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 $0.32 \mu\text{mol/l}$ but even a value as high as $0.7 \mu\text{mol/l}$ was found. In the donor urines the upper limit was $0.155 \mu\text{mol/l}$. The 24-hour urines showed an upper reference limit of $271 \mu\text{g}/24 \text{ h}$. 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.

Their results are summarised in Table 1, which shows that all authors gave reference values for total porphyrin of less than $350 \mu\text{g}/24 \text{ h}$.

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

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Table 1 Previous quantitative studies on porphyrins in normal urine

Authors	Year	Material	Method	Result (range)
Zieve <i>et al.</i>	1953	24-h urines from normal men (698) and women (50)	Acetic acid-ethyl acetate-HCl extraction with fluorimetric reading	100-300 $\mu\text{g}/24\text{ h}$ for men 200-275 $\mu\text{g}/24\text{ h}$ for women
Gutniak and Danciewicz	1957	24-h urines from 100 normal men	Technique of Zieve <i>et al.</i>	Mean $132 \pm 50\text{ }\mu\text{g}/24\text{ h}$ 95% limit 232 $\mu\text{g}/24\text{ h}$
Talman	1958	24-h urines from 9 healthy subjects	Acetic acid-ethyl acetate-HCl extraction and fluorimetric reading for coproporphyrin; Al_2O_3 adsorption and HCl elution for uroporphyrin	Coproporphyrin: range 81-222, mean 132 $\mu\text{g}/24\text{ h}$. Uroporphyrin: range 28-63, mean 39 $\mu\text{g}/24\text{ h}$. Total porphyrin: range 109-285, mean 671 $\mu\text{g}/24\text{ h}$.
Schwartz <i>et al.</i>	1960	No details	As above	Coproporphyrin 100-250 $\mu\text{g}/24\text{ h}$; uroporphyrin 10-30 $\mu\text{g}/24\text{ h}$; total porphyrin 110-280 $\mu\text{g}/24\text{ h}$
Rimington	1961	No details	Fractionate extraction with ether and cyclohexanone and spectrophotometric reading of HCl extracts	Coproporphyrin $166 \pm 45\text{ }\mu\text{g}/24\text{ h}$ for men, 134 ± 42 for women; uroporphyrin 5-80 $\mu\text{g}/24\text{ h}$ for both sexes. Total porphyrin upper limit 336 $\mu\text{g}/24\text{ h}$
Doss and Schmidt	1971	Random samples from 95 apparently normal persons (48 men and 47 women)	Ion exchange chromatography with Mills's (1961) method	$55 \pm 33\text{ }\mu\text{g}/\text{l}$ for men and $47 \pm 33\text{ }\mu\text{g}/\text{l}$ for women, upper 95% reference limit 121 $\mu\text{g}/\text{l}$
Sobel <i>et al.</i>	1974	24-h urines from 48 laboratory technicians	Ion exchange on a 200-400 mesh resin with fractionate extraction of copro- and uroporphyrin with ethanol-isopropanol-HCl mixtures and spectrophotofluorimetric reading	Coproporphyrin: range 35-235, mean 117 $\mu\text{g}/24\text{ h}$. Uroporphyrin: 15-60, mean 29 $\mu\text{g}/24\text{ h}$. Total porphyrin: 50-295 $\mu\text{g}/24\text{ h}$

Fig. 1 Five typical tracings: No. 1 shows a typical Soret peak ($e^* = 0.140$), No. 2 a low maximum ($e^* = 0.034$), No. 3 a still lower one ($e^* = 0.015$), No. 4 an inflection on a curve with concavity upwards ($e^* = 0.002$), and No. 5 a slightly concave curve without any inflection ($e^* = 0.001$).

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 $e^k = 2 \times e_{\text{max}} - (e^{380} + e^{430})$ 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).

Table 2 Acute porphyria urine from 15 AIP patients or carriers collected with buffering to pH 6-7

PBG ($\mu\text{mol}/\text{l}$)	Total porphyrin ($\mu\text{mol}/\text{l}$)	Molar ratio % Total porphyrin: PBG
11	0.13	1.19
120	0.24	0.20
10	0.07	0.75
5	0.01	0.22
92	0.37	0.40
194	0.57	0.31
67	0.14	0.21
39	0.24	0.62
227	0.35	0.15
136	0.61	0.45
27	0.25	0.93
650	1.61	0.25
269	0.55	0.20
33	0.60	1.82
7	0.06	0.85

The urines of 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 $\mu\text{mol}/\text{litre}$. PBG was determined by Mauzerall and Granick's (1956) 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 ($0.7 \mu\text{mol/l}$) 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 $0.23 \mu\text{mol/l}$. 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 $0.322 \mu\text{mol/l}$, and our day urines of blood donors showed the considerably lower figures 0.075 ± 0.047 , upper limit $0.155 \mu\text{mol/l}$. 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 $121 \mu\text{g/l}$, that is, $0.185 \mu\text{mol/l}$, in good agreement with our values for the donor urines.

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

Our studies on urines from known porphyric

Fig. 2 Total porphyrin in morning urine of 57 persons (laboratory personnel and spouses; 23 men and 34 women):

	Total	Men	Women
mean	0.128	0.116	0.137
σ	0.098	0.052	0.121
m + 2 σ	0.322	0.210	0.379

Fig. 3 Total porphyrin in arbitrary voided samples of 59 blood donors, 31 men and 28 women.

	Total	Men	Women
mean	0.075	0.083	0.066
σ	0.047	0.050	0.040
m + 2 σ	0.155	0.183	0.146

Fig. 4 Total porphyrin in 24-hour urine from 90 medical inpatients.

(a)	(b)
mean	67
σ	52
m + 2 σ	271

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 $\mu\text{mol/l}$ 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 porphyric 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 porphyric 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 porphyric attack.

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