Aberration of Porphyrin Metabolism in Iron-Deficient Anaemic Rats

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Iron-deficient rats excreted greater amounts of 5-aminolaevulinate in urine but the output of porphobilinogen was decreased considerably. In addition, activity of the enzyme 5-aminolaevulinate dehydratase was diminished in bone marrow and liver. The results suggest that in iron deficiency a defect occurs in porphyrin synthesis at the level of porphobilinogen formation.

Iron-deficiency anaemia is a condition in which the rate of erythropoiesis is arrested by limiting amounts of available iron. With the decline in haemoglobin formation protoporphyrin accumulates in erythrocytes (Heilmeyer, 1964; Dagg *et al.*, 1966; Langer *et al.*, 1972). However, the accumulation is quantitatively far less than would be theoretically expected (Prato *et al.*, 1968; Goldberg, 1971). It is therefore reasonable to assume that a concomitant decrease in the total synthesis of protoporphyrin occurs in this condition. Obviously the defect should occur in earlier stages of porphyrin biosynthesis. Therefore it was decided to determine the urinary excretion of 5-aminolaevulinate and porphobilinogen by iron-deficient rats. In addition, the activity of the enzyme 5-aminolaevulinate dehydratase (EC 4.2.1.24) was determined in several tissues in order to account for the diminished excretion of porphobilinogen in urine.

Materials and methods

Four-week-old male albino Norway rats weighing 30–40 g were used. The animals were fed on a synthetic iron-deficient diet (Sharma *et al.*, 1972) for 12 weeks, during which period they developed iron-deficiency anaemia, as evidenced by blood haemoglobin concentrations, haematocrit and plasma iron determinations, and confirmed by the presence of hypochromic erythrocytes in peripheral blood films and absence of stainable iron in liver sections and bone-marrow smears.

A 24 h urine sample was collected in 1.0 ml of 1% Na₂CO₃ and preserved frozen until assay. Porphobilinogen and 5-aminolaevulinate were determined by the method of Mauzerall & Granick (1956).

The rats were then killed under barbiturate anaesthesia and blood was collected from the heart. Liver was removed, chilled in crushed ice and homogenized in 0.14 M-KCl. The bone marrow was aspirated from femur, weighed and suspended in 2.0 ml of 0.14 M-KCl (containing 50 μ mol of 2-mercaptoethanol) at 37°C for 1 h to activate the 5-aminolaevulinate de-

hydratase. Blood and supernatants from liver homogenates were also incubated with mercaptoethanol. 5-Aminolaevulinate dehydratase activity was then determined in these samples by the method of Nandi *et al.* (1968), with the only difference that 20 μ mol of potassium phosphate buffer, pH 6.7, was used in place of Tris buffer. One unit of enzyme activity was defined as that amount which produced 1 μ mol of porphobilinogen in 60 min at 37°C.

Results

The severity of iron-deficiency anaemia in the rats of the experimental group may be gauged by the blood haemoglobin, haematocrit and plasma iron values: 6.2 ± 1.4 g/100 ml (control, 14.5 ± 1.2 g/100 ml), $28.4 \pm 3.8\%$ (control, $45.5 \pm 6.4\%$) and 56.8 ± 18.6 μ g/100 ml (control, 202.3 ± 30.1 μ g/100 ml) respectively, expressed as means $\pm$ S.D. for 15 control and 15 iron-deficient rats.

The urinary excretion of 5-aminolaevulinate and porphobilinogen is presented in Table 1. A highly significant fall in porphobilinogen ($P < 0.01$) and elevation in 5-aminolaevulinate ($P < 0.05$) excretion by iron-deficient rats was observed. 5-Aminolaevulinate dehydratase activities of liver ($P < 0.01$) and bone marrow ($P < 0.01$) were diminished, whereas that of blood ($P > 0.9$) was unaltered (Table 2). However, a significant difference was found when it was expressed as units/ml of packed erythrocytes ($P < 0.01$).

Discussion

The urinary excretion of 5-aminolaevulinate was increased and that of porphobilinogen decreased in iron-deficient rats. Heilmeyer (1964) reported an increase in 5-aminolaevulinate and a decrease in porphobilinogen excretion in the urine of iron-deficient subjects, these changes reverting to normal when the iron deficiency was abolished. A metabolic aberration at the level of porphobilinogen formation is thus indicated. The decreased activity of 5-aminolaevulinate dehydratase in bone marrow and liver

Table 1. *Urinary excretion of 5-aminolaevulinate and porphobilinogen by control and iron-deficient rats*

Urine samples were collected in 1.0ml of 1% Na₂CO₃ and preserved frozen until assay. Porphobilinogen and 5-aminolaevulinate were determined by the method of Mauzerall & Granick (1956). The values are expressed as means $\pm$ S.D. of 15 observations on different animals.

	5-Aminolaevulinate excretion (nmol/day)	Porphobilinogen excretion (nmol/day)
Control	130.4 $\pm$ 54.0	37.0 $\pm$ 13.9
Iron-deficient	190.6 $\pm$ 83.2	18.8 $\pm$ 9.4

Table 2. *Activity of 5-aminolaevulinate dehydratase in various rat tissues*

The bone marrow was suspended in 2.0ml of 0.14M-KCl (containing 50 μ mol of 2-mercaptoethanol) at 37°C for 1 h. Liver was chilled in crushed ice and homogenized in 0.14M-KCl. Whole blood and supernatants from liver homogenates were also incubated with mercaptoethanol to activate 5-aminolaevulinate dehydratase. The enzyme activity was determined in these samples by the method of Nandi *et al.* (1968), with the use of potassium phosphate buffer, pH 6.7. One unit of enzyme activity was defined as that amount which produced 1 μ mol of porphobilinogen in 60 min at 37°C. The values are expressed as means $\pm$ S.D. for 15 observations on different animals.

	5-Aminolaevulinate dehydratase activity			
	(units/g wet wt.)		(units/ml)	
	Bone marrow	Liver	Whole blood	Packed erythrocytes
Control	1.503 $\pm$ 0.784	0.717 $\pm$ 0.444	0.0158 $\pm$ 0.0073	0.033 $\pm$ 0.012
Iron-deficient	0.625 $\pm$ 0.200	0.443 $\pm$ 0.135	0.0157 $\pm$ 0.0069	0.056 $\pm$ 0.028

supports this contention. Although an inhibition phenomenon involving haem and 5-aminolaevulinate dehydratase obtained from human erythrocytes (Calissano *et al.*, 1966), mouse liver (Coleman, 1966) and *Rhodopseudomonas spheroides* (Nandi *et al.*, 1968) has been observed *in vitro*, it is unlikely to be operative *in vivo* because of compartmentalization of porphyrin biosynthesis (Sano & Granick, 1961; Sardesai *et al.*, 1965).

Reports on 5-aminolaevulinate dehydratase activity in human erythrocytes are opposite and conflicting (Rubino *et al.*, 1960; Lichtman & Feldman, 1963; Battistini *et al.*, 1971; Gutniak *et al.*, 1971; Mazza *et al.*, 1971). In iron-deficient rats the enzyme activity was found to be unaltered when expressed as units/ml of blood, but showed a significant increase when expressed as units/ml of packed erythrocytes. The contradictory findings in packed erythrocytes and blood may be explained on the basis of a relative increase in number of reticulocytes and a fall in packed cell volume in iron-deficient rats. However, the changes in bone marrow and liver may be more important in relation to porphyrin synthesis *in vivo* because these tissues possess the highest activities (Sardesai *et al.*, 1964). Moreover decreased 5-aminolaevulinate dehydratase activity in these tissues may also be responsible for impaired utilization of 5-

aminolaevulinate and its increased excretion in urine.

It seems that the decrease in 5-aminolaevulinate dehydratase activity is one of the mechanisms by which the porphyrin synthesis is restricted in iron deficiency in rats.

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