Porphobilinogen and Porphyrin Synthesis in Reticulocytes from Uraemic Patients

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Summary
Since no information exists concerning porphyrin metabolism in uraemic patients we have measured the activities of delta-aminolaevulinc acid dehydrase (D-ALA-D) and porphobilinogen desaminase (PBG-D) in reticulocytes from uraemic patients, anaemic patients without uraemia and in healthy subjects. Despite a severe anaemia uraemic patients had the same amount of reticulocytes/μl blood.

In uraemic patients D-ALA-D activity was reduced to about 20% compared to healthy subjects, PBG-D in uraemia was decreased to about 70%. It is concluded that porphyrin metabolism is altered in uraemic patients.

Introduction
Renal failure is usually associated with anaemia of a varying degree. The aetiology of uraemic anaemia has been ascribed to haemolysis and reduced erythropoiesis (Emerson and Burrows, 1959). Obviously the latter is caused by diminished erythropoietin production (Jacobson et al, 1957) and decreased responsiveness of haematopoietic tissue to erythropoietin (Bozini et al, 1966). The biochemical mechanism by which uraemia leads to depression of erythropoiesis has not been completely elucidated.

Normal erythropoiesis is based on undisturbed cell production and cell differentiation and on sufficient synthesis of haemoglobin in the developing cells. Haemoglobin synthesis involves three steps:
1. Production of protoporphyrin.
2. Insertion of iron into protoporphyrin under the influence of the enzyme haem synthetase.

Disturbances of each of these processes can influence haemoglobin synthesis. Iron deficiency, as many investigators have shown, is not responsible for uraemic
anaemia, although in many cases iron deficiency develops secondarily as a result of restricted diet or dialyser blood loss. In the latter patients, treatment with iron will improve erythropoiesis but without normalising anaemia (Brozovich et al, 1971; Carter et al, 1969).

According to Giordano et al (1972) uraemia influences globin synthesis by reticulocytes in vitro. To date no general information exists about porphyrin synthesis in uraemic patients, although it is well known that some other forms of anaemia (iron deficiency, side-roachrestic anaemia) are associated with alterations of porphyrin metabolism (Battistini et al, 1971; Heilmeyer, 1962).

From our own investigations we know that:

1. Anaemia of chronic uraemic rats can be improved by pretreating the animals with delta-aminolaevulinic acid.
2. Uraemic patients have higher plasma concentrations of delta-aminolaevulinic acid and porphobilinogen than patients with normal kidney function (Leber et al, 1974).

To clarify whether the higher plasma concentrations of porphyrin precursors are caused solely by their impaired renal excretion or additionally by disturbed utilisation for porphyrin synthesis we have measured two enzymes involved in porphyrin metabolism. The enzymes delta-aminolaevulinic acid dehydrase and porphobilinogen desaminase were measured in vitro in reticulocytes from uraemic patients and healthy subjects. Delta-aminolaevulinic acid dehydrase (D-ALA-D) catalyses the conversion of delta-aminolaevulinic acid to porphobilinogen, whereas porphobilinogen desaminase (PBG-D) brings about the further metabolism of porphobilinogen to uroporphyrinogen. Both enzymes are located in the cytoplasm and are still present and active in reticulocytes. According to Heimpel et al (1965) the enzymes in reticulocytes reflect the activities in hematopoietic cells of the bone marrow.

METHODS

The following groups of patients were investigated:

1. Patients on chronic intermittent haemodialysis, (2 x 10 hr/week, Gambro Lundia).
2. Patients with acute renal failure—blood was withdrawn before the first dialysis, 2—4 days after the onset of acute renal failure.
3. Healthy subjects.
4. Patients with different forms of anaemia without renal insufficiency.

Blood was drawn into heparinised syringes. Haemoglobin content, erythrocyte and reticulocyte counts were estimated using routine methods. The reticulocyte count was carried out in duplicate by two different co-workers. Haemolysis was achieved by adding 2 ml distilled water per millilitre of blood.
Delta-aminolaevulinic Acid Dehydrase

The enzyme activity was measured according to Gibson et al (1955). The incubation mixture contained in a final volume of 2.0 ml, 0.15 M phosphate buffer pH 7.0, 0.6 ml haemolysate. After a 60-min preincubation period under an atmosphere of nitrogen at 37°C the reaction was started by adding 20 μmoles delta-aminolaevulinic acid buffered to pH 7.0. After 0 and 60 min the reaction was stopped with 0.8 ml 10% TCA and 0.03 ml saturated CuSO₄ solution. After centrifuging porphobilinogen was estimated in the protein-free supernatant with Ehrlich’s reagent. Control samples contained buffer instead of haemolysate. Non-enzymatic conversion of delta-aminolaevulinic acid to porphobilinogen was negligible.

Porphobilinogen Desaminase

Porphobilinogen desaminase activity in peripheral blood cells was measured according to Miyagi et al (1971) by estimating the amount of porphyrins formed during the incubation period.

The incubation mixture contained (in a total volume of 2.0 ml) 125 μmoles phosphate buffer pH 7.4, 20 μmoles sodium-EDTA, 20 μmoles KCN, 0.2 μmoles porphobilinogen. The reaction was started by adding 0.8 ml haemolysate and continued in the dark at 37°C. After 0 and 120 minutes the reaction was stopped by the addition of 0.5 ml 25% TCA and 0.01 ml 1% I₂ in 96% ethanol. After 20 min in an ice bath the precipitate was centrifuged. 2.2 ml of the supernatant was mixed with the same volume of 3N HCl. The precipitate was washed twice with 1.0 ml 5% TCA in 1.5N HCl and these washes were combined with the first TCA-HCl supernatant and the whole was made up to 15 ml with 1.5N HCl.

The total porphyrin content was measured fluorometrically with uroporphyrin as standard (Schwarzet al, 1958). Non-enzymatic conversion of porphobilinogen to porphyrins under the stated conditions was negligible.

Since preliminary studies had shown that only reticulocytes contained D-ALA-D and PBG-D both enzyme activities were expressed as nmoles product/10⁷ reticulocytes x 60 min. Student’s ‘t’ test was used for statistical evaluations.

RESULTS

From Table 1 it can be seen that patients with acute and chronic uraemia had a severe anaemia. No differences exist between the three groups concerning the reticulocyte count. Figure 1 demonstrates the activity of D-ALA-D in the different groups. The highest values were found in healthy subjects. In patients with acute renal failure and on chronic intermittent haemodialysis the enzyme activity was decreased to about 22%. Orientating investigations with reticulocytes
TABLE I. Haematological Values of Healthy Subjects and Uraemic Patients
(p < 0.01, x against 0)

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th>Chronic uraemic patients</th>
<th>Acute renal failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 57</td>
<td>n = 42</td>
<td>n = 15</td>
</tr>
<tr>
<td>Haemoglobin (g/100 ml)</td>
<td>15.7 ± 1.1^x</td>
<td>7.4 ± 1.5^o</td>
<td>7.5 ± 1.8^o</td>
</tr>
<tr>
<td>10^6 erythrocytes/µl</td>
<td>5.1 ± 0.5^x</td>
<td>2.4 ± 0.4^o</td>
<td>2.5 ± 0.5^o</td>
</tr>
<tr>
<td>10^3 reticulocytes/µl</td>
<td>60.3 ± 28.2</td>
<td>71.5 ± 35.2</td>
<td>69.0 ± 38.2</td>
</tr>
</tbody>
</table>

Figure 1. Delta-aminolaevulinic acid dehydrase activity in the different groups of patients, p < 0.01. Uraemic patients against healthy subjects.

from anaemic patients without uraemia had different results. Though the latter groups are too small for statistical evaluation it seems likely that patients with haemolytic anaemia are in the normal range whereas patients with secondary anaemia (carcinoma, infection) or iron deficiency tended to have lower values.

There was no correlation between the activity of porphobilinogen synthesis and the degree of anaemia. When compared to haemoglobin content or erythrocyte count, no correlation could be found between degree of anaemia and reticulocyte count.

In Figure 2 D-ALA-D activity/10^7 reticulocytes × 60 min is plotted against the reticulocyte count/µl. A significant negative correlation exists between these two parameters: in both groups of patients the enzyme activity is higher, the lower the reticulocyte count measured. Therefore porphobilinogen production of each reticulocyte is higher in reticulocyte-poor blood than in reticulocyte-rich blood. Reticulocytes from healthy subjects synthesise more porphobilinogen than those from uraemic patients.
Figure 2. Correlation between enzyme activity and amount of reticulocytes/μl.

Figure 3. Porphobilinogen desaminase activity in the different groups of patients, p < 0.01. Uraemic patients against healthy subjects.

Figure 3 gives results concerning the conversion of porphobilinogen to porphyrins. The highest activity was found in healthy subjects, whereas uraemic patients had significantly lower values (about 70%). Reticulocytes from patients with haemolytic anaemia were in the normal range while those from patients with iron deficiency seemed to have higher activities than uraemic patients. Porphyrin production in reticulocytes from patients with anaemia due to carcinoma or infection was very variable.

Neither in healthy subjects nor in uraemic patients does a correlation exist between porphyrin production and the severity of anaemia as measured by
haemoglobin content and erythrocyte count. As with D-ALA-D a correlation exists (Figure 4) between the reticulocyte count/µl and porphyrin production/10^7 reticulocytes x 60 min. In healthy subjects the correlation tends more to the vertical than in uraemic patients. The reason for this correlation is not clear.

**DISCUSSION**

Our investigations have demonstrated that uraemic patients, despite severe anaemia, produce the same number of reticulocytes/µl blood as do healthy subjects. But reticulocytes from uraemic patients synthesise less porphobilinogen and porphyrins than healthy subjects when delta-aminolaevulinic acid and porphobilinogen are added in vitro.

Since in our experiments the substrates had been added in concentrations to saturate the measured enzymes it is concluded that reticulocytes from uraemic patients have a reduced capacity to utilize delta-aminolaevulinic acid and porphobilinogen for porphyrin synthesis, due to a decrease of the activity of the enzymes concerned.

As far as D-ALA-D is concerned reticulocytes from uraemic patients are comparable with those from patients with sideroachrestic anaemia (lead poisoning) or iron deficiency (Battistini et al, 1971; Heilmeyer, 1962) where reduced activities of D-ALA-D are found as well.

In contrast to the latter forms of anaemia, in uraemic patients a decreased PBG-D activity exists as well. Details concerning the mechanism by which the activities of the measured enzymes are diminished are unknown. In a previous paper we demonstrated that uraemic patients have higher delta-aminolaevulinic
acid and porphobilinogen plasma concentrations than patients with normal kidney function. According to our present results this is due not only to impaired renal excretion (Leber et al, 1974) but also to its disturbed utilisation for porphyrin synthesis.

Further investigations are needed to clarify to what extent the alterations of porphyrin metabolism described are responsible for the depression of erythropoiesis in uraemic patients.

References

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Open Discussion

M MCGEOWN (Belfast) You showed that there is reduction of enzymes in the patients with acute renal failure. How soon after the onset of renal failure did you measure them?

LEBER We studied these patients before the first haemodialysis, two to four days after beginning of renal failure.

MCGEOWN So it was a very rapid onset?

LEBER Yes

ESSERS (West Germany) I think you said that in uraemic patients the reticulocyte number is not reduced but that some enzymes are reduced, so you must expect a hypochromic anaemia. But this is not the case in most of our patients.

LEBER We have measured other enzymes of haemoglobin synthesis since the reticulocyte count was not significantly lowered in uraemic patients. It seemed to us that the function of the reticulocytes must be altered in uraemia.
ESSERS Did you measure the absolute count of reticulocytes? A normal man with a haemoglobin value of 14g/100ml must have 25,000 to 75,000 reticulocytes per ml. A patient who is anaemic must have far more reticulocytes for his hemo-
poiesis to compensate.
LEBER We found no large difference between these two groups in the absolute number of reticulocytes.