Control of δ -aminolaevulinate synthase and haem oxygenase in chronic-iron-overloaded rats

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The hepatic porphyrias are inborn errors of porphyrin and haem biosynthesis characterized biochemically by excessive excretion of δ -aminolaevulinate (ALA), porphobilinogen and other intermediates in haem synthesis. Clinical evidence has implicated iron in the pathogenesis of several types of these genetically transmitted diseases. We investigated the role of iron in haem metabolism as well as its relationship to drug-mediated induction of ALA synthase and haem oxygenase in acute and chronic iron overload. Acute iron overload in rats resulted in a marked increase in hepatic haem oxygenase that was associated with a decrease in cytochrome *P*-450 and an increase in ALA synthase activity. Aminopyrine *N*-demethylase and aniline hydroxylase activities, which are dependent on the concentration of cytochrome *P*-450, were also decreased. In contrast, in chronic-iron-overloaded rats, there was an adaptive increase in haem oxygenase activity and an increase in ALA synthase that was associated with normal concentrations of microsomal haem and cytochrome *P*-450. The induction of ALA synthase in chronic iron overload was enhanced by phenobarbital and allyl-isopropylacetamide, in spite of the fact that these agents did not increase haem oxygenase activity. Small doses of Co^{2+} were potent inducers of the haem oxygenase in chronic-iron-overloaded, but not in control, animals. We conclude that increased hepatic cellular iron may predispose certain enzymes of haem synthesis to induction by exogenous agents and thereby affect drug-metabolizing enzyme activities.

Hepatic cellular haem production is controlled principally by the activity of ALA synthase [succinyl-CoA:glycine *C*-succinyltransferase (decarboxylating), EC 2.3.1.37], the proposed rate-limiting enzyme in the haem biosynthetic pathway (Granick & Urata, 1963). A wide range of diverse substances such as steroids, drugs, alcohol and iron can cause a large increase in the activity of this enzyme in the liver leading to porphyrin accumulation and excretion (Kappas *et al.*, 1968; Meyer & Schmid, 1978). This regulatory enzyme can be induced as much as 300-fold by a combination of various porphyrinogenic drugs such as 3,5-diethoxycarbonyl-1,4-dihydrocollidine and allyl-isopropylacetamide (Whiting & Granick, 1976). ALA synthase is also sensitive to both feedback inhibition and to repression by haem (Burnham & Lascelles, 1963; Watson *et al.*, 1978). Stein *et al.* (1972) reported that the unusually high urinary secretion of ALA and porphobilinogen in some acute-intermittent-porphyria patients may be due to

excessive concentration of iron in the liver. It has also been shown that the induction of hepatic ALA synthase by certain drugs that produce hepatic porphyria is augmented by oral administration of iron as ferric citrate (Stein *et al.*, 1970; Bonkowsky *et al.*, 1981). Iron is also implicated in the pathogenesis of excess porphyrin production in porphyria cutanea tarda and its removal produces clinical and biochemical improvement (Kushner *et al.*, 1975).

When iron is administered acutely to experimental animals, the concentrations of cytochrome *P*-450 and microsomal haem are greatly suppressed and this effect is considered to be the result of an increased haem oxygenase activity and/or an inhibition of uroporphyrinogen decarboxylase (Ibrahim *et al.*, 1979; Bonkowsky *et al.*, 1979).

Haem oxygenase [haem, hydrogen-donor: oxygen oxidoreductase (α -methene-oxidizing, hydroxylating), EC 1.14.99.3] is the rate-limiting enzyme in haem catabolism and catalyses the oxidative degradation of haem to biliverdin (Tenhunen *et al.*, 1969; Maines & Kappas, 1977; Drummond & Kappas, 1981); the latter was subsequently reduced

Abbreviation used: ALA, δ -aminolaevulinate.

in the cytosol to bilirubin by biliverdin reductase (Tenhunen *et al.*, 1970). The induction of this regulatory enzyme in various systems is further modulated by certain hormones, haem and a variety of metal ions such as cobalt and iron (Bakken *et al.*, 1972; Bissell & Hammaker, 1976). Stimulation of this enzyme activity in the liver is believed to be inversely related to the size of the regulatory haem pool by the increase in the destruction of the microsomal haem. The acute administration of iron is known to stimulate the induction of ALA synthase and haem oxygenase (Ibrahim *et al.*, 1979).

The present study was designed to assess whether induction of ALA synthase in chronic-iron-overloaded rats was further modulated by certain drugs known to play a role in the clinical expression of the hepatic porphyrias or if any effect was noted secondary to the induction of haem oxygenase and depletion of the regulatory haem pool, thus depressing ALA synthase. The present study confirms the existence of elevated ALA synthase activities in acutely and chronically iron-overloaded rats and that iron plays an important role in the regulation of haem metabolism. Furthermore, unlike the acute iron overload, chronic iron overload by itself changes neither hepatic microsomal haem nor the mono-oxygenase system that metabolizes various drugs. A preliminary report of some of this work has been presented previously (Ibrahim & Levere, 1979).

Materials and methods

Reagents

NADP⁺, NAD⁺, glucose 6-phosphate, cycloheximide, ALA, haemin and bovine serum albumin were purchased from Sigma. Glucose 6-phosphate dehydrogenase was obtained from Boehringer Biochemicals. Aminopyrine and aniline were purchased from Aldrich Chemical Co. Iron dextran (Imferon) was obtained from Lakeside Laboratories Inc., and Dextran 40 was obtained from Coulter Laboratories. Allylisopropylacetamide and phenobarbital were gifts from Hoffman-La Roche.

Male Sprague-Dawley rats (125–150 g) were given 12 intraperitoneal injections of iron dextran over a 4-month period at a total dose of 100 mg/100 g body wt. Simultaneously, control rats were injected with an equal volume of dextran. The animals were kept in groups of four and received standard laboratory diets and tap water *ad libitum* during the treatment period and for an additional 2 months after the last injection (chronic iron overload). In the experiments with acute iron overload, rats were injected with 50 mg of iron dextran/100 g body wt. every other day over a period of 6 days. Phenobarbital was dissolved in saline (0.9% NaCl) and each rat was injected intraperitoneally at a dose

of 12 mg/100 g body wt. CoCl₂ was injected subcutaneously in saline at a concentration of 0.8 mg/100 g body wt. Control animals received equivalent volumes of vehicle. No difference in enzyme activities was seen between vehicle-treated and uninjected rats. The animals were killed as described in the schedules described in the legends to the Figures and Tables. The rats were killed by decapitation and livers were perfused *in situ* with 0.9% NaCl until totally blanched. The livers were removed and homogenized in 5 vol. of 0.25 M-sucrose/0.05 M-Tris/HCl buffer, pH 7.4. Portions were taken for assays of ALA synthase and ALA dehydratase. The tissue homogenate was centrifuged at 27 000 g for 10 min. The remaining supernatant was then centrifuged at 105 000 g for 90 min to yield the microsomal pellet. The microsomal fraction was suspended in 0.1 M-potassium phosphate buffer, pH 7.4, and used for determination of haem content, cytochrome P-450, aminopyrine N-demethylase, aniline hydroxylase and haem oxygenase.

ALA synthase assay

The activity of mitochondrial ALA synthase was determined by the method of Poland & Glover (1973) as modified by Sassa *et al.* (1979). In this colorimetric assay, enzymically formed ALA is detected after its conversion into a pyrrole, which forms a coloured complex with modified Ehrlich's reagent (Granick *et al.*, 1972). The absorbance of the sample was scanned from 450 to 650 nm with a model 14 Cary spectrophotometer. The amount of ALA formed was calculated by using an absorption coefficient of $58 \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$ at 553 nm for the coloured complex.

Aminopyrine N-demethylase activity

This was measured in the liver microsomes by the method of Alvares & Mannering (1970). The following mixture was used: NADP⁺, 2 μmol ; semicarbazide hydrochloride, 37.5 μmol ; MgCl₂, 10 μmol ; glucose 6-phosphate dehydrogenase, 2 units; glucose 6-phosphate, 20 μmol ; nicotinamide, 20 μmol ; aminopyrine, 2 mmol, dissolved in 1.15% KCl; 0.2 mmol of K₂HPO₄ buffer, pH 7.4; 1 ml of microsomal suspension equivalent to 5 mg of protein; and 1.15% KCl solution to a final volume of 5 ml. The reaction was stopped by the addition of 0.25 ml of 25% ZnSO₄ and 1.25 ml of saturated Ba(OH)₂ as described by Orrenius (1965). Formaldehyde produced from the demethylation reaction was measured by the method of Nash (1953).

Aniline hydroxylase activity

This was measured by following the formation of p-aminophenol from aniline, by a modification of the colorimetric method of Brodie & Axelrod (1948)

and described by Imai *et al.* (1966). The reaction mixture contained 5 mM-aniline, 0.32 mM-NADP⁺, 3 mM-glucose 6-phosphate, 2.0 units of glucose 6-phosphate dehydrogenase, 5 mM-nicotinamide, 100 mM-K₂HPO₄ buffer, pH 7.4, and 4–6 mg of microsomal protein in a final volume of 1.0 ml. The reaction was carried out for 20 min at 37°C with moderate shaking and terminated by addition of 1 ml of 20% trichloroacetic acid. The mixture was transferred to a conical centrifuge tube and centrifuged at 600 g for 10 min. Of the supernatant 1.0 ml was taken and 0.5 ml of 10% Na₂CO₃ was added, followed by 1 ml of 2% phenol in 0.2 M-NaOH. The resulting blue colour was measured at 630 nm after being left for 30 min.

Cytochrome P-450 content

This was measured by bubbling carbon monoxide through the solution for 1 min; then the sample was split equally between the sample and reference cuvettes. The sample was reduced with a few grains of sodium dithionite. The absorbance difference between 450 and 490 nm was used to calculate the cytochrome P-450 content, using an absorption coefficient of 91 mM⁻¹·cm⁻¹. Haem content was measured as described by Falk (1964).

Haem oxygenase activity

This was measured in the microsomal fraction by the method of Tenhunen *et al.* (1970) as modified in our laboratory (Ibrahim & Levere, 1980). The volume of the assay medium was 1.0 ml and contained 105 000 g microsomal supernatant fraction (3.0 mg of protein) as a source of biliverdin reductase and 17 μmol of haemin. The reaction was terminated after 15 min by addition of 0.1 ml of 0.01 M-HCl. The mixture was extracted three times with 1 ml of chloroform to remove bilirubin. The chloroform fraction was then evaporated under N₂, O₂-free, to a final volume of 0.8 ml and the extract

was scanned in a Cary model 14 spectrophotometer. The concentration of bilirubin was calculated from the difference in absorption between 465 nm and 530 nm utilizing an absorption coefficient of 40 mM⁻¹·cm⁻¹. Differing conditions used for the assay of haem oxygenase are outlined in the legends to the appropriate Figures and the Tables. Protein concentrations were determined by the method of Lowry *et al.* (1951).

Results

Effect of chronic iron overload on hepatic ALA synthase, haem oxygenase, microsomal haem and drug-metabolizing enzymes

ALA synthase, the rate-limiting enzyme that is ordinarily the most important factor in determining the rate of haem synthesis, showed increased activity in chronic iron overload (140% of control). As seen in Table 1 and Fig. 1, the activity of hepatic ALA synthase observed in control rats was 19.67 ± 3.12 nmol of ALA formed/g of tissue per h compared with 28.64 ± 3.63 nmol of ALA formed/g of liver tissue per h in chronic iron overload (*P* < 0.05). The activity of hepatic haem oxygenase in control animals was found to be 306 ± 29 pmol of bilirubin formed/10 mg of protein per min. This enzyme activity was also increased in iron overload (155% of the control). The microsomal haem content in control animals was 1.48 ± 0.13 nmol of haem/mg of protein, which was not significantly different from the microsomal haem content seen in chronic iron overload.

As shown in Table 1, the mean value of cytochrome P-450 in experimental rats was 0.88 ± 0.09 nmol/mg of protein, which was not significantly different from that of controls. The effects of iron on the cytochrome P-450-dependent mono-oxygenase drug-metabolizing enzymes aminopyrine *N*-demethylase and aniline hydroxylase were

Table 1. *Effect of chronic iron overload on haem metabolism and cytochrome P-450 drug-metabolizing enzymes in hepatic microsomes*

Iron dextran was given intraperitoneally to eight rats in divided doses to a total of 100 mg of iron/100 g body wt. over a period of 4 months. Measurements were made 2 months after the last injection. Rats were starved for 24 h before killing. Livers were perfused with 0.9% NaCl before homogenization and preparation of microsomes. Results are means ± S.E.M. for six different experiments.

Rats	Haem oxygenase activity (pmol of bilirubin formed/10 mg of protein per min)	Cytochrome P-450 content (nmol/mg of protein)	Aminopyrine <i>N</i> -demethylase activity (nmol of formaldehyde/mg of protein per min)	Aniline hydroxylase activity (nmol of <i>p</i> -aminophenol/mg of protein per min)
Control	306 ± 29	0.86 ± 0.11	5.74 ± 1.10	27.65 ± 3.12
Iron overload	476.5 ± 37*	0.88 ± 0.09†	4.72 ± 1.33†	23.42 ± 2.44†

* Significantly differs from control (*P* < 0.05).

† Mean is not significantly different from control.

studied and activities were not different from controls.

Influencing of acute iron overload on haem metabolism and haem-dependent drug-metabolizing enzymes

Rats were injected three times every other day with iron dextran at a total dose of 50 mg/300 g body wt. (Bonkowsky *et al.*, 1979). At 2 days after the last injection, the activities of ALA synthase, haem oxygenase and mono-oxygenase and the concentrations of cellular haem and cytochrome *P*-450 were measured. As seen in Table 2, acute single injections resulted in approx. 3-fold increase in haem oxygenase activity. This is contrasted with a modest increase of haem oxygenase activity in chronic iron overload. Associated with the increase in haem oxygenase is a concomitant decrease in microsomal cytochrome *P*-450 content in acute iron-treated rats (to 62% of the control value; $P < 0.001$). On the other hand, ALA synthase activity was increased about 2-fold as a result of acute iron effect (results not shown). The mono-oxygenase activities in the iron-treated rats were also decreased in proportion to the microsomal content of cytochrome *P*-450. As seen in Table 2, aminopyrine *N*-demethylase was decreased to 67% of the control value ($P < 0.05$) and aniline hydroxylase activity was decreased to 45% of the control ($P < 0.05$). These results are consistent with those of other investigators, who have also shown that acute iron treatment results in a decrease of cellular cytochrome *P*-450 and microsomal haem (Ibrahim *et al.*, 1979; Bonkowsky *et al.*, 1979).

Effect of allylisopropylacetamide, phenobarbital and cobalt on hepatic ALA synthase and microsomal haem content in chronically iron-overloaded rats

As shown in Fig. 1(a), the administration of allylisopropylacetamide in control rats resulted in a

43% decrease in microsomal haem and a concomitant 2.1-fold increase in ALA synthase. Phenobarbital increased microsomal haem by 90%, yet ALA synthase activity was increased by 81% above control values. Cobalt administration resulted in a 35% decrease in microsomal haem and yet caused

Fig. 1. Modifying effects of iron overload on phenobarbital (PB), allylisopropylacetamide (AIA) and CoCl_2 -induced (Co) alteration of hepatic ALA synthase and microsomal haem

The control animals and chronically iron-overloaded rats were injected intraperitoneally with phenobarbital (10 mg), allylisopropylacetamide (12 mg) and CoCl_2 (0.8 mg/100 g body wt.) subcutaneously for a period of 16 h. The control animals received saline treatment. ALA synthase (open columns) and microsomal haem (filled columns) were measured as described in the Materials and methods section. Each column represents the mean $\pm$ S.E.M. (indicated by the bar) of six different experiments. (a) Control; (b) chronic iron overload.

Table 2. Effect of acute iron overload on haem metabolism and cytochrome *P*-450 drug-metabolizing enzymes in hepatic microsomes

Six rats were injected three times every other day with iron dextran at a dose of 50 mg/300 g body wt. At 2 days after the last injection, activities of ALA synthase, haem oxygenase, aminopyrine *N*-demethylase and aniline hydroxylase and microsomal haem and cytochrome *P*-450 contents were measured. Controls were injected simultaneously with dextran 40.

Rats	Haem oxygenase activity (pmol of bilirubin formed/10 mg of protein per min)	Microsomal haem content (nmol content/mg of protein)	Cytochrome <i>P</i> -450 content (nmol/mg of protein)	Aminopyrine <i>N</i> -demethylase activity (nmol of formaldehyde/mg of protein per min)	Aniline hydroxylase activity (nmol of <i>p</i> -phenol/mg of protein per min)
Control	286 $\pm$ 36	1.41 $\pm$ 0.14	0.86 $\pm$ 0.11	6.11 $\pm$ 1.22	26.54 $\pm$ 3.34
Iron overload	851 $\pm$ 59*	0.79 $\pm$ 0.12*	0.53 $\pm$ 0.09†	4.14 $\pm$ 1.41†	11.93 $\pm$ 2.63†

* Value significantly different from control ($P < 0.001$).

† Value significantly different from control ($P < 0.05$).

little change in ALA synthase activity. In chronically iron-overloaded rats, as shown in Fig. 1(b), administration of allylisopropylacetamide caused a marked induction of ALA synthase activity (to 71 ± 5.6 nmol of ALA formed/g of liver per h). Phenobarbital-treated iron-overloaded animals had a greater increase in ALA synthase activity than phenobarbital-treated controls, without a corresponding increase in microsomal haem. The failure to increase microsomal haem despite the marked increase in ALA synthase is noteworthy. The microsomal haem was depleted to 64% of the control value in cobalt-treated rats with no significant change in ALA synthase. The combined action of cobalt and cobalt–protoporphyrin (Sinclair *et al.*, 1978; Igarashi *et al.*, 1978) on ALA synthase may prevent an increase in enzyme activity in spite of a decreased cellular haem.

Effect of phenobarbital, allylisopropylacetamide and cobalt on hepatic microsomal haem oxygenase and cytochrome P-450 in chronically iron-overloaded rats

In a series of experiments we tested the effect of phenobarbital, allylisopropylacetamide and cobalt on microsomal haem oxygenase activity and cytochrome P-450 concentration. As shown in Fig. 2, the administration of allylisopropylacetamide resulted in a 45% decrease in cytochrome P-450 in the control and 55% decrease in iron-treated rats. The further decrease in cytochrome P-450 observed with the injection of allylisopropylacetamide was not associated, however, with a greater induction of haem oxygenase. The administration of phenobarbital significantly increased cytochrome P-450 in

both normal and chronically iron-overloaded animals. But the increase in microsomal cytochrome P-450 content in chronic-iron-overloaded rats was not as pronounced as in control rats. However, microsomal haem oxygenase activity was not significantly different in chronic-iron-overloaded rats from the control as a result of phenobarbital administration. In comparison, cobalt administration resulted in a 70% increase in the haem oxygenase activity over that of iron-overloaded controls, and a

Fig. 2. Liver cytochrome P-450 and haem oxygenase were measured after treatment with various agents as indicated in Fig. 1

Various assays were conducted as described in the text. Open columns show cytochrome P-450 content and filled columns show haem oxygenase activity. Each column represents the mean $\pm$ S.E.M. (indicated by the bar) of six different experiments. (a) Control; (b) chronic iron overload. Abbreviations used: PB, phenobarbital; AIA, allylisopropylacetamide; Co, CoCl_2 .

Table 3. Effect of allylisopropylacetamide, phenobarbital and Co^{2+} on aminopyrine N-demethylase and aniline hydroxylase activities in control and iron-overloaded rats

Microsomal fractions (8–10 mg of protein/ml) from the control and treated male Sprague–Dawley rats were prepared. Aminopyrine N-demethylase and aniline hydroxylase activity were measured as described in the Materials and methods section. Results are means $\pm$ S.E.M. for six rats.

Conditions	Aminopyrine N-demethylase activity (nmol of formaldehyde liberated/mg of protein per min)	Aniline hydroxylase activity (nmol of p-aminophenol/mg of protein per min)
Control	6.83 ± 0.18	45.63 ± 2.32
+ Allylisopropylacetamide	$4.34 \pm 0.16^*$	$27.84 \pm 2.44^*$
+ Phenobarbital	12.93 ± 0.38	88.23 ± 4.13
+ Co^{2+}	$4.14 \pm 0.18^*$	$30.12 \pm 3.14^*$
Iron overload	6.29 ± 0.17	38.91 ± 2.82
+ Allylisopropylacetamide	$4.61 \pm 0.19^*$	$25.38 \pm 3.04^*$
+ Phenobarbital	10.31 ± 0.41	66.92 ± 4.83
+ Co^{2+}	$3.18 \pm 0.16^*$	$23.59 \pm 2.58^*$

* Significantly different from the respective control value ($P < 0.05$).

65% decrease in cytochrome *P*-450 in chronically iron-overloaded compared with overloaded controls, and a 35% decrease in cytochrome *P*-450 from that of normal controls.

Effect of allylisopropylacetamide, phenobarbital and cobalt on aminopyrine N-demethylase and aniline hydroxylase in chronically iron-overloaded rats

As seen in Table 3, aminopyrine *N*-demethylase and aniline hydroxylase activities were decreased by about 35% by administration of allylisopropylacetamide in both the control and in chronically iron-overloaded rats. Phenobarbital increased aminopyrine *N*-demethylase about 2-fold; aniline hydroxylase activity was increased 60% above the control. Significant decreases of aminopyrine *N*-demethylase and aniline hydroxylase activities were observed as a result of Co²⁺ treatment in chronically iron-overloaded rats.

Discussion

Although the precise mechanism of the role of iron in porphyrin and haem metabolism is not understood, it appears that both acute and chronic hepatic iron accumulation results in increased ALA synthase activity. In acute iron overload these studies confirm that there is increased ALA synthase activity concurrent with a decrease in microsomal haem content (Bonkowsky *et al.*, 1979; Ibrahim *et al.*, 1979) and an increase in haem oxygenase (Ibrahim *et al.*, 1979). Even though the prolonged increase in haem oxygenase may account for the decrease in the regulatory haem pool, other factors may affect microsomal haem by other mechanisms. (a) Iron may inhibit uroporphyrinogen decarboxylase and/or uroporphyrinogen III co-synthase (Kushner *et al.*, 1975; Bonkowsky *et al.*, 1981), as has been suggested in the pathogenesis of porphyria cutanea tarda, thus causing a decrease in regulatory haem; (b) iron may directly increase ALA synthase activity by a mechanism similar to the observed increase in the synthesis of the apoferritin in the presence of iron (Zaehring *et al.*, 1975); (c) iron may directly act as an inducer of haem oxygenase. As the haem oxygenase activity increases, the intracellular haem is depleted and ALA synthase is then induced by the release of haem feedback repression (Burnham & Lascelles, 1963; Sassa & Granick, 1970).

The study of the acute effects of iron on haem metabolism was also extended to cytochrome *P*-450 and the mono-oxygenase activities, which catalyse the metabolism of certain endogenous and exogenous environmental agents (Conney, 1967). The activities of aminopyrine *N*-demethylase and aniline hydroxylase were both decreased as a result of a lower cellular cytochrome *P*-450.

To gain further insight into the role of iron on haem metabolism, we studied the effect of iron on the liver over a long period of time (6 months). The results presented on chronically iron-overloaded rats indicate that, although there is an increase in haem oxygenase activity, there is an apparent adaptive increase in ALA synthase that results in the maintenance of a normal concentration of microsomal haem. The fact that hepatic ALA synthase in chronically iron-overloaded rats is considerably higher than in normal liver cells may not be completely explained simply by release of feedback repression of ALA by the depletion of the regulatory haem pool from the increased haem oxygenase. As in acute iron overload, there may be a partial inhibition of the haem pathway by iron at a point beyond ALA synthase. These results are consistent with the previous findings in human subjects demonstrating that the activity of ALA synthase was increased in the liver in porphyria cutanea tarda (Dowdle *et al.*, 1967), and returned to a normal value after treatment by phlebotomy. Brodie *et al.* (1979) have recently reported an increase in leucocyte ALA synthase activity in porphyria cutanea tarda patients. In such patients, there is a reduction in uroporphyrinogen decarboxylase in the liver, which has been confirmed by others (deVerneuil *et al.*, 1978). Our results are also compatible with those previously reported in patients with acute intermittent porphyria in that there is no evidence of a decrease in hepatic cytochrome *P*-450 (Pimston *et al.*, 1973). Furthermore, the present study demonstrates that the activities of aminopyrine *N*-demethylase and aniline hydroxylase, which are dependent on an adequate supply of microsomal cytochrome *P*-450, are not significantly different from normal in chronically iron-overloaded liver cells.

Because of the known effect of many exogenous agents on hepatic porphyria we studied the effect of phenobarbital, allylisopropylacetamide and cobalt on haem metabolism and the mono-oxygenase system in control and chronically iron-overloaded animals. The administration of allylisopropylacetamide to normal and iron-overloaded animals caused a decrease in microsomal haem and an associated significant increase in ALA synthase activity. The observed increase in ALA synthase in allylisopropylacetamide-treated animals was not associated with induction of haem oxygenase. Although the administration of phenobarbital in iron-overloaded animals resulted in a greater increase in ALA synthase compared with phenobarbital control rats, there was a smaller increase in cytochrome *P*-450. These phenobarbital- and allylisopropylacetamide-induced changes in haem synthesis were not associated with increases in haem oxygenase over controls. The mechanism by which

iron overload increases ALA synthase activity and impairs the increase in cytochrome *P*-450 observed in rats treated with phenobarbital is not understood. It is possible that the maintenance of the high activity of haem oxygenase in iron-overloaded rats may enhance the sensitivity of ALA synthase to an as yet unidentified inducer by a continuous depletion of the regulatory haem pool. Alternatively, an iron-dependent partial block of a sensitive enzyme in the haem enzymic pathway beyond ALA synthase may be present. Such a sensitive enzyme may require a combination of endogenous (steroid) or exogenous (allylisopropylacetamide and phenobarbital) environmental factors to manifest the sensitivity to iron. The mono-oxygenase activities were further increased by phenobarbital in both groups of animals, although to a lesser extent in chronically iron-overloaded rats. These results indicate that there was no major impairment of the inducibility of the oxidative drug metabolism in chronically iron-overloaded rats treated with phenobarbital. In contrast, the administration of small doses of cobalt, which affects the rate of haem synthesis in rodents (Sinclair *et al.*, 1978; Tephly *et al.*, 1978), resulted in marked elevation of haem oxygenase in iron-treated rats and depletion of the microsomal haem. In these animals, the mono-oxygenase activities were significantly decreased. The action of cobalt on haem synthetic and degradative enzymes and in turn total microsomal haem in iron-treated rats are very difficult to explain. One possibility is that iron may increase formation of cobalt protoporphyrin and result in inhibition of ALA synthase activity (Woods *et al.*, 1977; Sinclair *et al.*, 1978). Alternatively, iron may sensitize haem oxygenase induction by combination of cobalt and cobalt protoporphyrin with concomitant inhibition of ALA synthase.

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