Renal Mechanism for Excretion of Porphyrin Precursors in Patients with Acute Intermittent Porphyria and Chronic Lead Poisoning

By Robert Drayan, Birgitta Haeger-Aronsen, Wilfried Von Studnitz and Jan Waldenström

A rapid and accurate diagnosis of acute intermittent porphyria (AIP) became possible once suitable methods were available for measurement of urine content of δ-aminolevulinic acid (ALA) and porphobilinogen (PBG). Similarly, in lead poisoning, determination of ALA excretion has proven a useful chemical adjunct, both for diagnostic and therapeutic evaluation.

The biochemical defect(s) which result in increased excretion of porphyrin precursors in these two diseases remain unknown. At present, evidence favors overproduction of ALA and PBG in acute porphyria, and decreased utilization of ALA in lead intoxication. However, despite the direct role of the kidney, which provides the final excretory pathway for these metabolites, investigations of the renal mechanism for ALA and PBG excretion are sparse.

Both ALA and PBG are amino acids. Of pertinence are the reports of a generalized aminoaciduria occurring during experimental porphyria, clinical AIP, experimental lead intoxication, and clinical lead poisoning. In each instance, an aminoaciduria has been documented in association with an abnormally increased excretion of ALA. The possibility that renal abnormalities may contribute to increased ALA excretion in lead poisoning has been considered, but has not been investigated.

In this communication, we report studies of renal excretion of ALA and PBG based on measurements of their endogenous clearance. Subject material includes women with AIP, men with chronic lead poisoning, and normal controls. In addition, amino acid excretion among patients with AIP has been examined. A single report demonstrated general aminoaciduria in 12 patients; we have attempted to confirm and extend this observation.

**Materials and Methods**

Patients with AIP or chronic lead intoxication were ambulatory, except for patient I. O., who was hospitalized with paresis and tachycardia. All other patients were asymptomatic at the time that investigations were performed. Laborers, referred from industry, were chronically exposed to lead fumes.

Analyses for porphyrin precursors were made on samples of urine or serum immediately after collections were completed. ALA and PBG were separated and measured according to the method of Urata and Granick. One ml. urine samples, pH 5-6, were analyzed.*

*For control subjects, urine samples analyzed were 2.0 ml. For patients A. A., I. O., and E. S., all of whom had very high excretions of porphyrin precursors, 0.5 ml. samples of urine were used.

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For serum, a 3.0 ml aliquot was freed from protein by precipitation with 1.2 mM of trichloroacetic acid, and the supernatant solution was neutralized with 0.25 mM of sodium acetate and 0.4 mM of sodium hydroxide before application to a Dowex-1 column. Under these conditions, the following values were used to calculate ALA and PBG concentrations in urine and serum: 66.4 x E555 mg/PBG per L urine; 22.1 x E555 = mg. PBG per L serum; 49.4 x E525 = mg. ALA per L urine; 16.4 x E552 = mg. ALA per L serum. To measure PBG concentrations in serum from normal control subjects, readings were made in a 5 cm. cuvette, with appropriate corrections for path length. Creatinine in serum and in urine was measured by routine methods. Endogenous creatinine clearances were calculated from 24-hour urine collections, and clearance values were corrected for a standard body surface area of 1.73 M². Samples of urine for amino acid analysis, which contained 5 mg. of creatinine, were initially passed through a 3 cm. column of Zeo-Karb 225 resin to remove urea and reduce volume. Amino acids were then separated by high voltage electrophoresis on Whatman No. 3MM filter paper with formate-acetic acid buffer, pH = 1.2. The voltage gradient was 50 V per cm.; running time was three hours. Spots were located after spraying with ninhydrin and then copper reagent. Spots were then eluted from the filter paper with methanol, and quantitation was performed spectrophotometrically at 504 nm. Standard amino acid solutions and appropriate blanks were run together with each urine sample. Glycine, alanine, lysine, tryptophan, and tyrosine are resolved into discrete spots under these conditions. The following amino acids overlap: glutamine, glutamic acid, and asparagine; histidine and methyl histidine; serine and valine; threonine and leucine. These were further resolved by ascending paper chromatography, performed in a perpendicular direction after electrophoresis. Glacial acetic acid-n-butanol-water (1:4:5, v/v, upper phase) was employed as the solvent. Among the porphyric patients studied for aminoaciduria, the overlapping amino acids were in no instance present in abnormal concentration, and the data presented are for the spots separated by high voltage electrophoresis.

RESULTS

Measurements of creatinine clearance, serum ALA and PBG concentrations, and 24-hour excretions of ALA and PBG, as determined in 4 normal adults, 9 women with AIP, and 4 men with chronic lead intoxication are summarized in table 1. As expected, ALA and PBG excretion is increased among porphyric patients, and urine ALA is increased among the lead workers. ALA and PBG concentrations measured in serum have been reported, but in each instance, interfering substances were not removed prior to color development. In the present study, normal serum concentrations for porphyrin precursors are as follows: ALA = 0.172 mg. per L (95 per cent confidence limits: 0.043-0.301 mg. per L); PBG = 0.115 mg. per L (0.005-0.228 mg. per L). Among patients with AIP, serum concentrations of both porphyrin precursors are significantly increased; mean ALA = 0.534 mg. per L (p < 0.02); mean PBG = 0.580 mg. per L (p < 0.01). Among lead poisoned men, serum ALA concentration is also significantly increased, and the mean value for this group was 0.452 mg. per L (p < 0.05).

Endogenous clearance of creatinine, ALA, and PBG was measured in control subjects, and in patients with AIP. For lead workers, PBG clearance was not determined. Among the normal group, ALA clearance ranged from 3 to 7 per cent of that for creatinine, and similar values, 3 to 10 per cent, were measured for PBG clearance. If it is assumed that both ALA and PBG are filtered at the glomerulus, then significant tubular reabsorption occurs.
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Table 1.—Creatinine Clearance, Serum Concentration and 24-Hour Renal Excretion of 5-Aminolevulinic Acid (ALA) and Porphobilinogen (PBG):
Control Subjects, Patients with AIP and Patients with Chronic Lead Poisoning

<table>
<thead>
<tr>
<th></th>
<th>C_{Cr} (ml/min)</th>
<th>ALA (mg/L)</th>
<th>ALA (mg/24 hrs)</th>
<th>PBG (mg/L)</th>
<th>PBG (mg/24 hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KA</td>
<td>122.4</td>
<td>0.213</td>
<td>5.11</td>
<td>0.066</td>
<td>1.47</td>
</tr>
<tr>
<td>KB</td>
<td>154.4</td>
<td>0.148</td>
<td>4.05</td>
<td>0.141</td>
<td>0.79</td>
</tr>
<tr>
<td>RD</td>
<td>126.0</td>
<td>0.197</td>
<td>5.23</td>
<td>0.111</td>
<td>1.59</td>
</tr>
<tr>
<td>GP</td>
<td>114.5</td>
<td>0.132</td>
<td>1.60</td>
<td>0.141</td>
<td>0.76</td>
</tr>
<tr>
<td>AIP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>111.4</td>
<td>0.444</td>
<td>60.0</td>
<td>0.841</td>
<td>129</td>
</tr>
<tr>
<td>LR</td>
<td>29.1</td>
<td>0.378</td>
<td>10.3</td>
<td>0.509</td>
<td>17.1</td>
</tr>
<tr>
<td>GH</td>
<td>105.8</td>
<td>0.280</td>
<td>9.10</td>
<td>0.245</td>
<td>15.9</td>
</tr>
<tr>
<td>IO</td>
<td>129.4</td>
<td>1.02</td>
<td>20.5</td>
<td>0.797</td>
<td>170</td>
</tr>
<tr>
<td>DU</td>
<td>74.9</td>
<td>0.421</td>
<td>14.8</td>
<td>0.553</td>
<td>48.5</td>
</tr>
<tr>
<td>EU</td>
<td>90.3</td>
<td>0.592</td>
<td>57.8</td>
<td>0.664</td>
<td>67.8</td>
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<tr>
<td>IU</td>
<td>92.5</td>
<td>0.562</td>
<td>22.7</td>
<td>0.445</td>
<td>25.7</td>
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<tr>
<td>KS</td>
<td>110.0</td>
<td>0.658</td>
<td>74.5</td>
<td>0.445</td>
<td>64.8</td>
</tr>
<tr>
<td>US</td>
<td>71.1</td>
<td>0.658</td>
<td>36.0</td>
<td>0.751</td>
<td>41.7</td>
</tr>
<tr>
<td>Pb**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NL</td>
<td>114.1</td>
<td>0.559</td>
<td>48.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EL</td>
<td>97.9</td>
<td>0.444</td>
<td>55.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EPer</td>
<td>114.0</td>
<td>0.559</td>
<td>63.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EPet</td>
<td>126.6</td>
<td>0.247</td>
<td>20.6</td>
<td>-</td>
<td>-</td>
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</table>

AIP = acute intermittent porphyria
Pb** = chronic lead poisoning
- = not determined

under physiologic conditions, and is comparable, in magnitude, to values reported for α-amino acids. At increased serum concentrations, tubular reabsorption of porphyrin precursors decreases, and is actually calculated as zero for patient I. O. In figure 1, the relationship between serum concentrations of ALA and PBG, and their respective tubular reabsorptions is shown for all 3 patient groups studied. An inverse relationship between serum ALA concentrations and its tubular reabsorption applies equally to both porphyric patients and to lead intoxicated subjects, and within the parameters employed for this study, their renal excretion cannot be differentiated.

Amino acid analyses, performed on urines from 7 control subjects and 8 patients with AIP, are summarized in table 2. For the control group, mean values and 95 per cent confidence limits are given. Aminoaciduria was docu-
Fig. 1.—Tubular reabsorption of porphyrin precursors as a function of their serum concentrations. △—PBG, control subject; ▲—ALA, control subject; ○—PBG, patient with AIP; ●—ALA, patient with AIP; ■—ALA, lead poisoned patient; TR = Tubular reabsorption.

Commented for 3 of the 8 patients with AIP. In each porphyric patient, only a single amino acid was excreted in abnormal amounts. Patients L. C. and B. P. excreted excess tyrosine; patient G. H. showed a slight increase in tryptophan excretion. Total α-amino nitrogen, calculated as the sum of the amino acids measured, was within normal limits for all patients with AIP.

**DISCUSSION**

In AIP, serum ALA and PBG concentrations are increased, and are correlated with their renal excretion. Among laborers with chronic lead poisoning, a similar relationship occurs with respect to serum and urine ALA. In control subjects, whose concentrations of porphyrin precursors is significantly lower than the above groups, tubular reabsorption of ALA and PBG occurs; when glomerular filtration is estimated from endogenous creatinine clearance, 90 to 95 per cent of the filtered load of ALA and PBG is reabsorbed.

A previous report suggests that PBG is excreted by glomerular filtration alone, without tubular reabsorption2 and identical values were calculated for endogenous clearance of creatinine and PBG. In that study, 4 patients with AIP had serum PBG concentrations which ranged from 0.8 to 1.3 mg. per
Table 2.—Twenty-Four Hour Amino Acid Excretion in Urine from Control Subjects and Patients with AIP

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>CONTROL Mean</th>
<th>CONTROL Range</th>
<th>AA</th>
<th>LC</th>
<th>GS</th>
<th>KP</th>
<th>ES</th>
<th>IS</th>
<th>KS</th>
<th>US</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALANINE</td>
<td>0.86</td>
<td>0.10-1.89</td>
<td>1.11</td>
<td>1.20</td>
<td>0.52</td>
<td>1.32</td>
<td>0.71</td>
<td>0.48</td>
<td>0.70</td>
<td>0.94</td>
</tr>
<tr>
<td>ASPARAGINE</td>
<td>0.74</td>
<td>0.00-1.57</td>
<td>0.85</td>
<td>0.73</td>
<td>0.48</td>
<td>0.82</td>
<td>0.61</td>
<td>0.48</td>
<td>0.47</td>
<td>0.75</td>
</tr>
<tr>
<td>GLUTAMINE</td>
<td>4.08</td>
<td>1.61-6.55</td>
<td>2.23</td>
<td>2.86</td>
<td>2.71</td>
<td>7.20</td>
<td>5.75</td>
<td>4.80</td>
<td>4.13</td>
<td>2.82</td>
</tr>
<tr>
<td>GLUTAMINE</td>
<td>3.99</td>
<td>0.00-8.59</td>
<td>4.00</td>
<td>2.68</td>
<td>0.44</td>
<td>4.51</td>
<td>2.94</td>
<td>2.20</td>
<td>2.93</td>
<td>1.17</td>
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<tr>
<td>HISITIDINE</td>
<td>1.50</td>
<td>0.00-5.45</td>
<td>1.08</td>
<td>1.95</td>
<td>2.92</td>
<td>1.52</td>
<td>1.12</td>
<td>0.47</td>
<td>1.17</td>
<td></td>
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<tr>
<td>KETOSIDINE</td>
<td>0.85</td>
<td>0.00-1.78</td>
<td>1.17</td>
<td>0.89</td>
<td>0.50</td>
<td>1.17</td>
<td>1.13</td>
<td>0.81</td>
<td>0.49</td>
<td>1.17</td>
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<tr>
<td>TRYPTOPHAN</td>
<td>0.71</td>
<td>0.31-1.16</td>
<td>0.84</td>
<td>0.97</td>
<td>1.25</td>
<td>1.18</td>
<td>1.13</td>
<td>0.85</td>
<td>0.60</td>
<td>1.02</td>
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<tr>
<td>Tryptophan</td>
<td>0.60</td>
<td>0.22-1.10</td>
<td>0.72</td>
<td>1.35</td>
<td>1.23</td>
<td>0.76</td>
<td>0.76</td>
<td>0.87</td>
<td>0.88</td>
<td>0.88</td>
</tr>
<tr>
<td>HISURINE</td>
<td>15.86</td>
<td>4.59-25.11</td>
<td>15.11</td>
<td>17.01</td>
<td>10.75</td>
<td>19.72</td>
<td>15.57</td>
<td>12.48</td>
<td>11.40</td>
<td>10.05</td>
</tr>
</tbody>
</table>

All values are expressed as mg. amino acid per Gm. urine creatinine. Range is for 95 per cent confidence limits, calculated from “t” distribution with six degrees of freedom.

L, and urine excretions were 70 to 180 mg. per day. In the present study, patients A. A. and I. O. had serum PBG concentrations of this magnitude, and their PBG clearances were 92 and 108 per cent of their creatinine clearances. However, with a milder chemical abnormality, as manifested by a serum PBG concentration closer to the normal range, significant tubular reabsorption of PBG could be demonstrated in the remaining 7 porphyric patients.

Renal clearance of ALA was measured by Berlin et al. who studied a normal subject after the ingestion of 1.0 g. of C'-labelled ALA. Initial clearance was 94 ml. per minute, declining to 14 ml. per minute within 6 hours. However, initial serum ALA concentration, estimated by measurement of serum radioactivity (and neglecting possible contributions from C'-labelled metabolites) was 14 mg. per L. Among the porphyric and lead intoxicated patients in the present study, much lower serum ALA concentrations, i.e., 0.7-1.0 mg. per L, were associated with almost complete calculated renal clearance of ALA. As with PBG, however, lower concentrations of serum ALA, in both porphyric and lead intoxicated patients, were associated with demonstrable tubular reabsorption of ALA.

Using a less specific method for measuring serum and urine ALA, one of us (B. H.-A.) has collected a larger series of serum and urine ALA measure-
Repeated determinations of serum and urine ALA, performed on nineteen patients, resulted in a mean endogenous ALA clearance of 39.6 ml. per minute. Figure 2 shows the relationship between serum ALA concentrations and 24-hour urine ALA excretion using measurements from both the older and the present series.

The present data on renal excretion of porphyrin precursors documents an inverse relationship between tubular reabsorption of these compounds and their serum concentrations, and are in accord with the "overflow" model proposed for some aminoacidurias. Although renal tubular damage has been established as a consequence of chronic lead intoxication, our studies fail to show any difference in the mechanism of ALA excretion between patients with AIP and lead poisoning. In both groups, serum ALA concentration is correlated with urine ALA excretion, and comparable tubular reabsorptions, for a given serum ALA concentration, are obtained.

Neither the clinical symptoms nor signs of AIP or chronic lead poisoning bear any apparent relationship to the known pharmacologic effects of ALA or PBG. In fact, one documented pharmacologic effect of ALA, namely photosensitivity, is not observed in either AIP or lead intoxication. Our data may explicate this apparent discrepancy. Erythema in a photoexposed distribution followed ingestion of 0.075–0.210 mM of ALA per Kg. in normal subjects. Serum ALA, in 1 normal subject with erythema, measured 14 mg. per L immediately after ALA ingestion. Among our patients, the highest value for ALA measured in serum was 1.02 mg. per L in subject I. O. Thus,
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a fourteen-fold difference in serum ALA is shown between the pharmaco-
logically effective experiment and the natural disease.

The earlier report of aminoaciduria in patients with AIP is confirmed by
our findings, but neither the frequency nor the severity of aminoaciduria
reported by Mellinkoff et al.7 was duplicated by our patient group. Amino-
aciduria was earlier detected in 12 of 12 porphryic patients, with valine, β-
aminoisobutyric acid, serine, cysteic acid, and phenylalanine most frequently
excreted in increased amounts. Aminoaciduria in all patients involved several
amino acids. In the present study, only the aromatic amino acids tyrosine
and tryptophan were detected in excess, and of the 3 patients with amino-
aciduria, only a single amino acid was involved in each. Since significant
tubular reabsorption of ALA and PBG is seen under physiologic conditions,
excretion of porphyrin precursors in AIP by an “overflow” mechanism sug-
gests that aminoaciduria in patients with AIP could result from saturation
of a common reabsorption mechanism. Further measurements, with controlled
intake, would be necessary to evaluate this hypothesis.

SUMMARY

Serum concentrations of ALA and PBG have been measured in normal
subjects, patients with AIP, and lead workers. Both porphyrin precursors
are significantly increased in serum from porphryic patients, and serum ALA
is elevated in lead workers. Endogenous clearance measurements, when com-
pared with creatinine clearances, are consistent with significant tubular reab-
sorption of ALA and PBG under physiologic circumstances, and with an
“overflow” mechanism for increased excretion in AIP or lead intoxication.
Three of 8 porphryic patients showed an aminoaciduria; tyrosine was in-
volved in 2, tryptophan in the other 3. Evidence to suggest a specific renal
defect contributing to increased ALA excretion in chronic lead intoxication
was not obtained.

SUMMARIO IN INTERLINGUA

Le concentrationes seral de acido δ-aminolevulinic (AAL) e de porpho-
bilinogeno (PBG) esseva mesurate in subjectos normal, in patientes con acute
porphyria intermittente (API), e in travaliatores de plumbo. Ambe ille
precursores de porphyrina ha significativamente augmentate concentrationes
in le sero ab patientes con porphyria. In travaliatores de plumbo, solmente
le concentration seral de AAL es augmentate. Le mesurationes del clearance
endogene—quando comparate con le clearance de creatina—es congrue con
le these de un significative reaction tubular de AAL e PBG sub conditiones
physiologic e con un mecanismo de “effluxo in superabundantia” como
explication del augmentate excretion in API o in intoxication a plumbo. Tres
de 8 patientes con porphyria monstrava un certe grado de aminoaciduria. In
2 del casos le amino-acido eseva thyrosina, in le tertie, tryptophano. Esseva
obtenite nulle evidentia a indicar un specific defecto renal que contribuerea
al augmentate excretion de AAL in chronic intoxication a plumbo.
ACKNOWLEDGMENTS

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REFERENCES

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