Familial Porphyria Cutanea Tarda: The Pattern of Porphyrins Formed by Hemolysates

M. A. Alleman, J. H. P. Wilson, J. W. O. van den Berg, A. Edixhoven-Bosdijk, and L. M. H. van Gastel-Quist

Porphyria cutanea tarda is thought to result from an inherited deficiency of uroporphyrinogen decarboxylase (EC 4.1.1.37) in some patients. Present methods for determining uroporphyrinogen decarboxylase activity are time consuming, so we examined the pattern of porphyrins formed from porphobilinogen by hemolysates as a possible marker for hereditary porphyria cutanea tarda. After the hemolysates are incubated with porphobilinogen, the porphyrins are converted to their methyl esters and examined by liquid chromatography, with fluorometric detection. The porphyrinic patients examined, and some of their relatives, showed a characteristic pattern of porphyrin production, with high uroporphyrin/coproporphyrin and (uroporphyrin + heptacarboxylic porphyrin)/coproporphyrin ratios, at least partly ascribable to increased uroporphyrinogen I synthetase (EC 4.3.1.8) activity in patients' hemolysates, and also to a relative deficiency of uroporphyrinogen decarboxylase. Examination of the pattern of porphyrins produced from porphobilinogen by hemolysates is a suitable technique for detecting asymptomatic individuals with porphyria cutanea tarda.

Additional Keyphrases: heritable disorders - genetic screening - fluorometry - chromatography, liquid - urine - feces

Porphyria cutanea tarda (PCT)\(^2\) is a disease usually manifest in adult life as blistering and increased fragility of areas of the skin exposed to sunlight. Biochemically, PCT is characterized by marked increases in uro- and heptacarboxylic porphyrins in the urine, and some increase in urinary excretion of hexa-, pentacarboxylic- and coproporphyrin (1, 2). Large amounts of isocoproporphyrin are present in the feces (3). The cause of the disease is not completely understood. PCT is associated with exposure to alcohol (4, 5), estrogens (6, 7), iron excess (8), and polyhalogenated aromatic hydrocarbons such as hexachlorobenzene (9, 10) vinyl chloride (11), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (12). Liver disease, ranging from chronic hepatitis to cirrhosis or hepatoma, is often present (13–15). All this suggests that PCT is an acquired disease.

On the other hand, several authors (4, 16–20) have reported the familial occurrence of PCT. Kushner et al. (17) were able to demonstrate a deficiency of uroporphyrinogen decarboxylase (URO-D; uroporphyrinogen III carboxylase, EC 4.1.1.37) activity in liver tissue and erythrocytes of patients with PCT, and showed that some of their relatives had a similar deficiency of erythrocyte URO-D, despite a normal porphyrin excretion. This has been confirmed by others (21).

Patients with a negative family history may have normal URO-D activity in their erythrocytes but some such patients demonstrated low hepatic URO-D activity (21, 22).

Apparently there are two forms of PCT, one inherited, the other acquired. This inherited form is not always manifest, clinical or biochemical abnormalities being dependent on exposure to exogenous factors such as estrogens or alcohol.

Methods currently used for measuring URO-D activity involve pentacarboxylic porphyrinogen (21, 22) as substrate or preincubation with uroporphyrinogen synthetase (EC 4.3.1.8) (17, 18). Neither of these reagents is readily available. We therefore decided to examine the possibility that the pattern of porphyrins produced from porphobilinogen (PBG) by erythrocyte hemolysate might be characteristic of PCT and of use in family studies.

Materials and Methods

Material. Kieselgel 60 (70–230 mesh) and Merckosorb SI 100 (20 μm particle diameter) were obtained from Merck, Darmstadt, P.R.G.

The methyl esters of protoporphyrin, coproporphyrin I, and uroporphyrin I were obtained from Sigma Chemical Co., St. Louis, MO 63178; their purity was checked by measuring the molar absorptivity in chloroform by spectrophotometry and also by "high-pressure" liquid chromatography. The various porphyrins formed in the assays were quantitated by the latter method as their methyl esters. Because methyl ester standards of hepta-, hexa-, and pentacarboxylic porphyrins were not available, we standardized pentacarboxylic porphyrin against the coproporphyrin methyl ester, and the hepta- and hexaporphyrins against the uroporphyrin methyl ester.

It is essential that fluorometer readings be appropriately standardized and calibrated with porphyrin solutions of known concentrations and molar absorptivities (24). All other reagents used were of analytical grade.

Methods. Heparinized venous blood was washed three times with isotonic saline (NaCl, 9 g/L) at 4°C; the buffy coat was discarded. The erythrocytes were hemolyzed by freeze-thawing four times. The hemolysate, 0.25 mL, was incubated with 1 mL of Tris-PBG solution immediately afterwards for 60 min, at 37°C and in the dark. The final reaction mixture contained, per liter, 40 mmol of Tris·HCl (pH 8.0), 240 μmol of PBG, and about 150 mg of protein. The reaction was stopped by freezing in a mixture of acetone and solid CO\(_2\).

After the hemolysate was lyophilized overnight, we dissolved the residue in 1 mL of chloroform, added 5 mL of a solution of concd. H\(_2\)SO\(_4\) in methanol (100 mL/L), and incubated the mixture for 60 min in the dark at 37°C. We then added 10 mL of water, transferred the solution to a separatory funnel, and added chloroform to give a total volume of 35 mL. The two phases were vigorously mixed and centrifuged, and the water (upper) phase was removed. To neutralize the chloroform phase, we added 8 mL of a 50 g/L solution of NaHCO\(_3\) in water, and gently mixed. The procedure was re-
Repeated if the pH of the water phase was <7. The chloroform phase was then washed twice with 10 mL of water, filtered over paper, and evaporated under reduced pressure at room temperature.

The porphinyl methyl esters so produced were then dissolved in chloroform and applied to a silica gel column (Merckosorb SI 100, 20-μm particle size; Merck) for high-pressure liquid chromatography. A linear gradient of tetrahydrofuran/heptane, increasing from 1/4 by vol to 1/1 by vol in 15 min, was used as eluent. The porphyrins were measured with a fluorometric detector (L 1000; Perkin-Elmer, Beaconsfield, England); excitation 400 nm, emission 625 nm, band width 8 nm at half peak height.

We methylated 20 mL of lyophilized (for 48 h) urine, as described above. The feces were methylated as described above after overnight lyophilization. To remove other pigments of dietary and biliary origin, it is necessary to pass the extract through a column of kieselgel and wash with dichloromethane/ethyl acetate/methanol (99/5/0.5 by vol) as described previously (23).

Patients. We examined members of four generations of the families of two young women with PCT. The symptoms of PCT had become manifest while the patients were taking oral contraceptives. At the time of our investigation they had no symptoms, having stopped oral contraceptives and having been treated by repeated phlebotomy, but they showed the typical biochemical abnormalities of PCT in their urine.

Normal values were obtained for 10 ostensibly healthy subjects who were clearly free of PCT.

Results

The urine analysis of the two propositae (patients 5 and 6), who as we have said were clinically in remission, showed the typical pattern of PCT, with a predominant increase in uro- and heptacarboxyl-porphyrin (Table 1). Five other family

### Table 1. Urinary Porphyrins in PCT Patients and Their Relatives

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Urinary excretion rate, nmol/24 h</th>
<th>Uro</th>
<th>7-COOH</th>
<th>6-COOH</th>
<th>5-COOH</th>
<th>Copro</th>
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<tbody>
<tr>
<td>1</td>
<td>104</td>
<td>35</td>
<td>8</td>
<td>5</td>
<td>47</td>
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<td>2</td>
<td>20</td>
<td>14</td>
<td>11</td>
<td>0</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>18</td>
<td>10</td>
<td>trace</td>
<td>91</td>
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</tr>
<tr>
<td>4</td>
<td>18</td>
<td>11</td>
<td>5</td>
<td>3</td>
<td>56</td>
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<tr>
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<td>221</td>
<td>36</td>
<td>6</td>
<td>4</td>
<td>120</td>
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<td>26</td>
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<td>39</td>
<td>20</td>
<td>5</td>
<td>8</td>
<td>258</td>
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</tbody>
</table>

* From left to right, below: uroporphyrin, heptacarboxyl porphyrin, hexacarboxylic porphyrin, pentacarboxylic porphyrin, and coproporphyrin. * Patient with a typical pattern of PCT as judged by urinary porphyrin excretion. Patient no. is same as in Fig. 5.
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Fig. Clinically

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This difference of the mean values of normals and PCT subjects is statistically significant (p < 0.01, Wilcoxon test).

members had a slight increase of uroporphyrins and heptacarboxyl porphyrins in urine.

After incubation of hemolysates with PBG, a mixture of uro-, heptacarboxyl, hexacarboxyl-, pentacarboxyl-, and coproporphyrin was observed. There was a marked difference between the patterns for the normal controls and the patterns for the PCT patients and some of their relatives (Figure 1).

For the normal persons the ratio of uroporphyrin plus heptacarboxyl to coproporphyrin is 2.42 (SD = 0.45) and of uroporphyrin to coproporphyrin 1.52 (SD = 0.32). In the PCT group both these ratios were significantly greater (Figure 2). In this test system we demonstrated also a significant decrease in mean URO-D activity, but there was some overlap between normals and PCT subjects (Figure 3). Mean uroporphyrin I synthetase activity was significantly increased in the PCT group (Figure 4), but also in this case there was some overlap.

Each of these parameters alone will not safely discriminate between PCT and normals because of this overlap, but the ratio of porphyrins as described above can be used to discriminate between normals and persons with latent PCT who have minimal or no abnormalities in urine porphyrin excretion, for there was no overlap between these groups.

In this family we could establish the diagnosis of PCT in one of these forms in members of each of three generations (Figure 5).

Discussion

The increased ratio of uroporphyrin to coproporphyrin and of uro- plus heptacarboxyl porphyrins to coproporphyrin formed on incubation of hemolysates from PCT patients with PBG can partly be explained by a decrease in URO-D activity. Moreover, in the PCT group, uroporphyrin I synthetase activity was high as compared with normals, in agreement with Brodie et al. (25). These facts together can explain the relative increase in uro- and heptacarboxylic porphyrins seen in PCT. An increase in uroporphyrinogen I synthetase activity is also observed in liver tissue in hexachlorobenzene-induced PCT in rats (26).

Our results suggest that the pattern of porphyrins produced from PBG is characteristic for PCT. If so, this is a valuable
addition to clinical examination and biochemical analysis of urine. In our family study, eight persons in the PCT groups had abnormal urinary porphyrin excretion rates, while two had only a slight increase in heptacarboxyl porphyrin excretion. In subjects with only slight or no changes in urinary porphyrin excretion rates, this test will provide a diagnosis. This is important because it has consequences for these carriers.

Use of alcohol and of oral contraceptives is contraindicated. Because of the relative simplicity of using PBG as substrate, this test may be substituted for more complex procedures in the investigation of PCT patients and their families.

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References

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