Urinary total porphyrins by ion exchange analysis: Reference values for the normal range and remarks on preformed porphyrins in acute porphyria urine

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SUMMARY  Determination of total porphyrin with a rapid and easy method (ion exchange; spectrophotometry) was performed on 57 morning urine samples from laboratory personnel, 59 arbitrary day urine samples from blood donors, and 90 24-hour urines from medical inpatients. The upper reference limit for morning urine was 0·32 μmol/l but even a value as high as 0·7 μmol/l was found. In the donor urines the upper limit was 0·155 μmol/l. The 24-hour urines showed an upper reference limit of 271 μg/24 h. These values are in good agreement with values from the literature, mostly based on extraction analyses. Tracings of the absorption curves in the region of 380-430 nm were performed in all analyses and showed that the non-porphyrin absorption was close to linear in most cases. Studies of porphyric urines gave no support to the claim that preformed porphyrins not formed from porphobilinogen are excreted in this disease.

The reference values for urinary porphyrins in the normal range found in the literature are based on various techniques, but primarily on rather time-consuming solvent extraction methods, while satisfactory reference materials based on the more rapid and simple ion exchange analysis have not been presented. As we have recently (With and Pedersen, 1978) described an easy, rapid, and cheap ion exchange technique for the determination of total porphyrin in urine, we collected reference material of blood donors, laboratory personnel, and medical inpatients with this technique and included tracings of the absorption curve in the range 380-430 nm to get the best possible check on analytical accuracy.

We also included 10 patients with latent porphyria (acute intermittent type) from our material (With, 1969) to study the role of excretion of preformed porphyrins compared to porphobilinogen in this disease.

Previous studies

Several authors have published reference values on coproporphyrin based on the extraction technique and a few have used ion exchange chromatography.

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Their results are summarised in Table 1, which shows that all authors gave reference values for total porphyrin of less than 350 μg/24 h.

Material and methods

Total porphyrin was determined in the first morning urine of 57 laboratory personnel, 23 men and 34 women aged 20 to 65 years. Similar analyses were made on arbitrary day urine samples from 59 blood donors, 31 men and 28 women aged 18 to 60 years, and on 24-hour urines from 90 medical inpatients (61 men and 29 women), 56 suffering from heart disease (observation for coronary occlusion) and the remainder from diabetes, Addison's disease, Graves' disease, apoplexy, bronchial asthma, or rheumatoid arthritis. Patients with liver and blood diseases and infections were excluded.

The analytical method used was recently described in detail (With and Pedersen, 1978). The readings were performed in a Beckman Model 25 double beam recording spectrophotometer, and in all cases tracings in the region of 380-430 nm were performed to control the Soret maximum. These tracings give a picture of the linearity of the non-porphyrin absorption in the 380-430 nm interval, which is a condition for the Rimington-Sveinsson correction on which the calculations are based. One millilitre of urine
Table 1  Previous quantitative studies on porphyrins in normal urine

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Material</th>
<th>Method</th>
<th>Result (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zieve et al.</td>
<td>1953</td>
<td>24-h urines from normal men (698) and women (50)</td>
<td>Acetic acid-ethyl acetate-HCl extraction with fluorimetric reading</td>
<td>100-300 μg/24 h for men 200-275 μg/24 h for women</td>
</tr>
<tr>
<td>Gutniak and Dancewicz</td>
<td>1957</td>
<td>24-h urines from 100 normal men</td>
<td>Technique of Zieve et al.</td>
<td>Mean 132 ± 50 μg/24 h 95% limit 232 μg/24 h</td>
</tr>
<tr>
<td>Talman</td>
<td>1958</td>
<td>24-h urines from 9 healthy subjects</td>
<td>Acetic acid-ethyl acetate-HCl extraction and fluorimetric reading for coproporphyrin; A1203 adsorption and HCl elution for uroporphyrin</td>
<td>Coproporphyrin: range 81-222, mean 132 μg/24 h. Uroporphyrin: range 28-63, mean 39 μg/24 h. Total porphyrin: range 109-285, mean 671 μg/24 h.</td>
</tr>
<tr>
<td>Schwartz et al.</td>
<td>1960</td>
<td>No details</td>
<td>As above</td>
<td>Coproporphyrin 100-250 μg/24 h; uroporphyrin 10-30 μg/24 h; total porphyrin 110-280 μg/24 h</td>
</tr>
<tr>
<td>Rimington</td>
<td>1961</td>
<td>No details</td>
<td>Fractionate extraction with ether and cyclobexanone and spectrophotometric reading of HCl extracts</td>
<td>Coproporphyrin 166 ± 45 μg/24 h for men, 134 ± 42 for women; uroporphyrin 5-80 μg/24 h for both sexes. Total porphyrin upper limit 336 μg/24 h</td>
</tr>
<tr>
<td>Doss and Schmidt</td>
<td>1971</td>
<td>Random samples from 95 apparently normal persons (48 men and 47 women)</td>
<td>Ion exchange chromatography with Mills's (1961) method</td>
<td>55 ± 33 μg/l for men and 47 ± 33 μg/l for women, upper 95% reference limit 121 μg/l</td>
</tr>
<tr>
<td>Sobel et al.</td>
<td>1974</td>
<td>24-h urines from 48 laboratory technicians</td>
<td>Ion exchange on a 200-400 mesh resin with fractionate extraction of copro- and uroporphyrin with ethanol-isopropanol-HCl mixtures and spectrophotofluorimetric reading</td>
<td>Coproporphyrin: range 35-235, mean 117 μg/24 h. Uroporphyrin: 15-60, mean 29 μg/24 h. Total porphyrin: 50-295 μg/24 h</td>
</tr>
</tbody>
</table>

Fig. 1  Five typical tracings: No. 1 shows a typical Soret peak (ε* = 0·140), No. 2 a low maximum (ε* = 0·034), No. 3 a still lower one (ε* = 0·015), No. 4 an inflection on a curve with concavity upwards (ε* = 0·002), and No. 5 a slightly concave curve without any inflection (ε* = 0·001).

was used and eluted with 5 ml 3 M HCl. Most of the tracings exhibited a maximum or an inflection at 403 nm, the absorption maximum of coproporphyrin in 3 M HCl, and were linear outside the peak but some of the tracings were curved, showing an upward concavity. The corrected extinction ε* = 2 × ε*max = (ε380 + ε430) corresponding to such curves was often below zero. Typical curves are shown in Figure 1. The readings were converted to micromoles by using the molecular weight of coproporphyrin (654 Daltons).

The urines or our porphyric patients were collected in buffered flasks of pH 7 or above to avoid formation of porphyrin from porphobilinogen (PBG). We studied 15 urines from 15 different carriers of acute hepatic porphyria showing PBG excretion of 5-650 μmol/litre. PBG was determined by Mauzerall and Granick's (1936) method. The results are presented in Table 2.
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Results

Our results are presented in Figs 2, 3, and 4a, b. The maximal value in morning urine (0.7 μmol/l) was found in a healthy female laboratory technician aged 35. To exclude a latent porphyria we determined porphyrins and precursors in another urine sample two weeks later as well as in faeces. These analyses were normal, the total porphyrin being 0.23 μmol/l. This shows that normal urinary porphyrin can occasionally reach about twice the 95% limit (mean + 2 SD).

Our values for morning urine were 0.128 ± 0.098, upper reference value 0.322 μmol/l, and our day urines of blood donors showed the considerably lower figures 0.075 ± 0.047, upper limit 0.155 μmol/l. Our values are only strictly comparable with those of Doss and Schmidt (1971) as all other authors used 24-hour urine. They found a reference limit of 121 μg/l, that is, 0.185 μmol/l, in good agreement with our values for the donor urines.

Our values for 24-hour urines were 67 ± 52, upper reference limit 271 μg/24 h, corresponding reasonably well with the values in the literature where the upper reference limits vary between 230 and 336 μg/24 h.

Our studies on urines from known porphyrin carriers (Table 2) comprised 15 urines from different patients with a PBG content ranging from normal up to five times the upper normal limit. In all
instances the molar ratio total porphyrin:PBG was below 2%, and if PBG was above 50 μmol/l the molar ratio was always below 1%.

Discussion

As will be seen from the tracings in Fig. 1, it is meaningless to speak of a lower limit for normal urinary porphyrin because our absorption curves in several cases showed only faint inflections in the Soret region and occasionally were not linear, giving negative corrected extinctions. We have tried to get higher Soret peaks by using 5 ml of urine instead of 1 ml; but in urines without definite peaks the only result was an increase in the non-specific background absorption. As the analysis is more rapid with 1 ml urine we used this in our investigations.

Our results do not support the claim of Doss and Schermuly (1976) that preformed porphyrins exceeding normal excretion occur in porphyrin urines because only one of our urines, with 650 μmol PBG per litre, showed excretion exceeding our upper normal reference limit for total porphyrin. Therefore further documentation on urines from acute porphyrin patients with high PBG excretion collected at pH 7 or above is required to substantiate the claim that preformed porphyrins are excreted in excess during the porphyrin attack.

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References


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