Photosensitive or Congenital Porphyria with Hemolytic Anemia

I. Clinical and Fundamental Studies Before and After Splenectomy

By Robert A. Aldrich,* M.D., Violet Hawkinson, B.S., Moises Grinstein†, Ph.D. and Cecil James Watson, M.D., Ph.D.

PORPHYRIA is a metabolic disorder usually regarded as constitutional in origin. Three main types are recognized from a clinical standpoint. Photosensitive or “congenital” porphyria occurs in infancy or childhood and is very rare. It is characterized by red urine and sensitivity of the skin to sunlight, as a result of which vesicles (hydroa aestivale) and eventual scarring of exposed areas, are observed. Erythrodontia may be noted. Splenomegaly and anemia have been noted with relative frequency. The intermittent acute type of porphyria is observed in adults, more often females. This is the most common form. The major features include nervous manifestations, abdominal pain and intermittent excretion of dark or red urine. The presence of porphobilinogen is characteristic, while it is lacking in the photosensitive type. Mixed, or “chronic” porphyria is also seen in adults and is less frequent than the intermittent acute form. It combines some of the findings of the other two varieties. Many of these individuals present evidence of liver damage. Uro-type porphyrins appear in the urine of all three varieties. In the photosensitive form uroporphyrin I is found without admixture of a type III porphyrin, whereas, the Waldenström porphyrin, characterizing the urines of the intermittent acute type, is composed of uroporphyrin I in association with a type III porphyrin of as yet undetermined structure.

We have been afforded an unusual opportunity to study porphyrin and hemoglobin metabolism in a young girl with photosensitive porphyria. Splenomegaly and hemolytic anemia were marked and splenectomy has resulted in dramatic improvement. Some of the clinical and biochemical findings have been given in abstract form in preliminary reports. From the Departments of Medicine and Pediatrics, University of Minnesota Hospitals, Minneapolis. Aided by grants from the Division of Research Grants and Fellowships, U. S. Public Health Service (RG No. 345), and the Medical Research Fund of the Graduate School, University of Minnesota. Submitted by R. A. A. to the University of Minnesota Graduate School in partial fulfillment of the thesis requirement for the Ph.D. degree.

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PHOTOSENSITIVE PORPHYRIA WITH HEMOLYTIC ANEMIA. I.

CASE REPORT*

D. H., female, age 4 (on first admission to University of Minnesota Hospital, January 10, 1949).

**History of present illness:** During the winter of 1946–47, at 2 years of age, the patient's urine was observed to have a pink color. The color deepened to a portwine hue in three weeks and this persisted without variation until hospital admission. No discoloration of the stools was observed. The patient remained healthy until April 1947, at which time there appeared a vesiculo-bullous eruption limited to the parts of the skin exposed to light. Itching without erythema preceded the formation of the vesicles. They were filled with clear fluid which quickly clouded. Rupture occurred in a day or two leaving a superficial ulcer which often became secondarily infected. After healing slowly a depressed scar remained. The skin was easily bruised in these areas. There was slight photophobia and occasional mild epistaxis. During the summer the skin manifestations became severe and widely infected. Pallor was noted. The patient was admitted to a local hospital for therapy of the skin infections and of anemia. The skin healed in October and she was greatly improved. Tonsillectomy and adenoidectomy were performed without incident in January, 1948. The following spring the skin lesions and severe anemia reappeared. Since neither responded to treatment she was referred here for further study.

**Family history:** Both parents were of direct Norwegian descent. The father and 3 siblings, aged 12, 9, and 8 years were in good health. The mother had early symptoms of rheumatoid arthritis. There was no familial history suggestive of porphyria. The urine of the parents and siblings did not contain abnormal amounts or types of porphyrins.

**Past history:** Birth was normal on October 18, 1944, weight 9 lbs. 2 oz. She received formula of pasteurized milk with added carbohydrate. Orange juice was said to have been started at the second month; no cod liver oil was given. At 7 months scurvy developed with typical signs, symptoms, and x-ray findings. Prompt response followed ascorbic acid therapy. She was not vaccinated or immunized. Teeth first erupted at 4½ months and were not observed to be discolored. Development was normal and she was in good health until the onset of porphyria at the age of 2 years.

**Physical findings:** Weight 33 lbs., T. 101.0 (R), P. 106, R. 20, B. P. 106/60/50. She was listless and pale, but of normal size and nutrition for a child 4 years old. Her complexion was fair and her hair was a very light red. The skin over the exposed parts of the body was covered by long fine dark hair which was much more profuse than normal. The skin itself was thin and of poor consistency. Some areas were ulcerated and in several stages of healing. Those lesions which had healed were marked by small hard scars (milia). A few ecchymoses were present near ulcers. Fluid containing bullae were not seen on the day of admission, but they appeared subsequently. Her face and the backs of the hands and fingers were most severely involved. Some lesions appeared where the hair was parted and over the lower legs anteriorly. Examination of ears, eyes, nose and throat was normal. The teeth were a muddy brown shade most prominent at the gum margin. The lungs were normal. A soft systolic murmur was heard at the apex of the heart. It was attributed to the severe anemia. The spleen was enlarged and firm, extending 7 cm. below the left costal margin in the nipple line. The teeth and open skin lesions exhibited red fluorescence when exposed to a near ultraviolet light source.

**Laboratory findings:** Hematologic data are given in table 1 (this includes the second hospital admission). Routine urinalysis and culture were normal. X-rays of the chest, wrist, hand and skull were normal; blood urea nitrogen was 10 mg. per 100 cc.; Kahn test negative. Erythrocyte fragility to hypo- and hypertonic saline solutions was normal. Fractional serum bilirubin, 1–0.1 mg. per cent; total 1.0 mg. per cent. The total serum protein was 7.0 Gm. per 100 cc. The albumin was 4.3 Gm. and globulin 2.7 Gm. Sternal bone marrow† on February 10, 1949 showed normoblastic hyperplasia and very active

* The authors wish to thank Drs. M. D. Starekow and A. Canfield, Thief River Falls, Minn., for the opportunity to study their patient.
† Dr. E. N. Nelson made the sternal aspiration. Smears of the bone marrow and peripheral blood were interpreted by Dr. Dorothy Sundberg.
### Table 1.—Summary of Representative Hematologic Laboratory Data Before and After Splenectomy

<table>
<thead>
<tr>
<th>Date</th>
<th>Hgb. Gm. per cent</th>
<th>R.B.C. million per cu. ml</th>
<th>Retics. per cent</th>
<th>Platelets per cu. ml</th>
<th>Remarks and miscellaneous observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10-49</td>
<td>5.8</td>
<td>1.74</td>
<td>5.4</td>
<td>—</td>
<td>MCD—7.53 μ MCV—103.4 cu. μ MCH—30.46 μg. MCHC—29.44%; Hematocrit 18%.</td>
</tr>
<tr>
<td>1-20-49</td>
<td>4.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>1-25-49</td>
<td>4.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>2-3-49</td>
<td>4.3</td>
<td>1.70</td>
<td>—</td>
<td>—</td>
<td>Blood group A, Rh positive. Given 550 cc. whole blood intravenously in next 7 days.</td>
</tr>
<tr>
<td>2-12-49</td>
<td>8.9</td>
<td>—</td>
<td>7.6</td>
<td>—</td>
<td>End of first hospital admission. W.B.C. 3,600 per cu. mm. Normal differential.</td>
</tr>
<tr>
<td>2-17-49</td>
<td>6.0</td>
<td>1.74</td>
<td>—</td>
<td>—</td>
<td>Obtained while still at home.</td>
</tr>
<tr>
<td>4-5-49</td>
<td>3.3</td>
<td>1.47</td>
<td>3.2</td>
<td>84,000</td>
<td>Second hospital admission. Hematocrit—16%; given 825 cc. whole blood intravenously in next 5 days.</td>
</tr>
<tr>
<td>4-14-49</td>
<td>11.7</td>
<td>3.73</td>
<td>1.3</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>4-30-49</td>
<td>8.9</td>
<td>2.81</td>
<td>2.9</td>
<td>192,000</td>
<td>W.B.C. 4,050 per cu. mm. Normal differential, 1 normoblast per 100 leukocytes.</td>
</tr>
<tr>
<td>5-13-49</td>
<td>6.0</td>
<td>2.56</td>
<td>6.0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>5-18-49</td>
<td>5.5</td>
<td>1.71</td>
<td>10.0</td>
<td>—</td>
<td>Given 1,050 cc. whole blood intravenously between 5-18-49 and 5-25-49.</td>
</tr>
<tr>
<td>5-24-49</td>
<td>14.4</td>
<td>—</td>
<td>—</td>
<td>159,000</td>
<td>Normal bleeding, clotting, and prothrombin times. Serum protein 7.8 Gm. %</td>
</tr>
</tbody>
</table>

**Splenectomy 5-25-49**

<table>
<thead>
<tr>
<th>Date</th>
<th>Hgb. Gm. per cent</th>
<th>R.B.C. million per cu. ml</th>
<th>Retics.</th>
<th>Platelets per cu. ml</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-27-49</td>
<td>15.2</td>
<td>—</td>
<td>—</td>
<td>2.2</td>
<td>178,000 W.B.C.—23,400 per cu. mm.</td>
</tr>
<tr>
<td>6-21-49</td>
<td>14.4</td>
<td>5.11</td>
<td>0.7</td>
<td>—</td>
<td>W.B.C.—12,300 per cu. mm.</td>
</tr>
<tr>
<td>9-10-49</td>
<td>11.5</td>
<td>3.8</td>
<td>2.0</td>
<td>—</td>
<td>W.B.C.—12,000 per cu. mm.</td>
</tr>
<tr>
<td>9-14-50</td>
<td>12.2</td>
<td>4.13</td>
<td>0.5</td>
<td>316,000</td>
<td>W.B.C.—7,550 per cu. mm.</td>
</tr>
</tbody>
</table>

Red cell regeneration. Nuclei of the normoblasts showed degenerative changes. Macrophages were increased, many of them containing phagocytosed normoblasts, erythrocytes and pigment. Otherwise the marrow was not remarkable. Erythrocytes in the peripheral blood...
PHOTOSENSITIVE PORPHYRIA WITH HEMOLYTIC ANEMIA. I.

showed anisocytosis, polychromasia and poikilocytosis. Some were small and resembled spherocytes. Curious granulation was seen in the circulating erythrocytes and in the normoblasts of the marrow. Platelets were decreased in number.

Treatment other than whole blood transfusions (table 1), consisted only of a course of penicillin to overcome a streptococcal infection. She returned home temporarily on February 12, 1949.

The patient became more anemic at home, but felt well. A single skin lesion appeared on her nose twenty-four hours after a thirty minute exposure to sunlight. Samples of urine, feces and blood were sent to us by her physician.

Second hospitalization: She was readmitted to the pediatric service of the University of Minnesota hospitals April 4, 1949 with severe anemia, increasing numbers of new skin lesions and frequent epistaxis. T. 99.0 (R), P. 120, R. 24, B. P. 110/65/50. Pallor and weakness were prominent, but fewer skin manifestations were present than on the first admission. Physical examination was essentially unchanged except that the previously noted cardiac murmur was absent.

The general hematologic status was essentially unchanged except that the anemia was more severe (table 1). Porphyrin excretion was not significantly changed (table 2).

A diagnosis of hemolytic anemia complicating porphyria, was supported by the increased reticulocyte counts, normoblastic hyperplastic bone marrow and elevated excretion of fecal urobilinogen (table 2). It was repeatedly noted that after raising the level of circulating hemoglobin by transfusions, the patient again became severely anemic in four to five weeks. In addition the hemolytic nature of the process was confirmed by isotope studies with N\textsuperscript{15}. As described in paper II of this series, which follows, it was therefore advised in the hope that the hemolytic aspect of the disease might be eliminated. After preoperative transfusions an uncomplicated splenectomy and liver biopsy were performed by Dr. Clarence Dennis on May 25, 1949. Recovery from surgery was prompt and the patient left the hospital to return home on June 4, 1949.

Within a few days after operation the urine became a light pink color and after a few weeks was entirely normal in appearance. Fecal and urinary porphyrin and fecal urobilinogen excretion decreased markedly (table 2). The plasma porphyrin content similarly diminished (see special studies). Eighteen months after splenectomy the anemia has not recurred. Quite unexpectedly she has lost virtually all photosensitivity of the skin to sunlight. Epistaxis, bruising, and hirsutism are no longer present. The skin has a normal texture and color, but there are many scars of old healed lesions. Data for the follow-up period appear in tables 1 and 2. Characterization of the urinary and fecal porphyrins on several occasions after surgery has shown no change from the data shown in table 1. It is clear that while porphyria is still present a remarkable reduction in porphyrin excretion has taken place in association with striking clinical improvement.

METHODS

The porphyrins and stercobilin were isolated from the feces by the following modification of a previously described technique\textsuperscript{14}: The aqueous acetic residue after removal of the ether from the primary extract, was poured into several volumes of 1.5 N HCl. After neutralization of the HCl solution with sodium acetate and repeated ether extraction in the usual manner, the combined ether extracts were washed twice with 3 per cent aqueous sodium acetate solution; all of this was added back to the aqueous fraction which had been extracted by ether. The ether fraction contains the ether soluble porphyrins while the stercobilin is in the aqueous and is extracted from it by chloroform, and further purified as previously described. The final chloroform solution of stercobilin was dried by filtration through chloroform moistened filter paper, rather than by use of anhydrous sodium sulfate, as described in 1934. In addition, the
stercobilin was crystallized from chloroform-acetone, rather than chloroform, the latter being first largely removed on the water bath, after which several volumes of warm acetone were added. The substance was recrystallized from chloroform alone.

Table 2.—Quantitative Data on Urinary and Fecal Porphyrins and Fecal Urobilinogen, Before and After Splenectomy

<table>
<thead>
<tr>
<th>Collection dates</th>
<th>Urine</th>
<th>Feces</th>
<th>Feces urobilinogen mg./day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coproporphyrin mg./day</td>
<td>Uroporphyrin mg./day</td>
<td>Coproporphyrin mg./day</td>
</tr>
<tr>
<td>1-11-49</td>
<td>13.5</td>
<td>111.5</td>
<td>—</td>
</tr>
<tr>
<td>1-12-49</td>
<td>9.82</td>
<td>34.56</td>
<td>—</td>
</tr>
<tr>
<td>1-13-49</td>
<td>10.2</td>
<td>52.0</td>
<td>—</td>
</tr>
<tr>
<td>1-14-49</td>
<td>11.5</td>
<td>35.5</td>
<td>135.0</td>
</tr>
<tr>
<td>1-15-49</td>
<td>10.5</td>
<td>42.6</td>
<td>—</td>
</tr>
<tr>
<td>1-16-49</td>
<td>11.4</td>
<td>50.58</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>13.25</td>
<td>130.1</td>
<td>—</td>
</tr>
<tr>
<td>1-21-49</td>
<td>12.17</td>
<td>121.5</td>
<td>—</td>
</tr>
<tr>
<td>1-25-49</td>
<td>9.8</td>
<td>68.2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>8.82</td>
<td>89.2</td>
<td>—</td>
</tr>
<tr>
<td>2-2-49</td>
<td>10.4</td>
<td>86.3</td>
<td>—</td>
</tr>
<tr>
<td>2-6-49</td>
<td>11.2</td>
<td>75.6</td>
<td>—</td>
</tr>
<tr>
<td>Splenectomy 5-25-49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-27-49</td>
<td>3.25</td>
<td>20.1</td>
<td>—</td>
</tr>
<tr>
<td>6-1-49</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6-2-49</td>
<td>2.1</td>
<td>12.3</td>
<td>—</td>
</tr>
<tr>
<td>9-9-49</td>
<td>0.90</td>
<td>3.5</td>
<td>14.4/100 Gm.</td>
</tr>
<tr>
<td>9-14-49</td>
<td>49 μg per 298 μg per</td>
<td>5.23/100 Gm.</td>
<td>Neg.</td>
</tr>
<tr>
<td>100 ml.</td>
<td>100 ml.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Single dates represent 24 hour samples, otherwise dates are inclusive of collection period. † Ehrlich units.‡ Single samples.

The ether fraction mentioned above, after being washed with 3 per cent sodium acetate solution, was subjected to methods previously described for purifying and crystallizing copro- and protoporphyrin methyl esters.9, 10

Fecal uroporphyrin was extracted from the fecal residue after the ether extraction by means of methyl alcohol saturated in the cold with HCl gas. This effects esterification as well. From this point on the method was that usually employed.9, 10
The porphyrins, i.e., proto-, copro- and uro- were identified by means of absorption spectra and ester melting points in addition to characteristic solubility differences which permitted their fractionation.

The urinary porphyrins were isolated and purified by means of previously described methods. The coproporphyrin was first removed by ether extraction, and the uroporphyrin by precipitation. The concentration of the latter was so great that the majority of the porphyrin precipitated upon acidification of the urine with acetic acid. In contrast to the situation in intermittent acute porphyria, the porphyrins were not excreted as the zinc complex, hence were not readily adsorbed on talc. The urine did not contain demonstrable porphobilinogen at any time.

The porphyrins in the circulating (washed) erythrocytes were extracted and esterified simultaneously by methanolic HCl as described above. Further purification and crystallization was by the usual technics. Plasma uroporphyrin was crystallized in the same manner.

Uroporphyrin was isolated from the spleen as follows: the spleen was ground in a meat grinder and extracted repeatedly by grinding further in a mortar with a 1:3 mixture of glacial acetic acid and ethyl acetate. This was decanted and filtered through a sintered glass filter. After the first few extractions and decantations, the remaining pulp was transferred to the filter where further grinding and extraction was carried out. After tamping and drying the residue, it was thoroughly ground on the filter, with the suction off, with methyl alcohol saturated in the cold with HCl gas. The methanol-HCl extract contained the bulk of the uroporphyrin ester which was then chromatographed and crystallized according to the methods described above. Small amounts of proto- and coproporphyrin were present in the primary glacial acetic-ethyl acetate extract.

Protoporphyrin methyl ester from erythrocyte hemoglobin was prepared according to Grinstein. The relative proportion of the two coproporphyrin isomers was estimated by the method of Schwartz et al. Mesoporphyrin 9 was prepared by a modification of the method of Fischer and Kögl. The quantitative determination of the porphyrins was according to published methods.

The feces urobilinogen was determined by the ferrous hydroxide-petroleum ether method, and on random samples the simple Ehrlich method was used. The concentration of circulating hemoglobin was determined by the method of Evelyn. The percentage of reticulocytes was determined in the usual manner by supravital staining of blood smears with brilliant cresyl blue in a moist chamber for 10 minutes. Serum bilirubin was estimated by a modification of the method of Malloy and Evelyn.

Microfluorospectroscopy of the circulating erythrocytes, bone marrow, liver and spleen was carried out by means of a "spectral analytical microscope" (Steinheil, Munich), such as employed by Borst and Königsdörffer.

The portion of liver removed at surgery was investigated histochemically. Cytoplasmic ribonucleic acid was measured by use of basophilic staining and ribonuclease digestion technics. Toluidine blue was used as the basic stain.

* We are indebted to Dr. W. J. Williams of the Department of Anatomy for making the histochemical studies of the liver biopsy.
Fat was stained with sudan III. Organic and inorganic iron were demonstrated by the method of Glick. Glycogen was measured by the Bauer-Feulgen method and this procedure as well as the subsequent technics were taken from Lillie. Alkaline phosphatase determination was carried out on sections fixed in chilled acetone. Pigment stains were made on unstained sections fixed in neutral formalin, acid formalin, alcohol-formalin-acetic acid, and acetone. These sections were studied for hemofuscin and ceroid as well as organic and inorganic iron. Desoxyribonucleic acid was studied by the Feulgen technic. Hematoxylin and eosin stains and special stains were made for collagenous fibers, elastic fibers and reticular fibers.

Efforts were made to produce the skin lesions artificially by exposure to light from a near ultraviolet source† through a system of filters calculated to isolate various parts of the visible spectrum. The actual intensity of radiation striking the skin from any of the parts of the spectrum was measured by means of a thermopile. By varying the exposure time, each area of skin received the same amount of total radiation regardless of the wavelength used.

RESULTS OF SPECIAL STUDIES

1. Urine

Freshly voided urine fluoresced red in ultraviolet light. Spectroscopy revealed absorption maxima as follows: 612.5, 569, 534.8, and diffuse and absorption from 508 μ down. The porphyrin was therefore in the free state and not a metal complex. The zinc complex has absorption maxima at 537 and 578 μ. Porphobilinogen was absent. Porphobilin was not demonstrated. Uroporphyrin I, and coproporphyrin I and III methyl esters were isolated in crystalline form. Qualitative and quantitative data appear in tables 2 and 3. The crystal morphology is shown in figure 1. The ratio of copro-I to coproporphyrin III was 99:1.

2. Feces

A red fluorescence was noted when fresh feces were placed in ultraviolet light. Methyl esters of copro-I and III, uro-I, and protoporphyrin 9 (type III) were isolated in crystalline form. Qualitative and quantitative data are shown in tables 2 and 3. Stercobilin hydrochloride was also obtained in crystalline form (table 3). Porphyrin crystal morphology appears in figure 1.

3. Circulating Erythrocytes

Washed erythrocytes exhibited a strong red fluorescence in ultraviolet light. Prior to splenectomy, the methyl esters of uro-I, copro-I and coproporphyrin III were isolated in crystalline form. Characteristics of these esters are given in table 3, and their crystal morphology in figure 1. The ratio of copro-I to coproporphyrin III was 98:2.

The erythrocytes contained 363 μg. of free erythrocyte protoporphyrin and 145 μg. of coproporphyrin per 100 cc. of red cells, before splenectomy. Uroporphyrin was present in large amounts, but quantitation was grossly inaccurate because of rapid deterioration.

† Model BI-2 lamp, obtainable from Black Light Products, Inc., Chicago, Ill.
4. Plasma

Freshly separated plasma exhibited red fluorescence in ultraviolet light. Uroporphyrin I was crystallized as the methyl ester (table 3). Coproporphyrin was also present, but in quantities insufficient for crystallization. The isomers I and III were present in a ratio of 98: 2 respectively. Before splenectomy the plasma contained 50 μg. of coproporphyrin and 112 μg. of uroporphyrin per 100 cc.

5. Blood Porphyrins After Splenectomy

Primary fluorescence of plasma or erythrocytes in ultraviolet light disappeared within a month after splenectomy. Washed erythrocytes, sixteen months post-
operatively, no longer yielded uroporphyrin or coproporphyrin. The free erythrocyte protoporphyrin value at this time was 64.8 μg. per 100 cc. of red cells. Both uro- and coproporphyrin were present in the plasma in a concentration of 10.6 and 10.3 μg. per 100 cc. respectively.

6. Spleen

The spleen fluoresced red in ultraviolet light. Sections showed an increase in pulp and hyperplasia of follicles. The pulp contained much golden brown pigment. Polymorphonuclear leukocytes were numerous. The sinuses were filled with blood. A few megakaryocytes were seen. The histologic picture was not diagnostic, but was compatible with hemolytic anemia.

Uroporphyrin I methyl ester was crystallized from splenic pulp (table 3), and small amounts of proto- and coproporphyrin were also present.

<p>| Table 3.—Physical Characteristics of Porphyrins Isolated in Crystalline Form, from Urine, Feces, Erythrocytes, Plasma and Spleen |</p>
<table>
<thead>
<tr>
<th>Source</th>
<th>Substance isolated</th>
<th>Melting point degrees C.</th>
<th>Absorption maximum (chloroform) mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>Coproporphyrin I tetramethyl ester</td>
<td>252-254</td>
<td>622.8</td>
</tr>
<tr>
<td></td>
<td>Coproporphyrin III tetramethyl ester</td>
<td>140 remelt 170</td>
<td>622.8</td>
</tr>
<tr>
<td></td>
<td>Uroporphyrin I octamethyl ester</td>
<td>282-284</td>
<td>626.2</td>
</tr>
<tr>
<td>Feces</td>
<td>Coproporphyrin I tetramethyl ester</td>
<td>250-252</td>
<td>622.4</td>
</tr>
<tr>
<td></td>
<td>Coproporphyrin III tetramethyl ester</td>
<td>130 remelt 170</td>
<td>622.4</td>
</tr>
<tr>
<td></td>
<td>Uroporphyrin I octamethyl ester</td>
<td>282-286</td>
<td>626.0</td>
</tr>
<tr>
<td></td>
<td>Protoporphyrin 9 dimethyl ester</td>
<td>221</td>
<td>632.0</td>
</tr>
<tr>
<td></td>
<td>Stercobilin hydrochloride</td>
<td>146-9 (acetone) 125-7 (chloroform)</td>
<td></td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>Coproporphyrin I tetramethyl ester</td>
<td>254</td>
<td>622.3</td>
</tr>
<tr>
<td></td>
<td>Coproporphyrin III tetramethyl ester</td>
<td>—</td>
<td>622.3</td>
</tr>
<tr>
<td></td>
<td>Uroporphyrin I octamethyl ester</td>
<td>284-286</td>
<td>626.2</td>
</tr>
<tr>
<td>Plasma</td>
<td>Uroporphyrin I octamethyl ester</td>
<td>282-284</td>
<td>626.4</td>
</tr>
<tr>
<td>Spleen</td>
<td>Uroporphyrin I octamethyl ester</td>
<td>284</td>
<td>626.0</td>
</tr>
</tbody>
</table>

Mesoporphyrin methyl ester was prepared from the splenic vein blood hemoglobin. It melted at 205 C. (uncorrected), corresponding with mesoporphyrin 9 methyl ester (type III). This does not exclude the presence of mesoporphyrin 1 or 2 in small amounts in the mother liquor, but it is clear that at least the great bulk of the hemoglobin protoporphyrin was of type III configuration.

7. Histochemical Study of the Liver Biopsy

Hematoxylin and eosin stain of liver sections showed that the liver was essentially normal except for mild perivascular leukocytic infiltration in the portal spaces. Special stains revealed normal collagenous, elastic and reticular fibres in the stroma. There was no infiltration or deposition of fat. The biliary system appeared normal.

The glycogen content of more than half of the cells was normal. However,
there was a great range in the amount of intracytoplasmic glycogen in parenchymal cells without a specific lobular or any definite distribution of deficient cells.

The cytoplasm of parenchymal cells was strongly positive for alkaline phosphatase, much more so than is ever observed in animal livers or in other human biopsies containing apparently normal liver cells.

The specially fixed unstained sections showed large amounts of pigment within Kupffer cells and some in the parenchymal cells. These pigments were not soluble in acetone, alcohol or water and although they were basophilic, digestion with ribonuclease did not decrease the basophilia. Organic and inorganic iron in large amounts was found in Kupffer cells by the potassium ferricyanide staining method. There were some potassium ferricyanide positive granules in the parenchymal cells. Hemofuscin by the Mallory technic was present in the Kupffer cells and also positive granules were seen in parenchymal cells. The quantity of iron and hemofuscin was believed to be increased. Ceroid was not observed by a variety of methods including acid fast bacterial technic and basic fuchsin.

A generalized and uniform deficiency in ribonucleic acid was demonstrable in the cytoplasm of all hepatic parenchymal cells. There was obvious hypobasophilia following toluidine blue staining without appreciable reduction subsequent to incubation with ribonuclease.

Desoxyribonucleic acid appeared normal in the nuclei.

8. Photosensitivity of the Skin

We were unable to produce lesions of the skin with the amounts and wavelengths of light used.

9. Studies with the Microfluorospectrometer*

Unstained smears of the sternal bone marrow revealed intense red fluorescence which appeared to be coming from around the nucleus of erythropoietic cells. The fluorescence spectrum indicated that the fluorescence was due to porphyrin. Photographs of the fluorescence spectra suggested the presence of three separate porphyrins, the lines corresponding to those of proto-, copro-, and uroporphyrin.26

DISCUSSION

The porphyrin findings in the urine and feces together with the clinical manifestations of this patient, clearly support the diagnosis of photosensitive (congenital) porphyria. The types of porphyrin and their route of excretion are in agreement with the findings of Fischer and his associates in the celebrated case Petry.30, 31 One of us (C. J. W.) has studied the urine of 4 other patients of this type and in each instance the findings were similar to the present subject. These observations are in striking contrast to those made on patients with intermittent acute porphyria. In the latter the urine characteristically exhibits a zinc complex spectrum of the Waldenström uro-type porphyrin, in association with the Ehrlich reacting chromogen porphobilinogen.9, 10, 32

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The presence of hemolytic anemia associated with hepatosplenomegaly requires special consideration. Hematologic data obtained before splenectomy suggested hypersplenism. There was an increased rate of blood destruction as well as low platelet and leukocyte counts.

Splenomegaly has often been observed before in cases of this type. It was particularly emphasized by Mackey and Garrod\textsuperscript{33} who believed, however, that it did not appear until relatively late in the course of the disease, not in the first few years of life as in the present instance. Borst and Königsdörffer\textsuperscript{26} stated that the case Petry, later studied chemically in so much detail by Hans Fischer and his co-workers, died of pernicious anemia with splenomegaly. A study of the published data in this case, however, leaves one in doubt whether the patient actually had pernicious anemia with a megaloblastic bone marrow, or hemolytic anemia. The illustrations of the bone marrow in their monograph are more suggestive of the latter. Sato and Takahashi\textsuperscript{34} reported an infant with photosensitive porphyria and hemolytic icterus. The child expired, but they suggested in retrospect, that although a splenectomy had not been carried out it might have benefitted their patient. De Marval and Pons\textsuperscript{36} reported a case apparently identical with this, in which splenectomy was performed. Theirs is the only published instance that we have been able to find in which this was done. They stated that following operation the hemolytic activity was greatly reduced, and that subsequently the photosensitivity of the skin was partially improved. Without giving quantitative data they indicated that the metabolic defect of porphyria, and the amounts of porphyrin excreted, were unchanged. Since the porphyria was still present and the photosensitivity was reduced, they interpreted their observations to mean that there was no relationship between porphyria and photosensitivity. However, if quantitative data had been available they might have noted a reduction in the porphyrin excretion. Zuelzer and Kaplan\textsuperscript{37} relate the case of an infant with photosensitive porphyria and hemolytic anemia. Splenectomy carried out on their patient resulted in amelioration of the hemolytic anemia for approximately two years. The anemia reappeared at the end of this time and is now more severe than before. During the remission following splenectomy the underlying porphyria did not disappear, although again, quantitative data on porphyrin excretion are not available. The skin photosensitivity was apparently not altered.

Splenectomy in the present patient was followed by a marked decrease in the excretion of porphyrins (table 2). Hemolytic anemia disappeared and with it the skin photosensitivity. Nevertheless, examination of the excreta leaves no doubt that porphyria is still present. The change in the disease has only been quantitative. The marked benefit has now been maintained for eighteen months. No transfusions or other treatment have been indicated during this period. The patient is able to play out of doors for unlimited periods without developing vesicles, and in fact, during the past two summers she was able to become sun tanned without difficulty. The hemolytic anemia and photosensitivity appeared to vanish at the same rate. Both of these clinical changes appeared to parallel the fall in porphyrin excretion. As might be expected the plasma porphyrin simultaneously diminished. It is our opinion that the reduction in porphyrin excretion is related to diminished erythropoiesis following removal of the stimu-
PHOTOSENSITIVE PORPHYRIA WITH HEMOLYTIC ANEMIA.

The isolation of coproporphyrin I and uroporphyrin I from the circulating erythrocytes together with the microfluorospectrometric demonstration of the fluorescence spectrum of at least three porphyrins in erythroid cells of the bone marrow strongly suggest that the developing red cells of the bone marrow were an important, if not the only, site of formation of these porphyrins. If this is correct, this form of porphyria may properly be regarded as a blood dyscrasia.

The plasma porphyrins prior to splenectomy may have been derived in part from the destruction of circulating erythrocytes, especially in the spleen, with release into the circulation of the porphyrins contained in these cells. This very likely accounts too, for the relatively large amount of uroporphyrin found in the spleen. Similarly, the excreted porphyrins may well have been supplied in part from this source. However, results of isotopic studies with N15, as well as the appearance of the bone marrow and the huge amounts of porphyrin excreted, induce the belief that much of the uroporphyrin in the excreta was liberated in the bone marrow itself. It is quite conceivable that this liberation took place during maturation of young erythrocytes, and without their being destroyed. It seems rather unlikely that if excessive erythrocyte destruction had been taking place in the bone marrow, it would have been terminated by splenectomy. There is no reason to believe that the uroporphyrin I, as isolated from various sites, was derived from an abnormal hemoglobin having a type I rather than type III protoporphyrin in its molecule. The repeated isolation of protoporphyrin 9 (type III), and the failure to find other than a mesoporphyrin 9 after hydriodic acid reduction are evidence against such a possibility.

The skin photosensitivity in the present case appeared to be directly related to the concentrations of porphyrin in the plasma, urine and feces. Our unsuccessful attempt to reproduce the skin lesions by measured amounts of artificial light of suitable wavelength, is in accord with previous experience. Admittedly, the amount of radiation may have been inadequate. The histochemical evidence of some hepatic abnormality is of unknown significance, but is reported in the hope of stimulating similar or more extensive studies in future cases of this type. It may have been due simply to excessive hemolysis and anemia.

**Summary and Conclusions**

1. The clinical features, laboratory findings and special studies of a case of photosensitive (congenital) porphyria in a 4 year old girl have been presented. This case was of particular interest in view of severe hemolytic anemia with hepatosplenomegaly.

2. Copro- and uroporphyrin I were isolated from the urine and feces. The ratio of these porphyrins in the urine varied from 1:10, to 1:30 respectively, while in the feces the ratio was reversed at about 70:1. Coproporphyrin III was isolated in much smaller amount than the type I isomer, from both urine and feces. Isomer analyses of the coproporphyrins in the excreta indicated that approximately 98 per cent was type I.

3. Prior to splenectomy copro- and uroporphyrin I were isolated in crystalline
form for the first time from circulating human erythrocytes. Coproporphyrin III was also isolated in lesser amount. Uroporphyrin I was crystallized from the plasma, which also contained coproporphyrin I. Microfluorospectrometry of the bone marrow revealed large amounts of porphyrin in the developing red cells. The porphyrin fluorescence spectra indicated that at least three porphyrins were present.

4. Splenectomy was followed by disappearance of uro- and coproporphyrins from the erythrocytes, and a marked decrease in plasma, urine and feces without any essential change in the type of porphyrins excreted. The metabolic defect porphyria, was still present, but now latent in character. Reduction in porphyrin excretion was apparently related to elimination of hypersplenic hemolysis and compensatory increase of erythropoiesis. Anemia and dermal photosensitivity to sunlight disappeared simultaneously with the reduction in porphyrin excretion.

5. Efforts to reproduce the skin lesions by artificial light were unsuccessful.

REFERENCES

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Photosensitive or Congenital Porphyria with Hemolytic Anemia: I. Clinical and Fundamental Studies Before and After Splenectomy

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