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Iron and the Liver*

Acute and long-term effects of iron-loading on hepatic haem metabolism

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We have determined the dose–response curves (100–900mg of Fe/kg body wt.) and the time course over 84 days for the effects of a single injection of iron–dextran on rat hepatic 5-aminolaevulinate synthetase, cytochrome *P*-450, iron content, and GSH (reduced glutathione). Porphyrins in liver and urine have also been measured. (1) At 2 days after treatment, a dose of 500mg of Fe/kg produced a 20-fold increase in iron concentration, which was maintained for 14days. Total hepatic iron remained constant over 63 days, falling slightly by 84 days. (2) The activity of 5-aminolaevulinate synthetase was maximally increased (6-fold) 12–24h after iron treatment. By 48h the activity fell to less than twice the control value and and thereafter remained slightly above the control value (1.1–1.5-fold) until 84 days after iron treatment. Liver GSH concentrations were unaffected by iron. Porphyrins in liver and urine were either unchanged or decreased. (3) Hepatic cytochrome *P*-450 decreased after iron treatment to a minimum (63% of control) at 48h after iron administration and gradually returned to the control value by 28 days. (4) Iron–dextran potentiated 2-allyl-2-isopropylacetamide-induced synthesis of hepatic 5-aminolaevulinate. Potentiation occurred if the drug was given at the same time or 36h after iron administration, but did not occur if the drug was given 14 or 64 days after iron administration. (5) The results are discussed in relation to proposed mechanisms for the effects of iron on hepatic haem metabolism.

Iron overload affects hepatic haem metabolism. Examples of such effects of iron occur in the human disease porphyria cutanea tarda and the experimental porphyria produced by polyhalogenated aromatic hydrocarbons (Meyer & Schmid, 1978). Porphyria cutanea tarda usually occurs in persons with hepatic siderosis; the disease is regularly ameliorated by iron removal and may be exacerbated by readministration of iron. Similarly, iron-loading decreases the time of onset of toxic porphyria in rats induced by hexachlorobenzene, and increases the severity of the porphyria (Taljaard *et al.*, 1972; Louw *et al.*, 1977).

The biochemical mechanisms whereby iron overload affects hepatic haem metabolism are not completely understood. Enteral ferric citrate potentiates induction by 2-allyl-2-isopropylacetamide of 5-aminolaevulinate synthetase, the rate-controlling enzyme of hepatic haem synthesis (Stein *et al.*, 1970). More recently, we showed that ferric citrate increases the turnover of hepatic haem (Bonkowsky *et al.*, 1980*a*). Acute administration of iron–dextran, like ferric citrate, increases turnover of hepatic haem (De Matteis & Sparks, 1973; Ibrahim *et al.*, 1979). In several studies (Hanstein *et al.*, 1975; Ibrahim *et al.*, 1979; Maines, 1979), including our own (Bonkowsky *et al.*, 1979*a*), acute as against long-term effects of iron administration on haem metabolism have not been clearly distinguished.

In the present study we have compared acute (1–48h) and long-term (4–84 days) effects of a single intraperitoneal injection of iron–dextran on various parameters of hepatic haem metabolism. We have also studied the effect of varying the period between iron and 2-allyl-2-isopropylacetamide treatments on the potentiated induction of 5-

Abbreviation used: GSH, reduced glutathione.

* This is the second of a series of papers on this topic; paper I is Bonkowsky *et al.* (1979*a*).

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aminolaevulinate synthase. Our results indicate that the effect of iron on haem metabolism depends not only on the hepatic concentration of iron, but also on the time it has been within the liver. Portions of the present work have appeared in abstract form (Bonkowsky *et al.*, 1980*b*,*c*).

Methods and materials

Rat treatments

Male Sprague–Dawley rats from Charles River Laboratories (Cambridge, MA, U.S.A.) were maintained as previously described (Bonkowsky *et al.*, 1979*a*) and used when they had reached weights of 100–150g. For the dose–response study (Fig. 2 below) rats were starved for 24h before they were killed. For the other experiments, when rats were killed 24h or less after iron–dextran treatment, they were starved a total of 24h before they were killed. Otherwise rats were starved 24h before iron injection and 24h before they were killed. Water was allowed *ad lib* during starvation periods.

Iron–dextran (50mg of Fe/ml; Fisons Ltd., Holmes Chapel, Crewe, Cheshire, U.K.) was given intraperitoneally; controls received an appropriate volume of dextran-5 (Fisons Ltd.) (200mg/ml). Iron–dextran and dextran-5 were tolerated well; even at the largest dose used, rats showed no apparent ill effects and none died from the treatment. Solutions of 2-allyl-2-isopropylacetamide (15mg/ml) were prepared in 0.15M-NaCl. 2-Allyl-2-isopropylacetamide was given subcutaneously in a dose of 400mg/kg body wt. Control rats received appropriate volumes of solvents only. Details of treatment schedules are given in the Results section. For 24h urine collections, rats were placed into stainless-steel metabolic cages and given access to water but no food. Urine samples were collected into glass containers, which were covered with glass beads. Rats were killed by decapitation, their livers rapidly excised, rinsed in cold 0.15M-NaCl, blotted, weighed, and appropriate portions were taken for assays.

Assay procedures

The activity of 5-aminolaevulinate synthetase was determined with homogenates of freshly prepared liver by using the method of Abbritti & De Matteis (1971–1972). Concentrations of hepatic cytochrome *P*-450 were usually determined with 0.25ml portions of 20% (w/v) homogenates of whole liver by using the method of Estabrook *et al.* (1972), in which NADH (0.4mM final concn.) is added to homogenate, to reduce cytochrome b_5, the homogenate bubbled with CO, and a baseline established. The sample cuvette is then reduced with $Na_2S_2O_4$ and the reduced-minus-CO versus CO difference spectrum of cytochrome *P*-450 is recorded ($\Delta\varepsilon^{mM}_{450-510} = 100\,mM^{-1}\cdot cm^{-1}$). Homogenates could be frozen (−60°C) for at least 1 month without loss of cytochrome *P*-450. The patterns of results obtained with this method in treated as against control rats are the same as those obtained for isolated microsomal fractions by the method of Omura & Sato (1964). Hepatic concentrations of GSH were determined fluorimetrically, after reaction with *o*-phthalaldehyde (Hissin & Hilf, 1976). Liver pieces or homogenates (20%, w/v in 0.25M-sucrose/20mM-Tris/HCl/0.1mM-menadione, pH7.4) can be frozen (−60°C) for at least 1 month without change in GSH concentrations. Liver iron concentrations were determined spectrophotometrically by allowing iron to form a complex with bathophenanthroline, after wet-ashing 2–20mg of wet liver (Barry & Sherlock, 1971).

For determination of hepatic porphobilinogen, homogenates (as described above) were used. These may be frozen (−60°C) for at least a fortnight with no change in porphobilinogen content. To 2.0ml of homogenate in a 12mm × 75mm tube was added 0.2ml of 30% (w/v) $HClO_4$ and the homogenate vortex-mixed and centrifuged (1700*g*, 10min). A portion of the supernatant was mixed with an equal volume of modified Ehrlich's reagent (Mauzerall & Granick, 1956), left at room temperature for 15min, and the spectrum was scanned (500–650nm). The reference cuvette contained supernatant obtained from homogenate for a control rat that had not been induced for 5-aminolaevulinate synthetase. For calculation of porphobilinogen concentration, $\Delta\varepsilon^{mM}_{556-650} = 61\,mM^{-1}\cdot cm^{-1}$.

Liver porphyrin concentrations were determined by extracting 0.2ml of 20% (w/v) whole liver homogenate, prepared as above, with 2.3ml of 1M-$HClO_4$/methanol (1:1, v/v) (Granick *et al.*, 1975). Total porphyrin concentrations in urine were also determined spectrofluorimetrically by using the method of Schwartz *et al.* (1976); coproporphyrin served as a standard.

Proteins were determined by the method of Lowry *et al.* (1951).

Histological procedures

Portions of liver were fixed in formol/NaCl, processed routinely, and stained with Haematoxylin/Eosin and Masson's Trichrome and Prussian Blue (Lillie, 1965).

Statistical procedures

Student's two-tailed *t*-test was used to compare differences among mean values: $P < 0.05$ was considered significant.

Results

Acute effects of a single dose of iron–dextran (Figs. 1 and 2)

Fig. 1 shows that iron from a single intra-

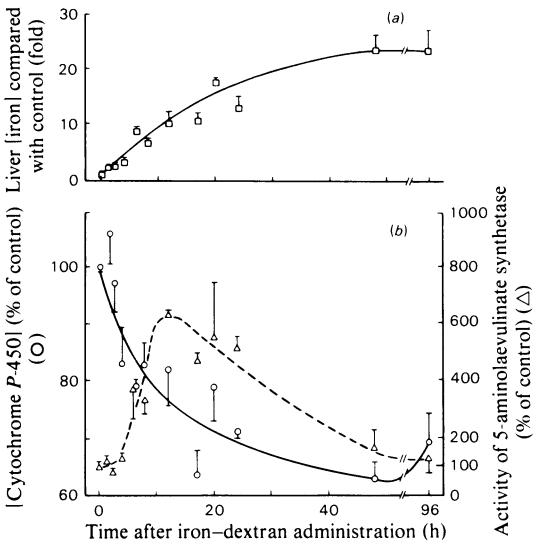

Fig. 1. *Early effects of iron-loading on (a) hepatic iron concentrations* (□) *and (b) hepatic cytochrome P-450 concentration* (○) *and 5-aminolaevulinate synthetase activity* (△)

A single intraperitoneal injection of iron–dextran (500mg of Fe/kg body wt.) was given, and rats in groups of four to seven animals were killed at various times thereafter as shown. Each data point represents the mean ± 1 S.E.M. compared with that for three to five control rats, which received an appropriate dose of dextran-5. Mean values for all controls (n = 25) were: liver iron concentration = 354 ± 97ng of Fe/mg dry wt. (range 240–550ng/mg); cytochrome P-450 = 191 ± 37pmol/mg of homogenate protein (range 129–261pmol/mg); 5-aminolaevulinate synthetase = 143 ± 45pmol of 5-aminolaevulinate formed/h per mg of protein (range 85–221pmol/h per mg).

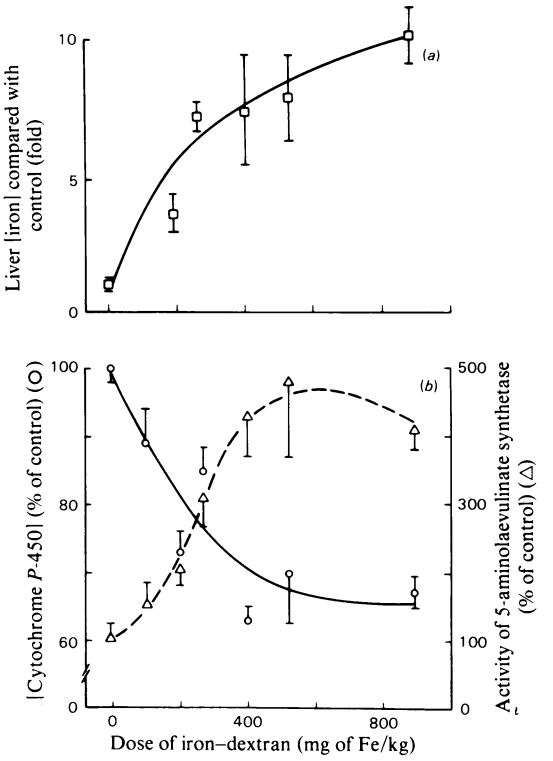

Fig. 2. *Dose–response curve for effect of iron on hepatic iron concentration* (□) *(a), and cytochrome P-450 concentration* (○) *and 5-aminolaevulinate synthetase activity* (△) *(b)*

A single intraperitoneal injection of iron–dextran was given and rats were killed 16h later. For each dose the result is the mean ± S.E.M. for four to seven livers, compared with three to five controls (controls received an appropriate dose of dextran-5). Absolute values for controls are given in the legend to Fig. 1.

peritoneal injection of iron–dextran (500mg of Fe/kg) entered the liver rapidly, the concentration of iron in the liver reaching a plateau (20–25-fold of control) by 48h after injection. Histological examination of liver obtained at 48h revealed marked deposition of Prussian Blue-positive granules throughout the liver, both in hepatocytes and Kupffer cells. No cell necrosis or inflammation was seen, although hepatocytes showed moderate vacuolization and ballooning. Deposition of iron in the liver was accompanied by a decrease in the concentration of cytochrome P-450 and an increase in 5-aminolaevulinate synthetase activity (Fig. 1*b*).

Other workers have reported a transient decrease in hepatic 5-aminolaevulinate synthetase activity, occurring within the first 4h after iron treatment (Ibrahim *et al.*, 1979). However, as shown in Fig. 1(*b*), we found no evidence for any significant decrease. At 1 and 2h after iron administration, 5-aminolaevulinate synthetase activity was the same as the control; by 4h it was already significantly (P < 0.03) above control. Maximal activities of the synthetase occurred 12–24h after iron treatment, but decreased to near control values by 48h, despite persistently decreased concentrations of cytochrome P-450 (Fig. 1*b*).

Fig. 2 shows the effect at 16h of increasing doses of iron–dextran on concentrations of liver iron (Fig. 2*a*) and on cytochrome P-450 and activities of 5-aminolaevulinate synthetase (Fig. 2*b*). Note the reciprocal relationship between the decrease in cytochrome P-450 and the increase in 5-aminolaevulinate synthetase at this time after injection of iron. These changes in both enzyme activities were not due to interference by iron with the assays used, since appropriate concentrations of iron added *in*

Table 1. *Rat weights and liver wet weights during long-term iron loading*
Rats were treated as described in the legend to Fig. 3.

Time after administration of iron–dextran or dextran (days) ...	Weight (g) (mean ± S.E.M.) 2	7	14	28	63	84
Rat						
Control	127 ± 4	131 ± 5	187 ± 3	282 ± 14	474 ± 25	512 ± 12
Iron-loaded	129 ± 5	134 ± 6	184 ± 9	298 ± 6	459 ± 15	470 ± 9*
Liver wet weight						
Control	4.9 ± 0.18	5.1 ± 0.30	7.0 ± 0.11	9.5 ± 0.40	12.2 ± 0.66	14.6 ± 0.39
Iron-loaded	5.1 ± 0.16	6.5 ± 0.31*	8.2 ± 0.45	11.5 ± 0.42*	14.0 ± 0.67	15.4 ± 0.62

* Iron-loaded significantly different from control ($P < 0.05$).

vitro (as ferritin, iron–dextran, or $FeSO_4$) did not influence the results obtained for homogenates from control rats.

Liver porphyrin concentrations were unaffected by iron treatment during the first 48h after treatment. Similarly, urinary excretion of porphyrin (measured on urine samples collected 0–24 and 24–48h after iron treatment) were unchanged, compared with samples collected just before treatment (results not shown).

It has been proposed that treatment with iron and some other metals affects hepatic concentrations of GSH (Maines & Kappas, 1976, 1977). We measured concentrations of GSH in liver 1, 2, 6, 20 and 48h after administration of iron–dextran and found that GSH concentrations were unchanged, in agreement with Maines (1979).

Long-term effects of iron–dextran

Rats treated with iron–dextran (500mg of Fe/kg) gained weight as rapidly as controls during the first month after treatment, but weighed slightly less at 84 days. Total liver weights of iron-loaded animals were the same as, or greater than, the control values throughout the experiment (Table 1).

Fig. 3(*a*) shows that the high iron concentrations reached by 2 days (Fig. 1) were maintained for 14 days. The concentration then fell because the liver weight increased. Total liver iron content was constant from day 14 to day 63, falling slightly by day 84. The decrease in liver iron concentration was confirmed microscopically; the intensity of Prussian Blue staining diminished in both Kupfer cells and (especially) hepatocytes. Inflammation, fibrosis and necrosis were not observed microscopically, and hepatocyte vacuolization was gone by 7 days after iron injection.

Between 2 and 14 days after treatment with iron–dextran, amounts of cytochrome *P*-450 returned towards control values (Fig. 3*b*). Note that

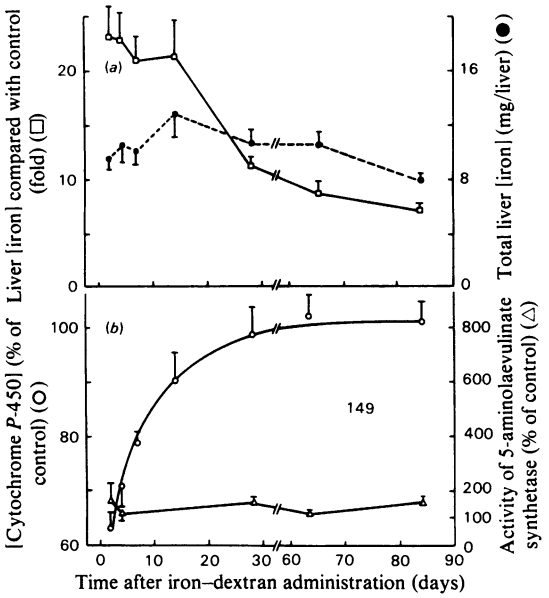

Fig. 3. *Late effects of iron-loading on total liver iron content* (●) *and iron concentrations* (□) (*a*) *and hepatic cytochrome P-450 concentration* (○) *and 5-aminolaevulinate synthetase activity* (△) (*b*)
Rats were killed 2–84 days after iron–dextran treatment. Each data point represents the mean ± S.E.M. for seven iron-loaded rats compared with a group of four controls killed at the same time. The range for control values is as given in the legend to Fig. 1. At 28 and 84 days after iron–dextran administration, the activity of 5-aminolaevulinate synthetase in iron-loaded livers (152 ± 18 and 149 ± 17) was significantly higher than control ($P < 0.025$ and $P < 0.05$ respectively). At 63 days, the activity in iron-loaded livers (111 ± 9) was also higher than control, but the difference did not reach statistical significance ($P < 0.2$). Units of 5-aminolaevulinate synthetase activity are in pmol of 5-aminolaevulinate formed/h per mg of protein.

liver iron concentration remained constant during this period (Fig. 3*a*). Activity of 5-aminolaevulinate synthetase remained slightly elevated (110–150% of control values) throughout the long-term study (days 2–84; Fig. 3*b*), confirming previous results (Ibrahim *et al.*, 1979).

The concentration of hepatic GSH, measured at 28 and 84 days, as well as 24h urinary excretions of porphyrins, measured at 7, 14, 28 and 63 days, were the same in control and iron-treated animals (results not shown). Total hepatic porphyrin concentrations were slightly, but significantly, decreased 14 and 63 days after iron treatment (at 63 days, control = 1.6 ± 0.05; iron-treated = 1.2 ± 0.07 nmol of porphyrin/g of wet liver). In both control and treated rats, liver porphyrin was predominantly (≥80%) protoporphyrin.

Potentiation of induction of 5-aminolaevulinate synthetase by iron–dextran and 2-allyl-2-isopropylacetamide

Iron, administered as ferric citrate by stomach tube, has previously been shown to potentiate the induction of 5-aminolaevulinate synthetase caused by porphyrogenic chemicals such as 2-allyl-2-isopropylacetamide (Stein *et al.*, 1970; Bonkowsky *et al.*, 1979*b*). As Fig. 4 shows, administration of iron as iron–dextran, given intraperitoneally, also potentiated the induction of 5-aminolaevulinate synthetase produced by 2-allyl-2-isopropylacetamide. The potentiation was maximal when iron–dextran was administered 36h before treatment with 2-allyl-2-isopropylacetamide. Note that the iron concentration increased from 0.5 to 2 days, remained at that value for at least 14 days, then fell during the 14–63 day period (Figs. 1*a* and 3*a*). Iron recently (≤2 days) deposited in the liver potentiates induction of 5-aminolaevulinate synthetase, whereas the same concentration of iron present for 2 weeks or more has less effect. Indeed, at 2 weeks after iron-loading, induction of 5-aminolaevulinate synthetase was not significantly greater than that produced by 2-allyl-2-isopropylacetamide alone ($0.05 < P < 0.07$). This suggests that the potentiation is not related simply to the concentration of hepatic iron, but also to the length of time that the iron has been in the liver.

The findings summarized in Fig. 5 indicate that the marked potentiation of induction of 5-aminolaevulinate synthetase by iron and 2-allyl-2-isopropylacetamide is reflected by hepatic accumulation of porphobilinogen. With the method used, porphobilinogen can be detected in the liver

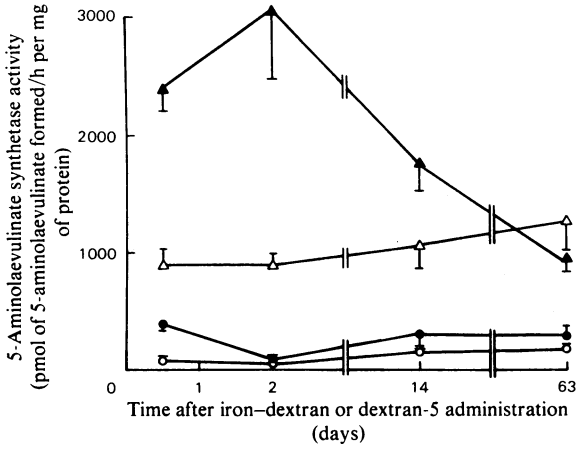

Fig. 4. *Effect of interval between administration of iron–dextran and 2-allyl-2-isopropylacetamide on the induction of 5-aminolaevulinate synthetase*

Rats in groups of three to six animals were given an intraperitoneal injection of iron–dextran or dextran-5 (controls) at zero time. At 12h before being killed, some rats were given either a subcutaneous injection of 2-allyl-2-isopropylacetamide (400mg/kg body wt.) or an appropriate volume of physiological saline (0.9% NaCl). Results are means ± S.E.M. ○, dextran-5 and saline; ●, iron–dextran and saline; △, dextran-5 and 2-allyl-2-isopropylacetamide; ▲, iron–dextran and 2-allyl-2-isopropylacetamide.

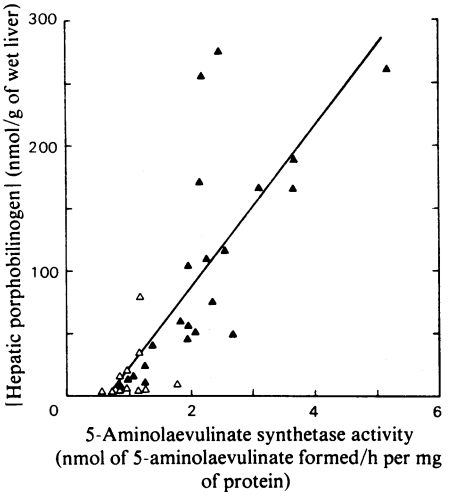

Fig. 5. *Correlation of liver porphobilinogen with 5-aminolaevulinate synthetase*

Concentrations of liver porphobilinogen were determined by using all liver homogenates from rats (shown in Fig. 4) treated with 2-allyl-2-isopropylacetamide. Each point represents a single homogenate. The line is the best fit obtained by linear-regression analysis of all the points ($y = 0.064x - 42$, $r = 0.79$). △, Treated with 2-allyl-2-isopropylacetamide; ▲, treated with iron–dextran and 2-allyl-2-isopropylacetamide.

only when 5-aminolaevulinate synthetase activity is increased 10-fold or more above control. Rats treated with only 2-allyl-2-isopropylacetamide usually had undetectable or barely detectable hepatic concentrations of porphobilinogen, whereas many rats treated with iron–dextran together with, or 36h before, 2-allyl-2-isopropylacetamide, had higher concentrations of this monopyrrole. As shown, there was a linear relationship between activity of hepatic 5-aminolaevulinate synthetase and concentration of porphobilinogen (Fig. 5). These results are not due to inhibition of porphobilinogen deaminase, since activity of this enzyme is unchanged by iron–dextran administration (Maines, 1979).

Discussion

The present studies show, both histochemically and quantitatively, that hepatic iron-loading (20–25-fold above control values) is achieved in rats after a single intraperitoneal injection of iron–dextran. The maximal iron concentrations after injection of 500mg of Fe/kg body wt. are similar to those seen in haemochromatosis (Pollycove, 1978). This dose of iron–dextran affects hepatic haem and haemoprotein metabolism, increasing activity of 5-aminolaevulinate synthetase, the first and normally rate-limiting step of haem synthesis and decreasing amounts of cytochrome *P*-450, a major hepatic haemoprotein (Meyer & Schmid, 1978; Bonkowsky *et al.*, 1979*b*) (Figs. 1 and 2). An increase in activity of hepatic 5-aminolaevulinate synthetase after iron–dextran injection confirms results of others (De Matteis & Sparks, 1973; Ibrahim *et al.*, 1979). In addition, the present results show that iron–dextran itself not only induces 5-aminolaevulinate synthetase, but also potentiates the induction of this enzyme produced by the porphyrogenic chemical 2-allyl-2-isopropylacetamide (Figs. 4 and 5).

The detailed time courses (Figs. 1, 3 and 4) show that the dual effects of iron–dextran on 5-aminolaevulinate synthetase and cytochrome *P*-450 are complex. For example, although the dose–response curve at 16h (Fig. 2) suggests a reciprocal relationship between induction of 5-aminolaevulinate synthetase and decrease in cytochrome *P*-450 after iron loading, the time course (Figs. 1 and 3) shows clearly that peak induction of the synthetase activity is transient (12–24h) compared with the decrease in cytochrome *P*-450, which persists for 1 week. Thereafter the amount of cytochrome *P*-450 gradually increases, reaching control concentrations 3–4 weeks after iron treatment. During the time that cytochrome *P*-450 was increasing back to control values and the activity of 5-aminolaevulinate synthetase was only slightly above control (110–150%) whereas both the concentrations and content of liver iron were constant (Fig. 3). In agreement with previous results in man (Bonkowsky & Pomeroy, 1977; Bonkowsky *et al.*, 1979*b*), effects of long-term hepatic iron overload in rats include a slight but persistent induction of 5-aminolaevulinate synthetase activity (Fig. 1). The effects of iron–dextran on 5-aminolaevulinate synthetase and cytochrome *P*-450 are not related simply to the hepatic content or concentration of iron. Similarly, the potentiation by iron–dextran of induction of 5-aminolaevulinate synthetase produced by 2-allyl-2-isopropylacetamide was not only related to the hepatic concentration of iron but also to the time elapsed after iron injection (Figs. 1, 3 and 4). The potentiation was greatest when iron was given 12 or 36h before 2-allyl-2-isopropylacetamide; it decreased to less than significant values when iron was given 14 days before, and it did not occur at all when iron was given 63 days before 2-allyl-2-isopropylacetamide (Fig. 4).

In previous studies it has been shown that a single dose of ferric citrate given enterally does not, by itself, induce 5-aminolaevulinate synthetase (Stein *et al.*, 1970; Bonkowsky *et al.*, 1979*b*). Nevertheless, ferric citrate enhanced hepatic haem turnover (Bonkowsky *et al.*, 1980*a*) and potentiated the induction of 5-aminolaevulinate synthetase produced by porphyrogenic chemicals (Stein *et al.*, 1970; Bonkowsky *et al.*, 1979*b*), even though hepatic iron concentrations increased by only 40–50%. Our results with iron–dextran suggest that induction of 5-aminolaevulinate synthetase by iron alone may require, in addition to rapid and recent iron deposition in the liver, a higher concentration of hepatic iron than can be achieved by a single treatment with ferric citrate.

The present results indicate that studies to assess long-term effects of iron loading should be designed to avoid confusion from acute effects of iron administration. This point has not received sufficient attention in the past (Hanstein *et al.*, 1975; Bonkowsky *et al.*, 1979*a*; Ibrahim *et al.*, 1979; Maines, 1979). A period of 3–4 weeks should elapse after administration of large doses of iron–dextran if chronic effects of iron overload are to be distinguished clearly from acute effects. The use of lower doses of iron–dextran may decrease the duration of the acute effects of iron-loading, but this has not been demonstrated.

Mechanisms of iron action on hepatic haem metabolism

There is much indirect evidence supporting the hypothesis that activity of hepatic 5-aminolaevulinate synthetase is controlled by a regulatory haem pool (Meyer & Schmid, 1978; Bonkowsky *et al.*, 1979*b*). This hypothetical pool is thought to be small, to turn over rapidly and to exchange with cell haemoproteins (e.g. cytochromes b_5 and *P*-450,

catalase and tryptophan pyrrolase). This may also be the haem pool that controls activity of haem oxygenase, the rate-limiting enzyme of haem catabolism. Activity of 5-aminolaevulinate synthetase, the first and rate-limiting step in haem synthesis, is believed to be inversely related to the size of the regulatory haem pool.

The mechanism of action by which hepatic iron overload affects hepatic haem synthesis has been proposed to be by depleting the regulatory haem pool thus derepressing 5-aminolaevulinate synthetase. Two different mechanisms have been proposed (Bonkowsky *et al.*, 1979*b*): (i) inhibition of haem synthesis due to decreased activity of enzymes of the haem pathway (Kushner *et al.*, 1972, 1975; Maines, 1979); and (ii) increase in haem turnover (De Matteis & Sparks, 1973; Ibrahim *et al.*, 1979; Bonkowsky *et al.*, 1980*a*).

(i) *Inhibition of haem synthesis.* Previous results do not support the hypothesis that either acute (Bonkowsky *et al.*, 1979*b*) or long-term (Taljaard *et al.*, 1972; Louw *et al.*, 1977) iron loading inhibits hepatic uroporphyrinogen decarboxylase. In support of this lack of inhibition, in the present experiments, urinary excretions and hepatic concentrations of porphyrins in iron-loaded rats were never increased above control values; they were unchanged or decreased, in agreement with other recent findings (Maines, 1979).

A slight (25%) decrease in activity of hepatic 5-aminolaevulinate dehydratase has previously been found in iron-treated rats (Stein *et al.*, 1970; Maines, 1979). The physiological importance of this decrease is not established, since the maximal activity of 5-aminolaevulinate dehydratase in rat liver is about 1000 times those of 5-aminolaevulinate synthetase and porphobilinogen deaminase (Meyer & Schmid, 1978). Thus, when 5-aminolaevulinate synthetase has been markedly induced, as after administration of iron–dextran and 2-allyl-2-isopropylacetamide, porphobilinogen deaminase becomes the rate-limiting enzyme of hepatic haem synthesis (Elder, 1976). In support of this conclusion, livers from such animals contain higher concentrations of porphobilinogen (250–300 nmol/g of wet liver, Fig. 5) than of porphyrins (2.0–2.6 nmol/g of wet liver). Activity of prophobilinogen deaminase is said to be unaffected in animals treated with iron–dextran (Maines, 1979). Thus it does not appear that short- or long-term iron loading induces 5-aminolaevulinate synthetase as the result of inhibition of enzymes of the haem-synthetic pathway.

It has been proposed that iron itself, rather than haem (iron-protoporphyrin) inhibits haem synthesis by repressing 5-aminolaevulinate synthetase (Maines & Kappas, 1977). However, the present studies show that iron affects 5-aminolaevulinate synthetase in ways quite different from those caused by haem (Waxman *et al.*, 1966; Hayashi *et al.*, 1972; Whiting & Granick, 1976; (*a*) iron–dextran, unlike haem, even at the earliest times studied (1–4 h) did not decrease activity of 5-aminolaevulinate synthetase (Fig. 1); (*b*) iron treatment, unlike haem, potentiated the induction of 5-aminolaevulinate synthetase produced by 2-allyl-2-isopropylacetamide (Fig. 4). Elsewhere we have presented other results that make unlikely the suggestion that iron rather than haem is the proximate regulator of 5-aminolaevulinate synthetase (Bonkowsky *et al.*, 1979*b*).

(ii) *Increase in haem turnover.* Iron overload may decrease the size of the regulatory haem pool by increasing the rate of haem catabolism. This mechanism of action is supported by direct demonstrations of increased hepatic haem turnover shortly after rats had been given iron–dextran (Ibrahim *et al.*, 1979) or ferric citrate (Bonkowsky *et al.*, 1980*a*). Short-term treatment of rats with iron–dextran increases haem oxygenase activity (Maines & Kappas, 1976; Ibrahim *et al.*, 1979), which may be responsible for the increase in haem turnover, although this point is controversial (Paine & Legg, 1978; Bissell & Guzelian, 1980).

The decrease in cytochrome *P*-450 caused by iron treatment (Fig. 1*b*) may be due to insufficient haem being available for continual synthesis of this cytochrome. However, the time course (Figs 1 and 2), suggests that the regulatory haem pool controlling the activity of 5-aminolaevulinate synthetase is largely repleted 2 days after iron treatment, since the activity almost returns to control values. Cytochrome *P*-450 does not increase until 4–7 days after treatment, suggesting that the haem supply for cytochrome *P*-450 synthesis may be limiting. If the persisting decrease in cytochrome *P*-450 is indeed due to a limitation of the supply of haem, the present findings would imply either that there is not necessarily free haem exchange between the regulatory haem pool and cytochrome *P*-450, or that apo-cytochrome *P*-450 cannot necessarily remove haem from the regulatory pool. Alternatively, and perhaps more likely, iron treatment may affect the amount of apo-cytochrome(s) *P*-450 (independent of effects on haem metabolism). We have recently shown that iron–dextran treatment decreases both the haem and the apoprotein of cytochrome *P*-450 (J. F. Sinclair, J. F. Healey, R. A. McAllister, H. L. Bonkowsky & P. R. Sinclair, unpublished work).

The persisting slight increase in activity of hepatic 5-aminolaevulinate synthetase, long after iron treatment (Fig. 3*b*), is probably secondary to increased hepatic haem demand as the result of an increased rate of haem turnover (Ibrahim *et al.*, 1979, Bonkowsky *et al.*, 1980*a*).

Iron-catalysed lipid peroxidation could

theoretically deplete the regulatory haem pool (De Matteis & Sparks, 1973; Bonkowsky *et al.*, 1979*b*). Among mechanisms whereby lipid peroxidation may act to deplete hepatic haems are the following: (i) lipid peroxidation may damage microsomal, mitochondrial and other membranes, leading to denaturation of haemoproteins (e.g., of cytochromes b_5 and *P*-450) and increased rates of breakdown of their haem moieties; and (ii) cytochrome *P*-450 may itself act as a peroxidase, disposing of the lipid peroxides, but with destruction of its haem in the process (Hrycay & O'Brien, 1971). However, iron enhancement of hepatic lipid peroxidation *in vivo*, after administration of either ferric citrate or iron–dextran, has not been demonstrated, in contrast with the known stimulatory effects of iron *in vitro*. The present results, showing no effect of iron–dextran treatment on hepatic concentrations of GSH, and other recent results in livers of humans with haemochromatosis (Selden *et al.*, 1980), suggest that there may be little lipid peroxidation *in vivo* in iron-loaded liver. GSH concentrations would have been expected to have fallen if lipid peroxidation were appreciably increased (Högberg *et al.*, 1975; Selden *et al.*, 1980).

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