light and electron microscopy, were identical to those found in congenital dyserthropoietic anemia type I and are similar to those described in other patients with congenital erythropoietic porphyria. The red-orange nuclear fluorescence was the only feature not usually associated with congenital dyserthropoietic anemia type I. Massive porphyrinuria was present, but the pattern of porphyrin excretion was atypical for porphyria. The red-orange nuclear fluorescence was the described in other patients with congenital dyserythropoietic anemia type I and are similar to those studied with light and electron microscopy. were identical to those found in congenital dyserthropoietic anemia type I. Massive porphyrinuria was present, but the pattern of porphyrin excretion was atypical for congenital erythropoietic porphyria. Hepta carboxyl (7-COOH) porphyrin was the major urine porphyrin. and there was also a large amount of uroporphyrin present. Both

CONGENITAL ERYTHROPOIETIC PORPHYRIA is by far the least common of the porphyrias, having been reported in only about 70 persons. Clinically, the disease is characterized by onset in infancy, pronounced cutaneous photosensitivity, erythrodontia, hirsutism, anemia of variable severity, and the excretion of dark red urine containing excessive amounts of uroporphyrin I, a biologically useless isomer that cannot be converted to heme.

Studies with fluorescence microscopy have suggested that the excess porphyrin is formed in red cell precursors in the bone marrow, and suppression of erythropoiesis by transfection of packed erythrocytes results in a marked decrease in porphyrin production and excretion. Anemia has been a significant clinical feature in the majority of the reported patients with congenital erythropoietic porphyria. Because of the rarity of the disease, little is known about the pathogenesis of the anemia. The kinetic features of the anemia have been studied in several cases. These studies have suggested that both a shortened red cell survival and ineffective erythropoiesis play a role in the pathogenesis of the anemia. Kinetic studies have suggested that both abnormal and normal erythrocytes are produced in the same subject.

Morphological descriptions of the blood and bone marrow have been reported in detail in very few patients with congenital erythropoietic porphyria. Anisocytosis, poikilocytosis, polychromasia, basophilic stippling, and nucleated erythrocytes appear to be fairly common features noted on Romanovsky-stained preparations of the peripheral blood. Morphological abnormalities of the bone marrow, when described at all, have ranged from erythroid hyperplasia to degenerative changes in erythropoietic cells. When looked for, nuclear inclusions containing hemoglobin have been found.

Studies of the marrow morphology, using light and fluorescence microscopy, have suggested the coexistence of normal and abnormal erythropoietic cells. The dual nature of the erythroid precursors was supported by ultrastructural morphology in a single brief report.

The pattern of heme precursor overproduction in congenital erythropoietic porphyria is consistent with a defect in the conversion of porphobilinogen to uroporphyrinogen III, the isomer of uroporphyrinogen that is converted to heme. The biosynthesis of uroporphyrinogen III involves a complex series of reactions requiring two enzymes: uroporphyrinogen I synthase and uroporphyrinogen III cosynthase (Fig. 1). Uroporphyrinogen III cosynthase activity was not subnormal, but uroporphyrinogen decarboxylase activity was 50% of control values. A family study revealed other relatives with the same degree of subnormal erythrocyte uroporphyrinogen decarboxylase activity, but none with congenital erythropoietic porphyria or anemia. This is the first reported case of congenital erythropoietic porphyria associated with deficient activity of uroporphyrinogen decarboxylase, and it suggests that congenital erythropoietic porphyria may be a syndrome rather than a disease due to a specific enzymic defect. The family study in this case suggested that two defects may be required for clinical expression of the porphyria. One is an enzymic defect in the heme biosynthetic pathway and the other is dyserthropoietic anemia, indistinguishable on morphological grounds from congenital dyserthropoietic anemia type I. The morphological changes do not appear to be a consequence of the heme biosynthetic defect.

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The generation of intermediates with 7.6 and 5 carboxyl groups patients with congenital dyserythropoietic anemia type boxylic compound coproporphyrinogen is accompanied by the coproporphyrinogen III to compounds of the isomer I type. The decarboxylation of the pathway is diverted from the normal synthesis of coproporphyrinogen. In the absence of uroporphyrinogen III cosynthase, the coproporphyrinogen III is transformed nonenzymically to uroporphyrinogen III by uroporphyrinogen III cosynthase, which produces both ring closure and the proper orientation of the "D" pyrrole. Pre-uroporphyrinogen is converted to uroporphyrinogen III cosynthase activity in erythrocytes and cultured skin fibroblasts obtained from several patients with congenital erythropoietic porphyria. One is an enzymatic defect in the heme biosynthetic pathway at the uroporphyrinogen III step. This suggestion has been supported by the findings of Levin and coworkers, who reported subnormal activity of uroporphyrinogen III cosynthase. The adsorbed porphyrins were eluted and esterified with BF3-methanol and transferred to dichloromethane. An aliquot of the dichloromethane solution was taken to dryness, and the porphyrin-esters eluted with methanol:chloroform (1:5, v/v). The bands corresponding to the methyl-esters of 8,7,6,5 and 5-COOH porphyrins were scraped off the silica gel thick layer plates, which were then developed with petroleum ether:chloroform (1:5, v/v), and the purity of each band established by high performance liquid chromatography (HPLC) with a Waters (Milford, Mass.) HPLC system using a µ-Porasil column and a solvent mixture of heptane:ethyl acetate (55:45, v/v). The purified porphyrin-esters were hydrolyzed overnight in 6N HCl, taken to dryness, and the isomer composition of each was determined by a modification of the method of Englert et al. with reverse phase HPLC using an acetonitrile:phosphate buffer system. Retention times of the I and III isomer peaks (Table 1) were compared with those of standards obtained from Porphyrin Products, Logan, Utah. Baseline resolution between peaks was achieved. Purity of the standards was confirmed both in the reverse phase system and by esterification and analysis on µ-Porasil with the heptane:ethyl acetate solvent system.

**Materials and Methods**

**Porphyrin Determinations**

Urine porphyrins were quantified by adjusting the pH of a 100-ml aliquot to 3.5 and then adding approximately 0.5 g of Talc, USP. The adsorbed porphyrins were eluted and esterified with BF3-methanol and transferred to dichloromethane. An aliquot of the dichloromethane solution was taken to dryness, and the porphyrins were dissolved in chloroform and quantified spectrophotometrically. The remaining dichloromethane solution was applied as a broad band on silica gel thick layer plates, which were then developed with petroleum ether:chloroform (1:5, v/v), and the purity of each band established by high performance liquid chromatography (HPLC) with a Waters (Milford, Mass.) HPLC system using a µ-Porasil column and a solvent mixture of heptane:ethyl acetate (55:45, v/v). The purified porphyrin-esters were hydrolyzed overnight in 6N HCl, taken to dryness, and the isomer composition of each was determined by a modification of the method of Englert et al. with reverse phase HPLC using an acetonitrile:phosphate buffer system. Retention times of the I and III isomer peaks (Table 1) were compared with those of standards obtained from Porphyrin Products, Logan, Utah. Baseline resolution between peaks was achieved. Purity of the standards was confirmed both in the reverse phase system and by esterification and analysis on µ-Porasil with the heptane:ethyl acetate solvent system.

**Erythrocyte, plasma, and stool porphyrins were quantified by fluoroscopy of the methyl esters separated by thin-layer chromatography (TLC) as described by Day et al.** HPLC analysis of...
plasma porphyrins was done by a modification of the method of Heller et al. Washed packed erythrocytes were freeze-thawed 5 times and the lysate diluted 1:10 in 0.05M Tris, pH 8.2. A 0.5-ml aliquot of the diluted lysate was incubated for 1 hr at 37°C with 750 nmole of porphobilinogen (Porphyrin Products, Logan, Utah) and sufficient buffer to bring the reaction volume to 2.0 ml. Reactions were stopped by the addition of 2.5 ml of 25% TCA and the precipitated protein removed by centrifugation. The supernatant was diluted 1:1 with 3N HCl and the porphyrin content determined spectrophotometrically. Results are expressed as µg uroporphyrinogen generated /ml RBC/ hr.

**Uroporphyrinogen I Synthase**

Assays were done by a modification of the method of Magnussen et al. Washed packed erythrocytes were freeze-thawed 5 times and the lysate diluted 1:10 in 0.05M KPO₄, pH 7.6, and sonicated 4 times for 30 sec each with a Bronson Model (Stamford, Conn.) W-140 Sonifier. The lysate was then diluted 1:10 in 0.05M KPO₄, pH 7.6, and sufficient buffer to bring the total volume to 2.0 ml. Reactions were stopped by the addition of 2.5 ml of 25% TCA and the precipitated protein removed by centrifugation. The supernatant was diluted 1:1 with 3N HCl and the porphyrin content determined spectrophotometrically. Results are expressed as µg uroporphyrinogen generated /ml RBC/ hr.

**Uroporphyrinogen III Cosynthase**

Assays were done by a modification of the method of Romeo and Levin. Washed packed erythrocytes were freeze-thawed 5 times, the lysate diluted with an equal volume of 0.05M KPO₄, pH 7.6, and sonicated 4 times for 30 sec each with a Bronson Model (Stamford, Conn.) W-140 Sonifier. The lysate was then diluted with sufficient buffer to yield a final hemoglobin concentration of 7 g/dl. Forty nanomoles of porphobilinogen was added to sufficient uroporphyrinogen I synthase, prepared from *R. spheroides*, to generate 10 nmole of uroporphyrinogen I. Once this empiric determination had been done, aliquots of the erythrocyte lysate (0.01-0.20 ml) were added to a series of test tubes in an ice bath. Forty nanomoles of porphobilinogen, the appropriate amount of uroporphyrinogen I synthase, and sufficient 0.05M KPO₄, pH 7.6, to bring the total volume to 2.0 ml were then added to each tube. The tubes were transferred to a 37°C water bath and incubated for 30 min. Reactions were stopped by the addition of 0.5 ml of 0.6N HCl, the precipitated protein brought down by centrifugation, and the supernatants saved. The pellets were reextracted with 0.5 ml of 0.3N HCl and the two supernatants combined and transferred to Carius combustion tubes (1.2 x 21.5 cm with a wall thickness of 0.2 cm). The tube walls were rinsed with an additional 0.5 ml of 0.3N HCl, evacuated, sealed, and heated to 180°C for 4 hr. The tubes were opened, centrifuged, and the liquid supernatant carefully aspirated from the tarry residue. The residue was extracted with 1.0 ml of 0.3N HCl, the supernatants combined and taken to dryness. The dry residue was dissolved in 100 µl of acetonitrile:phosphate buffer (15:85, v/v) and the isomer composition of the resulting isomers of coproporphyrin in each sample determined by reverse phase HPLC (see above). A plot was then made with the percent of the III isomer of coproporphyrin on the vertical axis and volume of lysate on the horizontal axis.

**Uroporphyrinogen Decarboxylase**

Assays were done by a modification of a previously published method. The modification included the enzymic generation of unlabeled uroporphyrinogen I as a substrate and separation and quantification of the reaction products by HPLC.

**Light Microscopy**

Light microscopic studies were done on smears of peripheral blood and bone marrow (obtained by sternal puncture) stained with Romanovsky stain. The iron content of marrow cells was evaluated with the Prussian blue reaction. Benzidine staining of the marrow was done by the method of Garcia-Blanco et al.

**Electron Microscopy**

Unstained smears of the bone marrow aspirate were examined with a Zeiss (Oberkochen, W. Germany) Photomicroscope II with epifluorescence attachments and xenon light source. Smears were illuminated with light in the Soret band by using a Zeiss B.P. 400-440 nm excitation filter and photographed with Kodak Ektachrome ASA 400 daylight film.

**CASE REPORT**

**History and Physical Examination**

G.B., a 51-yr-old white male was admitted to the clinical research center at the University of California, Davis Medical Center, in February 1980 for evaluation of congenital erythropoietic porphyria (Günther’s disease).

He was first noted to have red-colored urine as an infant. At age 4, he began to develop “huge water blisters” on sun exposed areas of the skin. At age 5, a diagnosis of congenital erythropoietic porphyria was made and avoidance of sunlight was advised. An anemia was noted and a brief period of therapy with oral iron was begun.

The skin blisters continued to occur and they often ruptured spontaneously and became infected. Several hospitalizations were required for treatment of these infections. At age 12, a portion of his left ear was surgically resected because of a persistant infection involving the cartilage.

During his teen-aged years he spent much of his time out of doors, and severe disfiguring scars developed at the sites of infected blisters. By the age of 30 he had lost all of his fingers and major portions of both ears, the nose, and upper lip. He subsequently attempted to avoid sunlight but continued to have severe and extensive blistering on his hands and head, frequently complicated by secondary infections. Skin grafting was required for the repair of an extensive lesion on the scalp.

His anemia persisted in spite of both iron and folic acid therapy and, over the years, he received several blood transfusions. Jaundice had never been noted and he had no symptoms to suggest the presence of gall stones.

<table>
<thead>
<tr>
<th>Table 1. Reverse Phase Chromatography of Porphyrin Isomers</th>
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<tr>
<td></td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>8-COOH†</td>
</tr>
<tr>
<td>7-COOH</td>
</tr>
<tr>
<td>6-COOH</td>
</tr>
<tr>
<td>5-COOH</td>
</tr>
<tr>
<td>4-COOH</td>
</tr>
<tr>
<td>3-COOH</td>
</tr>
<tr>
<td>2-COOH</td>
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*0.01M Na₂HPO₄, pH 6.85, with 0.0005M EDTA.
†Flow rate 1.5 ml/min using a Waters C-18 Bondapak column.
There was no family history of anemia or photosensitive dermatosis. His father is English and his mother was of Armenian extraction.

Physical examination revealed striking mutilation of the face and hands. The skin over the head was thickened and scarred (Fig. 2). There were erosions, ulcerations, and crusted areas on the scalp as well as several intact blisters. Male pattern baldness was present but, in addition, there were widespread areas of cicatricial alopecia. Most of the nose and most of both ears were absent. The upper lip was absent, exposing the gingiva and upper incisors. The teeth exhibited a pink fluorescence when illuminated with a Wood’s light. He was unable to completely close his eyes because of bilateral lower lid ectropia. All of the phalanges and some of the distal metacarpals were missing (Fig. 2). There was marked scarring on the dorsal aspects of the hands. Aside from a generalized increase in body hair, the skin on sun-protected areas appeared normal.

Fig. 2. Patient GB. The skin is thickened and scarred. Note absence of most of the nose, the upper lip and most of both ears. The teeth fluoresced when illuminated with a Wood’s light. Photomutilation has resulted in the loss of all phalanges and of the distal ends of some metacarpals.

Laboratory Findings

Blood counts revealed a VPRC of 23.7%; Hb 7.6 g/dl; MCV 82 fl; MCH 26.7 µg; MCHC 32.3%; WBC 12.9 x 10⁹/liter with 83% segmented neutrophils, 5% bands, 4% monocytes, and 8% lymphocytes; platelets 169 x 10⁹/liter; reticulocyte count 2.2%. The acidified serum hemolysis test (Ham’s test) was negative. No agglutination was noted when the patient’s erythrocytes were incubated with either anti-A or anti-B antibodies.

Serum chemistries were normal with the exception of the unconjugated bilirubin, which was 1.2 mg/dl, and the uric acid, which was 10.3 mg/dl. The serum iron concentration was 216 µg/dl. The serum ferritin was 184 µg/ml. The iron binding capacity was 240 µg/ml (normal 150-250). A 24-hr urine collection contained 21,200 µg of porphyrins (normal <250). HPLC analysis of the urinary porphyrins, as their methyl-esters, revealed 7-COOH porphyrin to be the major constituent and uroporphyrin to be the next most abundant compound (Fig. 3). Quantification and isomer determination of the individual urinary porphyrins are given in Table 2. The daily excretion of isomer III porphyrins was 18,370 µg compared to 2829 µg of isomer I porphyrins.

Stool porphyrin content was 351.3 µg/g dry weight (normal <140). Thin layer chromatographic (TLC) analysis revealed coproporphyrin to be the major fecal porphyrin with isocoproporphyrin representing about 25% of the total fecal porphyrins (Table 3).

Plasma porphyrin content was markedly elevated at 83.8 µg/dl (normal 0-0.48). HPLC analysis revealed prominent peaks corresponding to uroporphyrin, 7,6 and 5-COOH porphyrins (Fig. 3). In addition, TLC analysis of the plasma revealed both isocoproporphyrin (approximately 12% of the plasma porphyrins) and an unspecified porphyrin with migration characteristics as described by Day et al.²⁹ (Table 3). Erythrocyte porphyrin content was moderately elevated at 187.8 µg/100 ml RBC (normal 5-44). The excitation

Fig. 3. HPLC tracing of porphyrin methylester standards (A) and methylesters of porphyrins extracted from the urine (B) and the plasma (C) of patient G.B. The verticle axis represents absorbance at 405 nm. The solvent mixture was heptane:ethyl acetate (55:45, v/v). the flow rate 2.0 ml/min, and the column a Waters Associates µ-Poracl. The numbers over the peaks refer to the number of carboxyl groups on each porphyrin (2 = 2-COOH or protoporphyrin, 4 = 4-COOH or coproporphyrin, 5,6, and 7 represent intermediate compounds, and 8 = 8-COOH or uroporphyrin). The major urine constituent is 7-COOH porphyrin (B). Uroporphyrin, 7, 6, and 5-COOH porphyrins are present in the plasma (C).
Table 2. Urinary Porphyrins

<table>
<thead>
<tr>
<th>Porphyrin</th>
<th>Sample 1</th>
<th>Percent Isomer III (µg/24 hr)</th>
<th>Percent Isomer I (µg/24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uroporphyrin</td>
<td>5.046</td>
<td>65 (3,280)</td>
<td>35 (1,766)</td>
</tr>
<tr>
<td>7-COOH porphyrin</td>
<td>13.483</td>
<td>97 (13,078)</td>
<td>3 (404)</td>
</tr>
<tr>
<td>6-COOH porphyrin</td>
<td>1.569</td>
<td>86 (1,349)</td>
<td>14 (202)</td>
</tr>
<tr>
<td>5-COOH porphyrin</td>
<td>742</td>
<td>86 (1,338)</td>
<td>14 (104)</td>
</tr>
<tr>
<td>Coproporphyrin</td>
<td>360</td>
<td>7 (25)</td>
<td>93 (335)</td>
</tr>
<tr>
<td>Total porphyrins</td>
<td>21,200</td>
<td>87 (18,370)</td>
<td>13 (2,829)</td>
</tr>
</tbody>
</table>

*Normal values: uroporphyrin, <50 µg; 7,6,5-COOH porphyrins, trace; coproporphyrin, <200 µg.

Table 3. Stool and Plasma Porphyrins

<table>
<thead>
<tr>
<th>Porphyrin</th>
<th>Stool (µg/g dry wt)</th>
<th>Plasma (µg/100 ml)</th>
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<tbody>
<tr>
<td>Uroporphyrin</td>
<td>47.7</td>
<td>8.3</td>
</tr>
<tr>
<td>7-COOH porphyrin</td>
<td>33.7</td>
<td>17.2</td>
</tr>
<tr>
<td>6-COOH porphyrin</td>
<td>24.5</td>
<td>16.5</td>
</tr>
<tr>
<td>5-COOH porphyrin</td>
<td>15.9</td>
<td>12.2</td>
</tr>
<tr>
<td>Isocoproporphyrin</td>
<td>82.0</td>
<td>10.1</td>
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<tr>
<td>Coproporphyrin</td>
<td>107.1</td>
<td>75.5</td>
</tr>
<tr>
<td>Porphyrin unspecified</td>
<td>—</td>
<td>3.5</td>
</tr>
<tr>
<td>3-COOH porphyrin</td>
<td>40.4</td>
<td>5.9</td>
</tr>
<tr>
<td>Protoporphyrin</td>
<td>40.4</td>
<td>10.1</td>
</tr>
<tr>
<td>Total</td>
<td>351.3</td>
<td>83.8</td>
</tr>
</tbody>
</table>

*Normal values: stool <140 µg/g dry wt; plasma <0.48 µg/100 ml.

and emission maxima in methanol (420 nm and 588 nm, respectively) were consistent with the porphyrin being present as zinc protoporphyrin. Virtually all of the erythrocyte porphyrin was protoporphyrin with only a trace of uroporphyrin detected.

A percutaneous liver biopsy weighing 20.2 mg (wet weight) did not fluoresce. Only trace amounts of porphyrin could be extracted in 3N HCl.29 Microscopic examination was unremarkable. There was no evidence of hepatic siderosis. Liver iron content, determined by atomic absorption spectrophotometry, was 152 µg iron/100 mg dry weight (normal range 30–140). Unfortunately, the biopsy specimen thawed in shipment from Davis to Salt Lake City and enzyme assays were not possible.

RESULTS

Enzyme Assays

Uroporphyrinogen I Synthase

The patient’s erythrocyte uroporphyrinogen I synthase activity was 75 µg/ml RBC/hr. This value is greater than twice the highest value for normal subjects in our laboratory (normal range 20–34 µg/ml RBC/hr).

Uroporphyrinogen III Cosynthase

The uroporphyrinogen III cosynthase activity of the patient’s erythrocytes and the activity of the erythrocytes from 9 normal subjects are shown in Fig. 4. The enzymic activity in the patient’s cells was greater than in any of the normal cells. Cosynthase assays were done with the generation of 8, 10, 15, and 20 n mole of substrate, and in each case, the enzymatic activity in

Uroporphyrinogen Decarboxylase

The uroporphyrinogen decarboxylase activity of the patient’s erythrocytes was 0.08 U/mg Hb. The enzyme in normal cells has a mean activity of 0.18 U/mg Hb (range 0.15–0.22). Thus, uroporphyrinogen decarboxylase activity in the patient’s erythrocytes was approximately 50% of the lower limits of normal.

Morphology

Light Microscopy

A representative area of the peripheral blood smear is shown in Fig. 5A. In addition to the anisocytosis and poikilocytosis, moderate polychromasia and focal coarse basophilic stippling were also noted. Abnormal erythrocyte forms included tear drops, ovalocytes, and occasional schistocytes. An occasional nucleated erythrocyte with an irregular, lobulated nucleus was seen. The platelets appeared normal in number but several
Phages were loaded with ingested, dyserythropoietic normoblasts (Fig. 5C).

The Prussian blue stain revealed a marked increase in marrow reticuloendothelial iron stores. Most of the normoblasts contained more than three siderotic granules in the cytoplasm, and there were many ringed sideroblasts. The benzidine stain of the marrow aspirate revealed from one to several dark inclusions in the nucleus of many of the dyserythropoietic normoblasts.

Fluorescence Microscopy

Fluorescence microscopy revealed a red-orange fluorescence limited to the dyserythropoietic, polychromatophilic, and orthochromic normoblasts (Fig. 5D). The fluorescence was much stronger in the nuclei than in the cytoplasm.

Electron Microscopy

Approximately 25% of the marrow normoblasts exhibited no ultrastructural abnormalities. This corresponded to the percentage of normal cells seen with the light microscope. The majority of the normoblasts, however, exhibited multiple, often bizarre, ultrastructural nuclear abnormalities. Most of the nuclei had irregular contours ranging from shallow indentations and deep clefts, to splits and blebs (Fig. 6). Intranuclear inclusions, corresponding in appearance to the cytoplasm, gave many nuclei a spongy appearance (Fig. 7). In several normoblasts the abnormalities were so bizarre that it looked as if an explosion had taken place within the nucleus. The nuclear envelope had lost its integrity, resulting in an outpouring of nuclear material into the cytoplasm (Fig. 8). Cytoplasmic invagination between chromatin masses, with apparent dissolution of the chromatin, was observed. Lobulation and multinucleation of normoblast nuclei were noted (Fig. 8B). A population of normal-appearing normoblasts was also present (Fig. 8C). The perinuclear space was often dilated. Within the cytoplasm, vacuoles of varying size and electron density were seen. These vacuoles contained degenerated mitochondria, iron, myelin figures, segments of membranes, and apparent nuclear material (Fig. 8D).

Iron was frequently seen within the normoblast mitochondria. The mitochondria were usually short and plump (Figs. 7 and 8D), in contrast to the iron-laden mitochondria of primary sideroblastic anemia, which are often dilated and demonstrate degenerative changes.46

Macrophages containing numerous dyserythropoietic normoblasts were noted. The macrophage lysosomes appeared to be laden with iron. No ultra-

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Fig. 5. (A) Peripheral blood smear demonstrating anisocytosis, poikilocytosis, and focal hypochromia. Romanovsky stain (x 1100). (B) Bone marrow aspirate demonstrating dyserythropoietic normoblasts. Many normoblasts have irregular lobulated nuclei. Romanovsky stain (x 1100). (C) Bone marrow aspirate demonstrating a macrophage loaded with ingested dyserythropoietic normoblasts. Prussian blue stain (x 980). (D) Unstained bone marrow aspirate viewed with a fluorescence microscope. Intense red-orange nuclear fluorescence (reproduced in white) is seen. Note the lobulated nucleus in one of the fluorescent dyserythropoietic normoblasts (x 1100).
Family Studies

The patient's mother was deceased and his only brother was unavailable for study. Red cell enzyme assays, urine, plasma, and fecal porphyrins were studied in specimens obtained from his father, his spouse, and his 7 children. The pedigree is shown in Fig. 9. Uroporphyrinogen decarboxylase activity was subnormal in two sons, III-1 (0.12 U/mg Hb) and III-2 (0.11 U/mg Hb), but was normal in all other pedigree members studied with the exception of the proband. Uroporphyrinogen III cosynthase activity was indistinguishable from control values in all individuals studied except the proband. Uroporphyrinogen I synthase activity was elevated (51.7 μg/ml RBC/hr) in III-2 and in the proband but was within the normal range in all others.

Total urine porphyrins, total fecal porphyrins, and HPLC analysis of urine and fecal porphyrins were within normal limits in all pedigree members except the proband. Total plasma porphyrins were also normal, but HPLC analysis of plasma porphyrins in III-1 revealed prominent peaks corresponding to uroporphyrin, 7-COOH porphyrin, and coproporphyrin (Fig. 10).

DISCUSSION

The patient described had all of the typical clinical features of congenital erythropoietic porphyria. The disease was first manifested in infancy, and mutilating cutaneous photosensitivity eventually developed. Massive porphyrinuria was present and the source of the excess porphyrin production was the erythron, as demonstrated by fluorescence microscopy of the bone marrow. The liver did not fluoresce and only trace amounts of porphyrin could be extracted from the liver biopsy specimen. There was a marked anemia, bizarre erythroid hyperplasia of the marrow, indirect hyperbilirubinemia, and splenomegaly.

The laboratory finding that suggested that this was a distinctly atypical case of congenital erythropoietic porphyria was the chromatographic pattern of the urinary porphyrins. In congenital erythropoietic porphyria, the urinary porphyrins are comprised primarily
Fig. 7. Electron photomicrograph of the bone marrow aspirate. A dyserthrypoietic, orthochromic normoblast is shown with intranuclear inclusions (IN) representing penetration of the cytoplasm into the nuclear area. The perinuclear space is dilated. The cytoplasm contains iron-laden mitochondria (MI) and autophagocytic vacuoles ($\times 18,000$).

of uroporphyrin 1. Smaller amounts of coproporphyrin, again predominantly of the isomer I type, are also found. HPLC analysis has not revealed large amounts of porphyrins with 7, 6, and 5 carboxyl groups. The predominant urinary porphyrin in this patient was 7-COOH porphyrin. Large amounts of uroporphyrin and 6-COOH porphyrin were present as well (Fig. 3). Virtually all of the 7-COOH porphyrin was of the isomer III type (Table 3). This pattern of urinary porphyrin excretion is much more typical of that seen in porphyria cutanea tarda. Although uroporphyrin is generally the predominant urine porphyrin, we have observed several patients with porphyria cutanea tarda in whom the amount of 7-COOH porphyrin excreted exceeded that of uroporphyrin.

Analysis of fecal porphyrins revealed the presence of a significant quantity of isocoproporphyrin (Table 3). This alternate product of the metabolism of 5-COOH porphyrinogen is present in trace amounts in stool samples obtained from normal subjects, but is present in greatly increased amounts in patients with porphyria cutanea tarda. A second similarity between the findings in the present case and those in porphyria cutanea tarda is that the erythrocyte uroporphyrinogen decarboxylase activity was reduced by approximately 50%.

Uroporphyrinogen III cosynthase activity, expected to be subnormal in congenital erythropoietic porphyria, was actually higher than control values (Fig. 4). The increase in cosynthase activity may be due to circulating erythrocytes of a younger mean age than that in the control group. In their original studies, Romeo and Levin found the erythrocyte cosynthase activity in a patient with pyridoxine responsive sideroblastic anemia and a high reticulocyte count to be approximately two times higher than control values. Those authors based their activity calculations on the separation of the I and III isomers of uroporphyrinogen by a method now realized to be suboptimal, although the results do roughly parallel those found with methods that separate coproporphyrin isomers.
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Fig. 8. Electron photomicrographs of the bone marrow aspirate. (A) Focal disintegration of a normoblast nuclear envelope with an outpouring of nuclear material (arrows) (x 10,000). (B) Lobulation and apparent multinucleation of a normoblast nucleus. Disintegration of the nuclear envelope has resulted in a mixture of chromatin strands (CR) within the cytoplasm (x 110,000). (C) Two normal-appearing normoblasts. Compare the size of the pores (P) in the nuclear envelope with disruptions in the dyserythropoietic cells (x 8000). (D) Severe lobulation of the nucleus (N) and multifocal breaks in the nuclear envelope. Iron-laden mitochondria and vacuoles (V) are seen in the cytoplasm (x 10,000).

Fig. 9. The pedigree of patient G.B. The proband is indicated by the arrow. With the exception of the two individuals not studied, open circles and squares indicate individuals with normal uroporphyrinogen decarboxylase activity and normal concentrations of urine, fecal, and plasma porphyrins.

Fig. 10. HPLC tracing of the methylesters of plasma porphyrins from the patient's son (individual III-1, Fig. 5) (lower panel) and the patient's wife (upper panel). The details of the chromatography system and the identification of the peaks are as in Fig. 3. The pattern of plasma porphyrins in the upper panel (the patient's wife) is normal. The pattern in the lower panel demonstrates peaks corresponding to uroporphyrin (8), 7-COOH porphyrin (7), and coproporphyrin (4).

Of interest is the finding of higher than normal erythrocyte uroporphyrinogen I synthase activity in our patient. Increased uroporphyrinogen I synthase activity has been reported in several cases of congenital erythropoietic porphyria, and Miyagi et al. have proposed that a genetically determined increase in uroporphyrinogen I synthase activity may be the primary enzymic defect in this disease. No evidence based on family studies exists to support this hypothesis, and it remains to be determined if increased activity of uroporphyrinogen I synthase in patients with congenital erythropoietic porphyria is due to a young population of circulating erythrocytes, a primary genetic abnormality, or a phenomenon secondary to diminished activity of other heme biosynthetic enzymes (e.g., uroporphyrinogen III cosynthase or uroporphyrinogen decarboxylase).

Our patient was thus biochemically similar to patients with porphyria cutanea tarda, but he clearly did not have that disease. His porphyria began in infancy, the erythron and not the liver appeared to be the source of the excess porphyrin production, and he...
had a severe dyserythropoietic anemia associated with marked splenomegaly. These features also help to distinguish our case from hepatoerythropoietic porphyria, a very rare form of porphyria. In hepatoerythropoietic porphyria, the liver fluoresces and is heavily laden with porphyrins.57-59 Anemia, if present at all, is mild,57 60 dyserythropoietic changes and fluorescence have not been noted in the marrow,57 evidence of liver disease and cirrhosis may be found,57-60 and of rare form elevations of erythrocyte protoporphyrin content marked splenomegaly. These features also help to had a severe dyserythropoietic anemia associated with Kushner ET AL. 734 to homozygosity for the gene producing due massive splenomegaly has not been reported.57,60 Mcrerescence have not been noted in the marrow,57 evidence 60 porphyria,57 protoporphyrin content were prominent in the marrow of our case the activity was 50% of normal, but in our patient. Hepatoerythropoietic porphyria appears to be due to homozgyosity for the gene producing a defective uroporphyrinogen decarboxylase.62 In hepatoerythropoietic porphyria, erythrocyte uroporphyrinogen decarboxylase activity is only 7% of normal, but in our case the activity was 50% of normal, suggesting heterozygosity for the enzymic defect.

Two other patients with clinically typical congenital erythropoietic porphyria, but with a pattern of urinary porphyrin excretion similar to porphyria cutanea tarda, have been reported.63,64 Eriksen and Eriksen suggested that a defect in uroporphyrinogen decarboxylase might be present in one of these cases,65 and in the other, porphyria cutanea tarda and congenital erythropoietic porphyria were found in the same pedigree.64

Diminished activity of uroporphyrinogen decarboxylase in families with porphyria cutanea tarda appears to be inherited as an autosomal dominant trait.29,32,34,52 The finding of enzyme activity 50% of normal in our case is compatible with heterozygosity for the defect. In support of this is the finding of diminished uroporphyrinogen decarboxylase activity in only two, and not all, of his offspring. Both of these offspring were phenotypically normal, with no clinical evidence of porphyria or of anemia. One (III-1), however, did demonstrate a minor abnormality of plasma porphyrins on HPLC analysis.

In families with porphyria cutanea tarda, many pedigree members demonstrate the uroporphyrinogen decarboxylase defect but do not clinically express the porphyria.29,32,34,52 A coexistent liver abnormality, hepatic siderosis, appears to be required for clinical expression of the porphyrnic syndrome.66 In the present case the enzymic defect was presumably transmitted from the proband’s mother and to two of his offspring, yet only the proband had the clinical syndrome of congenital erythropoietic porphyria. He, of course, was the only pedigree member with dyserythropoietic anemia.

It thus appears that three distinctly different clinical disorders may be associated with an inherited deficiency of uroporphyrinogen decarboxylase activity. Heterozygosity for the enzyme defect with coexisting hepatic siderosis results in porphyria cutanea tarda. Homozygosity for the enzyme defect results in hepatoerythropoietic porphyria. Heterozygosity for the enzyme defect with coexisting dyserythropoietic anemia, as observed in the present case, results in the clinical picture of congenital erythropoietic porphyria.

The abnormal morphological findings in the peripheral blood of our patient include anisocytosis, poikilocytosis, basophilic stippling, polychromasia, and occasional nucleated erythrocytes. Abnormalities in the marrow include erythroid hyperplasia, fluorescent normoblasts, nuclear inclusions that stain with benzidine, and striking dyserythropoiesis. All of these abnormalities have previously been noted in patients with congenital erythropoietic porphyria.1,6 The coexistence of anemia, a normal reticulocyte count, erythroid hyperplasia of the marrow, and evidence of increased pigment turnover suggests that ineffective erythropoiesis is the major kinetic abnormality responsible for the anemia in this case.

Light and electron microscopy revealed the presence of two lines of erythroid precursors, one with normal morphology and one with quite bizarre morphology. The latter exhibited striking dyserythropoietic changes in the orthochromic and polychromatophilic normoblasts. The presence of two morphologically distinct cell lines has also been described in other patients with congenital erythropoietic porphyria.1,4,15,16

The intense nuclear fluorescence of the morphologically abnormal normoblasts has also been noted by others.4,15,67-69 and it has been suggested that the normoblast nucleus is the site of excess porphyrin production in congenital erythropoietic porphyria.15 This seems unlikely, as the localization of the defective enzymes (uroporphyrinogen cosynthase22,24 and, in this case, uroporphyrinogen decarboxylase) is the cytoplasm.70 It is not clear why nuclear fluorescence is prominent, but the finding on electron microscopy of deep cytoplasmic invaginations into the nucleus suggests that the fluorescence emanates from the porphyrin-rich cytoplasm "invading" the nucleus. The nuclear membrane surrounding these invaginations may have a particular affinity for porphyrins, thus concentrating the fluorescent molecules. The benzidine-positive nuclear inclusions in this case, and others,
appear to be due to the cytoplasmic hemoglobin present in the invaginations.

The morphological abnormalities observed in our patient are similar not only to those reported in other patients with congenital erythropoietic porphyria, but also to those described in patients with congenital dyserythropoietic anemias. The morphological features we observed most closely resemble those found in congenital dyserythropoietic anemia type I. As in this type of congenital dyserythropoietic anemia, erythroid hyperplasia with megaloblastoid erythropoiesis was evident in the marrow. The erythroid precursors showed prominent nuclear budding and fragmentation, suggesting abnormal nuclear division. In many cells the nuclear envelope had lost its integrity, and there were large cytoplasmic invaginations between nuclear chromatin masses. Cytoplasmic inclusions such as myelin figures, membrane residues, and vacuoles were frequent, as were iron-laden mitochondria. Pronormoblasts and basophilic normoblasts were normal, but cells at later stages were grossly abnormal. In addition, as with congenital dyserythropoietic anemia, both light and electron microscopy revealed a population of erythroid precursors with no evidence of morphological aberration at any state of the maturation sequence.

Linear structures parallel to the nuclear membrane, the ultrastructural hallmark of congenital dyserythropoietic anemia type II, were not found in our case. In addition, in congenital dyserythropoietic anemia type II, the acidified serum hemolysis test is usually positive, whereas it was negative in our patient. In contrast to congenital dyserythropoietic anemia type III, marked erythroid multinuclearity, “gigantoblasts,” and prominent macrocytosis are expected and were not observed in our case.

Two possible explanations can be offered for the coexistence of an enzymic defect in porphyrin biosynthesis and the dyserythropoietic anemia. The first is that abnormal porphyrin biosynthesis is the primary defect and the dyserythropoietic anemia is a secondary phenomenon. It has been suggested that the nuclear abnormalities in the normoblasts are due to the photodynamic action of light on the porphyrins intimately associated with the nuclear membrane, and that the lifespan of circulating erythrocytes may be shortened by exposure to sunlight. How light reaches the marrow normoblasts has not been explained.

Several observations make the hypothesis that the morphological abnormalities are secondary to the biochemical defect implausible. First, although the inherited biochemical abnormality is presumably present in all cells, morphological abnormalities are present only in normoblasts. Second, as shown in the family studies in this case, the defect in uroporphyrinogen decarboxylase activity was present not only in the erythrocytes of the proband, but also in the erythrocytes of two of his offspring. There was no evidence of deranged erythropoiesis in the offspring. Finally, the presence of a morphologically normal population of erythroid precursors would not be expected if an inherited enzymatic abnormality was solely responsible for the anemia.

The second possible explanation, which we favor, is that the enzymatic defect and the dyserythropoietic anemia are inherited independently. This would be compatible with the extreme rarity of congenital erythropoietic porphyria. Congenital dyserythropoietic anemia type I is an extremely rare disorder and appears to be inherited as an autosomal recessive trait. Based on the studies of Levin and coworkers, and on the isomer pattern of the excreted porphyrins in most of the reported cases, it appears that congenital erythropoietic porphyria is usually associated with deficient activity of uroporphyrinogen III cosynthase, also inherited as an autosomal recessive trait. The concordance of homozgyosity for two rare recessive traits in a single individual would be unusual and in keeping with the extreme rarity of congenital erythropoietic porphyria.

In support of the hypothesis that two defects are required for the expression of erythropoietic porphyria are occasional case reports of erythropoietic porphyria first appearing in adult life. In most cases, individuals with no prior history of hematologic disease developed anemia and clinical and biochemical evidence of erythropoietic porphyria at the same time. The excreted porphyrins were predominantly of the isomer I type, suggesting the subnormal activity of uroporphyrinogen III cosynthase was the primary biochemical abnormality, and in two individuals, the erythrocyte enzyme was assayed and found to have activity of only 18% of normal. It is possible that a somatic mutation occurred in all of these cases, producing a dyserythropoietic cell line deficient in uroporphyrinogen III cosynthase activity, but an equally plausible explanation is that an acquired anemia occurred in individuals with inherited, but clinically silent, enzymatic defects. An inherited deficiency of uroporphyrinogen III cosynthase activity in two adult-onset cases was demonstrated by appropriate family studies.

It appears that congenital erythropoietic porphyria (Günther's disease) may be a syndrome rather than a specific disease entity. The case reported here is the first to offer direct evidence that the abnormality in porphyrin biosynthesis may be associated with enzymatic defects other than deficient activity of uropor-
uporphyrinogen III cosynthase. The anemia, in most cases, appears to be similar, if not identical, to congenital dyserythropoietic anemia type I. In a few cases, an acquired dyserythropoietic anemia may be present. It is proposed that in our patient congenital erythropoietic porphyria resulted from the coexistence of two defects. One was deficient activity of uroporphyrinogen decarboxylase, transmitted as an autosomal dominant trait. The other was congenital dyserythropoietic anemia, either inherited as an autosomal recessive disorder or due to a somatic mutation.

REFERENCES

CONGENITAL ERYTHROPOIETIC PORPHYRIA

Congenital erythropoietic porphyria, diminished activity of uroporphyrinogen decarboxylase and dyserythropoiesis

JP Kushner, NR Pimstone, CR Kjeldsberg, MA Pryor and A Huntley