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# Iron and the Liver: Acute Effects of Iron-Loading on Hepatic Heme Synthesis of Rats. Role of Decreased Activity of 5-Aminolevulinate Dehydrase.

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# Iron and the Liver: Acute Effects of Iron-loading on Hepatic Heme Synthesis of Rats

## ROLE OF DECREASED ACTIVITY OF 5-AMINOLEVULINATE DEHYDRASE

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ABSTRACT Acute iron loading of rats, by intraperitoneal administration of iron-dextran (500 mg Fe/kg body wt 18–20 h before killing) decreased by 30% the rate of conversion of 5-amino-[$^{14}$C]levulinate ([$^{14}$C]ALA) into heme as measured with a recently described procedure for liver homogenates (1981. *Biochem. J.* **198**: 595–604). The decrease in conversion of labeled ALA into heme caused by iron loading was shown to be due to a 70–80% decrease in activity of ALA dehydrase. The decrease in activity of ALA dehydrase caused by iron loading was not associated with a decrease in hepatic concentrations of GSH, nor could it be reversed by addition of dithiothreitol, $Zn^{2+}$ or chelators of $Fe^{2+}$ and $Fe^{3+}$. Addition of $FeSO_4$, ferric citrate, or ferritin to homogenates of control liver had no effect of activity of ALA dehydrase. The decrease in activity of ALA dehydrase, caused by iron-dextran, was mirrored by a reciprocal increase in ALA synthase. Iron-dextran potentiated the induction of ALA synthase by allylisopropylacetamide. However, this potentiation could be dissociated from the decrease in ALA dehydrase caused by iron loading.

## INTRODUCTION

After administration of a single dose of iron-dextran to rats, there is a transient increase in activity of hepatic 5-aminolevulinate (ALA)[1] synthase, the rate-limiting enzyme in hepatic heme biosynthesis (1). The peak of the increase (four- to sixfold above control) occurs 12–24 h after treatment; by 48 h the activity of ALA synthase decreases to ~1.5-fold greater than control, and persists for several weeks (1, 2).

According to current concepts, a regulatory heme pool exerts endproduct negative feedback control on synthesis of ALA synthase (for recent reviews, see refs. 3–5). Decreases in this heme pool may arise as the result of enhanced heme breakdown or of decreased heme synthesis. It has been proposed by some investigators that iron decreases hepatic heme synthesis by inhibiting uroporphyrinogen decarboxylase (6). However, others have reported no effect (7) or activation (8) of the decarboxylase by ionic iron. We have also failed to find evidence for a decrease in activity of uroporphyrinogen decarboxylase in iron-loaded liver (1, 9).

In this paper, we use a procedure recently developed by our laboratory (10) to show that the rate of heme synthesis from ALA is diminished in liver homogenates of iron-loaded rats. We further show that the decrease can be accounted for by a decrease in activity of ALA-dehydrase that occurs in iron-loaded livers.

A portion of this work has appeared in abstract form in 1981. *Hepatology.* **1**: 497.

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[1] *Abbreviations used in this paper:* AIA, allylisopropylacetamide; ALA, 5-aminolevulinate; [$^{14}$C]ALA, 5-amino-[4-$^{14}$C]levulinic acid; BHT, butylated hydroxytoluene; DTT, dithiothreitol; PBG, porphobilinogen.

## METHODS

*Animals and preparation of liver homogenates.* Male Sprague-Dawley rats (150–200 g) were kept as previously described (11) and starved 24 h before killing. Rats treated with iron-dextran >24 h before killing were starved 16 h before injection with iron-dextran, refed after injection, and starved again 24 h before killing. All controls received dextran-5 (2 g/kg) in normal saline (200 mg/ml). Both iron-dextran and dextran-5 were tolerated well as reported previously (1).

Rats were killed by decapitation and their livers quickly removed, weighed, and homogenized (20% wt/vol) in ice-cold 0.25 M sucrose/0.02 M Tris/HCl (pH 7.4). Where indicated, these 20% homogenates were frozen (−60°C) before use for measurements other than ALA synthase. In most experiments, we immediately added the antioxidant, butylated hydroxytoluene (BHT) (90 μM final), to homogenates with thorough mixing. For studies that included a determination of ALA synthase activity, we took a separate piece of the liver and homogenized it in a different buffer (see next section). We homogenized livers 15–20 s with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) at low speed and, just before use, diluted homogenates to 7.7% wt/vol, with sucrose/Tris buffer.

*Assay procedures.* Activities of ALA synthase and concentrations of hepatic GSH and iron were measured as previously described (1). The conversion of [$^{14}$C]ALA into heme and heme precursors, and the extraction of unconjugated bilirubin were performed as described earlier (10). We measured malondialdehyde (MDA) by the method of Buege and Aust (12).

Activities of ALA dehydrase were routinely measured in 7.7% (wt/vol) homogenates containing ALA (5 mM), dithiothreitol (DTT) (10 mM), and BHT (45 μM). DTT was sometimes omitted, as described in Results. Tubes containing a total volume of 0.65 ml were incubated for 30 min at 37°C in a shaking water bath. We stopped the reaction by placing the tubes in a slurry of water and ice and added 2 vol of 7.5% TCA:0.05 M $HgCl_2$ with mixing. After centrifugation (1,000 *g*, 10 min), we mixed 1 ml of supernatant with 1 ml modified Ehrlich's reagent (13), and after 15–25 min, scanned the mixture from 500–680 nm against a "blank" using an Aminco DW2 Spectrophotometer (American Instrument Co., Silver Springs, MD). The "blank" consisted of homogenate to which TCA had been added at zero time and that had been kept on ice for 30 min. We calculated porphobilinogen concentrations using a $\Delta\xi$(555–650 nm) of 61,000 (13).

Activities of uroporphyrinogen decarboxylase were measured in 20% (wt/vol) homogenates by determining the rate of decarboxylation of pentacarboxylate porphyrinogen III to coproporphyrinogen III (14).

*Use of BHT as antioxidant.* In previous studies, $FeSO_4$, in the presence of the antioxidant menadione, increased the rate of formation of heme from ALA by homogenates of rat liver, although menadione alone decreased the rate of conversion of ALA into heme (10). We now find the antioxidant, BHT, is also effective in preventing iron-stimulated lipid peroxidation, as assayed by accumulation of MDA. Furthermore, unlike menadione, BHT alone does not decrease accumulation of labeled heme from [$^{14}$C]ALA. Therefore, we used BHT routinely in the present studies.

*Reagents.* Unless otherwise noted, chemicals were dissolved just prior to use in the sucrose/Tris buffer, pH 7.4. 4,6-Dioxoheptanoic acid, and ferritin (Cd-free from horse spleen) were from United States Biochemical Corp. (Cleveland, OH). BHT (Aldrich Chemical Co., Milwaukee, WI) was dissolved in ethanol (10 mg/ml). Allylisopropylacetamide (AIA) (15 mg/ml), a gift from Hoffmann-LaRoche (Nutley, NJ), was dissolved in hot 0.15 M NaCl. After the solution had cooled, it was administered subcutaneously in a dose of 400 mg/kg body wt. Controls received saline. [4-$^{14}$C]ALA (51.5. mCi/mmol) was purchased from New England Nuclear (Boston, MA) and prepared as described previously (10).

Unlabeled ALA (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.1 M sodium phosphate buffer and titrated to pH 7.0. Menadione and ferrozine were from Sigma Chemical Co. We dissolved zinc sulfate (Sigma Chemical Co.) in distilled water, and ferrous sulfate (ACS grade, Fisher Scientific Co., Pittsburgh, PA) in 1 mM HCl. 2-Thiobarbituric acid was from Sigma; desferrioxamine mesylate (Desferal) was from CIBA-Geigy (Summit, NJ). Dextran-5 was dissolved in normal saline (200 mg/ml final concentration). Iron-dextran and dextran-5 were gifts from Fisons, Ltd. (Holmes Chapel, Cheshire, England). Pentacarboxylic porphyrin III and mesoporphyrin IX were gifts from Professor A. H. Jackson, University College, Cardiff, England.

## RESULTS

### *Conversion of [$^{14}$C]ALA into heme and porphyrins by iron-loaded liver*

As shown in Table I, liver homogenates of rats treated with iron-dextran 20 h before killing, when incubated with [$^{14}$C]ALA, accumulated significantly less radioactivity in protoporphyrin and heme than did controls. There are three possible explanations for this difference. In homogenates of iron-loaded rats, compared to controls, there may be: (*a*) increased breakdown of labeled heme; (*b*) greater dilution of the added [$^{14}$C]ALA by endogenous ALA; or (*c*) decreased rates of synthesis of heme or heme precursors.

*Does decreased heme radioactivity occur due to breakdown of labeled heme during incubation of liver homogenates?* Fig. 1 shows that heme was stable in vitro for 30–90 min in homogenates of control and iron-loaded liver with BHT. A decrease in radioactivity in heme with time occurred in homogenates of iron-loaded liver only when BHT was omitted. In other studies in which higher concentrations of ALA were used, we assumed heme was also stable, as in Fig. 1.

*Is the decreased heme radioactivity due to dilution of [$^{14}$C]ALA by endogenous ALA?* Administration of iron-dextran to rats is known to increase hepatic ALA synthase (1, 15, see Fig. 2). An increase in this enzyme could increase the endogenous hepatic pool of ALA sufficiently to dilute exogenously added [$^{14}$C]ALA and thereby decrease the accumulation of radioactivity in heme and protoporphyrin. To determine the contribution of endogenous ALA, we incubated homogenates with two different concentrations of unlabeled ALA keeping the amount of labeled ALA constant. Table II shows the results of a typical experiment, performed with homogenates of iron-loaded livers that

TABLE I

*Decreased Accumulation of Radioactivity in Heme and Protoporphyrin by Liver Homogenates of Iron-loaded Rats Incubated with [$^{14}$C]ALA*

| Treatment of rats | n | Heme | Protoporphyrin | Σ[protoporphyrin + heme] |
|---|---|---|---|---|
| | | | *dpm × 10$^{-3}$* | |
| Control | 3 | 84.3±6.2 | 11.5±0.7 | 95.8±6.0 |
| Iron loaded | 4 | 56.4±8.3° | 4.0±0.9° | 60.4±7.8° |

Iron-loaded rats received iron-dextran, 500 mg Fe/kg body wt, in a single i.p. injection 20 h before killing. Control rats received an appropriate amount of dextran-5 i.p. Incubations (30 min) of 0.65 ml of 7.7% wt/vol homogenates of individual livers were carried out as described (10), in the presence of 45 μM BHT, 0.2 μCi of [$^{14}$C]ALA and a final ALA concentration of 22 μM. Protoporphyrin was the only porphyrin that accumulated to a measurable extent (10). Differences between mean values were compared using the students two-tailed *t* test.

° Significantly different from control ($P < 0.005$).

had a 5.7-fold increase in ALA synthase. The observed ratios of b/a in the table provide a means for estimating the endogenous pools of ALA. The endogenous pool of ALA is slightly higher in homogenates of iron-loaded than of control livers. However, the increase (0.8 nmol/50 mg liver), calculated from the observed ratios, is small relative to the amount of exogenous ALA (26 nmol) routinely used in homogenate incubation. Thus, dilution of [$^{14}$C]ALA by endogenous ALA

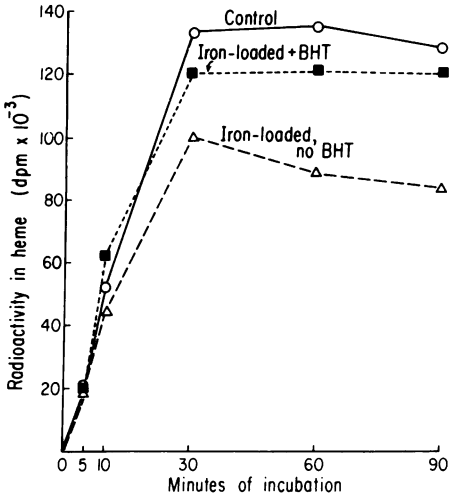


FIGURE 1 Stability of labeled heme synthesized from [$^{14}$C]ALA by homogenates of control and iron-loaded livers. Freshly prepared homogenates of liver (15.4% wt/vol), from rats treated as described in the legend to Table I and killed 24 h after treatment, were incubated with 0.1 μCi (1.9 nmol) [$^{14}$C]ALA. Total volume of incubation was 0.65 ml. In these studies, an additional extraction step was carried out to remove any bilirubin that might otherwise have remained in the heme fraction (10). The concentration of BHT was 70 μM. The recovery of labeled heme was 74% (10).

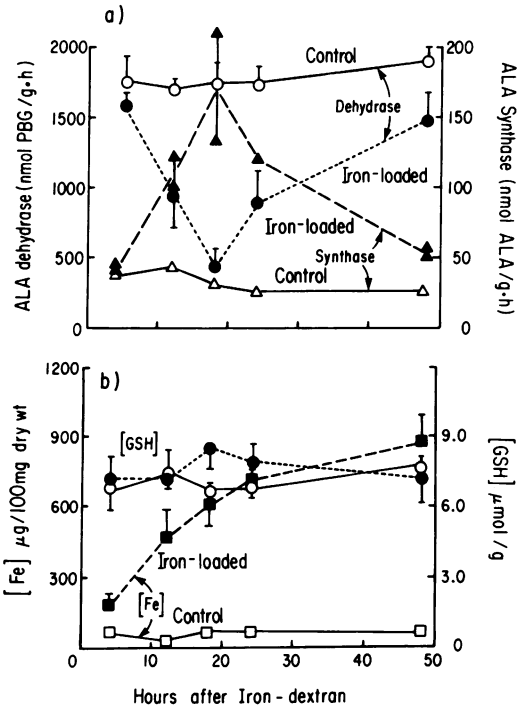


FIGURE 2 Effects of iron loading on hepatic ALA synthase, ALA dehydrase, GSH, and total iron. Rats, treated as described in Methods, were killed at various times after injection. Assays of ALA dehydrase, GSH, and Fe were performed on homogenates of individual livers; $n$ = 3–5 for both controls and iron-loaded animals, except for assays of liver iron in control rats that were performed on portions of single livers. Assays of ALA synthase in homogenates of iron-loaded livers were performed on two separate pools of liver containing two to three livers per pool, except for the 24-h time point, which was done on a single pool of three livers. For ALA dehydrase, GSH and Fe, results are mean±1 SD; for ALA synthase, the values for each pool are presented. Open symbols, control; closed symbols, iron-loaded.

TABLE II

*Effect of Changing the Concentration of Exogenous Carrier ALA on Incorporation of [$^{14}$C]ALA into Heme by Homogenates of Rat Liver*

| Type of homogenate | n | a<br>21.8 μM ALA | b<br>43.6 μM ALA | b/a<br>Observed | b/a<br>Theoretical, assuming no dilution |
|---|---|---|---|---|---|
| | | *Radioactivity in heme* [*dpm* × $10^{-3}$] | | | |
| Control | 3 | 116.0±4.5 | 63.2±2.6 | 0.546±0.004 | 0.500 |
| Iron loaded | 4 | 68.7±11.0 | 39.6±5.7 | 0.577±0.013* | 0.500 |

Rats were injected intraperitoneally with dextran-5 (control) or iron-dextran (iron-loaded) and killed 18 h later. Homogenates (7.7% wt/vol) of liver were incubated, in the presence of $FeSO_4$ (0.25 mM) and BHT (45 μM), with two preparations of ALA, a and b, both of which contained the same amount of [$^{14}$C]ALA (0.2 μCi). However, preparation b contained twice the total amount of ALA as did a. The extent to which the observed ratios of radioactivity in heme (column 3) differ from the theoretical, assuming no dilution of added ALA by endogenous ALA (column 4), provides an estimate of the relative sizes of the endogenous pools. Results are mean±SD. In the homogenates used, activities of ALA synthase were: control, 30 nmol ALA/g·h; iron-loaded, 171 nmol ALA/g·h; and activities of ALA dehydrase were: control, 2060 nmol PBG/g·h; iron-loaded, 559 nmol PBG/g·h.

* Significantly greater than control value ($P$ = 0.011).

could only contribute a small fraction of the change observed.

*Is decreased heme radioactivity due to a decreased rate of synthesis of heme or heme precursors?* The elimination of (*a*) and (*b*) coupled together with data of Table I imply there is a decreased rate of synthesis of labeled heme and heme precursors from [$^{14}$C]ALA in homogenates of iron-loaded rats. Previously, it had been found that administration of ferric citrate (16) or iron-dextran (17) to rats decreased activity of ALA dehydrase. Therefore, we made further studies of the effect of iron-loading on this enzyme, particularly on the time sequence and on heme synthesis in vitro.

## *Effect of iron loading on hepatic ALA dehydrase*

Iron loading produced a marked decrease in activity of ALA dehydrase that was mirrored by a reciprocal increase in ALA synthase (Fig. 2a). A transient decrease in activity of ALA dehydrase was observed after iron loading, in five separate experiments, although there was some variability in the maximal degree of the decrease (50–80%) and the time after iron treatment at which the nadir occurred (12–20 h).

*Kinetics of ALA dehydrase in iron-loaded livers.* The major effect of iron loading on ALA-dehydrase was to decrease the maximum velocity ($V_{max}$) (At 18 h, control = 1,600 nmol porphobilinogen (PBG)/g liver/h; iron-loaded = 394 nmol PBG/g liver per h). The extent of the decrease depended on the time of iron treatment. The Michaelis constant ($K_M$) of the enzyme was approximately the same for control and iron-loaded livers (control = 105 μM; iron-loaded = 91 μM ALA).

The following may be responsible for the decrease in activity of ALA dehydrase: (*a*) effects on sulfhydryl groups, (*b*) a deficiency of zinc, (*c*) direct inactivation of enzyme by iron, (*d*) lipid peroxidation, and (*e*) accumulation of a soluble inhibitor of the enzyme. (*a*) ALA dehydrase is well known to require sulfhydryl groups for activity (18); two observations indicate that the decrease we observed is not due to depletion of hepatic thiols. First, hepatic concentrations of GSH were not decreased by iron treatment in vivo (Fig. 2b). Second, omission of DTT from the assay for ALA dehydrase produced only a 10–15% decrease in activity in homogenates of both control and iron-loaded livers. (*b*) ALA dehydrase also requires $Zn^{2+}$ for activity (18); thus we tested the effect of the addition of $Zn^{2+}$ (0.1–1.0 mM) with or without 20 min of preincubation, on activity of the enzyme in homogenates of iron-loaded liver. When added to liver homogenates from control rats or from rats treated with iron-dextran, $Zn^{2+}$ either had no effect (at 0.1 mM) or decreased activity by 50% (at 0.5 mM) or 80% (at 1.0 mM). (*c*) Another possibility is that some iron compound inhibits ALA dehydrase directly. We investigated this possibility in rat liver homogenates in vitro, by adding either different iron compounds to control homogenates or chelators of iron

to homogenates from rats treated with iron dextran. $FeSO_4$, iron-dextran, ferric citrate or ferritin (each at 0.25, 1.5, and 5 mM Fe) had no appreciable effect on activity of the dehydrase in homogenates of control rat liver. (The concentration of iron in 10% homogenate of iron-loaded liver 18–24 h after treatment is ~1 mM). When liver homogenates from rats treated with iron-dextran 12 or 18 h before killing were treated with desferrioxamine (7 mM), a chelator of $Fe^{3+}$ (19), ferrozine (0.25 mM), a chelator of $Fe^{2+}$ (20), or a combination of the two, there was no increase in activity of ALA dehydrase. At these or lower concentrations, the chelators alone did not affect activity of ALA dehydrase in homogenates of control livers. (Concentrations of ferrozine >0.25 mM decreased activity of dehydrase in homogenates of control livers.) (*d*) The decrease in dehydrase activity in homogenates of iron-loaded livers could not be attributed to iron-catalyzed lipid peroxidation in vitro since addition of BHT to the assay, which totally prevented formation of malonaldehyde, had no effect upon dehydrase activity. Also, the addition of iron salts, with or without BHT, to homogenates of control liver had no effect on activity of ALA dehydrase, regardless of iron-catalyzed lipid peroxidation. (*e*) Equal volumes of homogenates of control and iron-loaded livers were mixed together. The activity of ALA dehydrase was the theoretical additive amount expected from each component of the mixture. Such an experiment provided no evidence for a soluble inhibitor in iron-loaded livers.

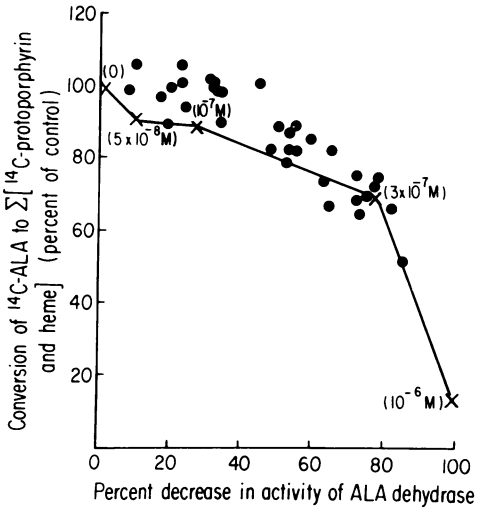


FIGURE 3 Similar effects of two ways of decreasing activity of ALA dehydrase upon conversion of ALA to Σ[protoporphyrin + heme] by liver homogenates. Activity of ALA dehydrase was decreased by (*a*) injecting rats with iron-dextran, 500 mg Fe/kg, at various times (4–48 h) before killing, or (*b*) adding increasing concentrations (shown in parentheses) of 4,6-dioxoheptanoic acid to homogenate of normal liver. Assays for ALA dehydrase and conversion of [$^{14}$C]ALA to Σ[protoporphyrin + heme] were as described in Methods. Each point is the result for a single rat given iron-dextran; results of three separate experiments are summarized together. × —— × gives results for homogenates to which 4,6-dioxoheptanoic acid was added 5 min before assay.

### *Effect of decreased activity of ALA dehydrase on conversion of [$^{14}$C]ALA to heme and protoporphyrin by iron-loaded livers*

We next asked to what extent the decrease in activity of ALA dehydrase would decrease the conversion of ALA to protoporphyrin and heme. With increasing concentrations of 4,6-dioxoheptanoic acid, a potent inhibitor of ALA dehydrase (21), the dehydrase was decreased to different extents and the resulting decrease in accumulation of radioactivity into Σ(protoporphyrin and heme) from [$^{14}$C]ALA is shown in Fig. 3. The percent decrease in ALA dehydrase was always much greater than the decrease in accumulation of radioactivity. As shown in Fig. 3, the various decreases in dehydrase activity caused by iron loading led to decreases in accumulation of radioactivity identical to those caused by 4,6-dioxoheptanoic acid. The curves obtained were superimposable and show that the decreased accumulation of radioactivity into Σ(protoporphyrin and heme) in the homogenates of the iron-loaded liver can be explained solely by the decrease in ALA dehydrase.

The kinetics of the incorporation of ALA into Σ(protoporphyrin + heme) were determined using homogenates of control and iron-loaded livers. The activity of ALA dehydrase in the homogenate of the iron-loaded liver was decreased by 75%. The concentration of ALA needed to achieve half-maximal rates of formation of Σ(protoporphyrin + heme) was increased from 5 μM (control) to 9 μM (iron-loaded).

The inhibition of Σ(protoporphyrin + heme) in Fig. 3 was obtained using an initial concentration of 40 μM ALA and 30-min incubations. Decreasing the initial concentration of ALA to 5 μM increased the inhibition of heme synthesis, whereas increasing the initial concentration of ALA to 100 μM decreased the inhibition (Table III). Also, the inhibition of heme formation was greater for all concentrations of ALA when the incubations were stopped at 5 min. These results indicate that there is a lag in the production of labeled heme, which probably is due to lag in PBG synthesis.

We next looked for evidence of inhibition of other steps of the pathway between ALA and heme. Homogenates of control and iron-loaded liver were incubated for 1 h with a high concentration of unlabeled ALA (1 mM), and the amount and type of porphyrins that accumulated were measured. In this study, we

TABLE III

*Effect of Different Initial Concentrations of [$^{14}$C]ALA on Initial Rates of $^{14}$C-Heme Synthesis by Homogenate of Iron-loaded vs. Control Livers*

| Duration of incubation | Initial concentration of ALA, μM: Ratio of the rate of labeled heme formation, iron-loaded/control | | |
|---|---|---|---|
| | 5 | 40 | 100 |
| *min* | | | |
| 5 | 0.30 | 0.42 | 0.47 |
| 10 | 0.41 | 0.50 | 0.55 |
| 15 | 0.43 | 0.52 | 0.55 |

Homogenates from rats described in Fig. 4 were incubated in the presence of $FeSO_4$ (0.25 mM) and BHT (45 μM) with three concentrations of [$^{14}$C]ALA. Heme was extracted from the samples and counted as described in Methods. For ease of comparison, the accumulation of label in heme by homogenate of iron-loaded liver is presented as a fraction of that by homogenate of control liver.

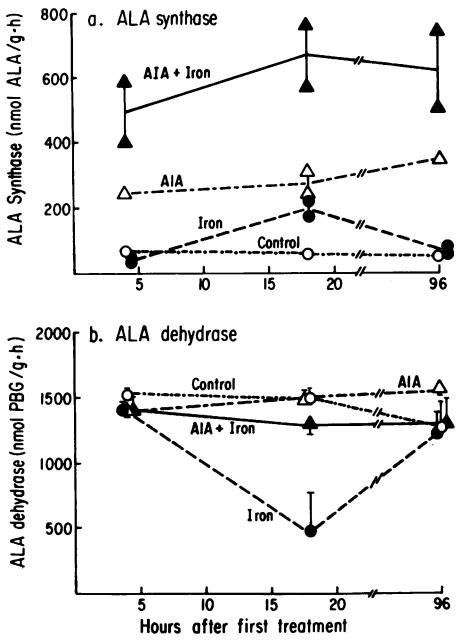


FIGURE 4 Effect of administration of iron-dextran and AIA on hepatic ALA synthase and ALA dehydrase. Rats were starved for 12 h before their first treatment. Rats killed 4 or 18 h after treatment (first and second time points) were given iron-dextran i.p. (500 mg Fe/kg), AIA s.c. (400 mg/kg), or the combination, and starvation was continued until killing. Rats killed 96 h after first treatment (third time point) were first given iron-dextran, after which they were refed for 66 h. Then food was again withheld until killing, and some were given AIA 18 h before killing. Control rats received appropriate amounts of dextran-5 and normal saline in place of iron-dextran and AIA, respectively. ALA synthase was measured on pooled homogenates of two or three separate livers. Each point is the value for a single pool. ALA dehydrase was measured on homogenates of individual livers. Each point is the mean±SD of three to six livers.

added $FeSO_4$ (0.25 mM) to control homogenate to provide optimal substrate for ferrochelatase (10). Addition of $FeSO_4$ to iron-loaded homogenate did not enhance accumulation of labeled heme. Both kinds of homogenate accumulated similar amounts of porphyrin, 0.8 nmol/g of uro- plus coproporphyrin, and 2.0 nmol/g of protoporphyrin. Thus, iron loading does not cause accumulation of heme precursors in the biosynthetic pathway leading to protoporphyrin. Furthermore, treatment of rats with iron-dextran had no effect on activity of hepatic uroporphyrinogen decarboxylase, measured 4 and 18 h after treatment (results not shown).

### *Effect of iron-dextran, AIA, and the combination on activities of hepatic ALA synthase, ALA dehydrase, and uroporphyrinogen decarboxylase*

Administration of ferric citrate (9, 16) or iron-dextran (1) to rats potentiates the induction of hepatic ALA synthase produced by the porphyrogenic chemical AIA. To test whether this potentiation by iron is related to a decrease in activity of ALA dehydrase, or uroporphyrinogen decarboxylase, we performed experiments of the type summarized in Fig. 4. AIA, given simultaneously with iron-dextran, prevented the decrease in activity of ALA dehydrase produced by iron-dextran alone (Fig. 4b, 18 h time point). AIA treatment, with or without iron-dextran, did not affect hepatic uroporphyrinogen decarboxylase measured 4 and 18 h after treatment (results not shown). Thus, iron-mediated potentiation of AIA induction of ALA-synthase occurred despite no decrease in the activities of ALA-dehydrase or uroporphyrinogen decarboxylase.

## DISCUSSION

In this paper we have presented new evidence for effects of iron on enzymes of the heme biosynthetic pathway. We have observed transient effects of a single iron dose, namely, a decrease in ALA dehydrase and a reciprocal mirrored increase in ALA synthase (Fig. 2a). The effect of the iron loading on heme synthesis in vivo was estimated by studying the ability of homogenates of the livers to convert [$^{14}$C]ALA to heme and protoporphyrin. The rate of the conversion was decreased in liver homogenates from animals 18–20 h after being loaded with iron, even when the homogenates were incubated in the presence of the anti-

oxidant, BHT, which suppresses lipid peroxidation (Table I). We concluded the decreased heme synthesis could be attributed solely to the decreased activity of ALA dehydrase for the following reasons: (*a*) Decreased heme synthesis was not due to dilution of added [$^{14}$C]ALA by an expanded endogenous pool (Table II) nor by increased breakdown of heme (Fig. 1); (*b*) No increase or decrease in accumulation of porphyrins was observed when homogenates of iron-loaded livers were incubated with excess ALA. These results are consistent with there being no decrease in PBG deaminase as reported by others (17), nor decrease in later enzymes of the pathway. Furthermore, using a method (14) other than used previously (9, 22), we still failed to detect any decrease in uroporphyrinogen decarboxylase in iron-loaded liver homogenates; (*c*) Decreases in ALA dehydrase, either by iron-loading in vivo or by adding 4,6-dioxoheptanoic acid, caused identical decreases in conversion of [$^{14}$C]ALA to heme by homogenates (Fig. 3).

The cause of the transient decrease in activity of ALA dehydrase mediated in vivo by iron treatment remains unknown. We could find no evidence that it was due (*a*) to direct action of either some iron compound or other soluble inhibitor; (*b*) to iron-catalyzed lipid peroxidation in vitro; (*c*) to depletion of GSH in vivo; or (*d*) to a deficiency of zinc. Our inability to inhibit ALA dehydrase in vitro with ferric citrate differs from previous findings (16) possibly because of the different assay methods used.

The decrease in activity of ALA dehydrase caused by iron loading was rapid and transient (Fig. 2a). If the half-life of the dehydrase molecules in normal rat liver is similar to the half-life of the mouse liver enzyme (5–6 d) (23), our results imply either that acute iron loading increases the catabolism of the dehydrase molecules, or that iron loading decreases the activity of the dehydrase molecules. The effect is short lived (Fig. 2a) despite the fact that the concentration of iron within the liver continued to increase (Fig. 2b). These findings provide additional support for the transient occurrence of an "active" pool of iron (1).

*Effect of decreased activity of ALA dehydrase on hepatic heme synthesis.* Because the activity ($V_{max}$) of ALA dehydrase in most mammalian tissues is 20–100 times higher than that of ALA synthase or PBG deaminase (3, 24), it is often thought that activity of the dehydrase rarely limits the rate of heme synthesis (24). We show that a 50–70% decrease in ALA dehydrase has only a small effect on hepatic heme synthesis when measured in vitro (Fig. 3). However, larger decreases in ALA dehydrase affects heme synthesis drastically (Fig. 3). Furthermore, the effect of a decrease in dehydrase activity on heme synthesis from ALA depends on the initial concentration of ALA (Table III) since this will affect the rate of accumulation of PBG. Therefore, during iron loading, the in vivo activity of ALA dehydrase can decrease the concentration of PBG sufficiently to decrease the rate of conversion of ALA to heme. Despite the decrease in the $V_{max}$ of ALA dehydrase, restoration of a normal rate of heme synthesis remains possible if the rate of PBG formation is sufficient. This condition may be met because we found that iron loading led to an increase of ALA synthase (Fig. 2, ref. 1), and probably to an increase in the hepatic concentration of ALA.

The effect of decreased activity of ALA dehydrase on heme synthesis in vivo is further complicated since ALA and PBG can leak out of hepatocytes, especially when hepatic production and accumulation are increased. This leakage occurs in human acute porphyrias, in certain experimental porphyrias (3, 5) and in cultured liver cells (25). Because leakage of ALA evidently occurs at concentrations well below the $K_m$ of ALA dehydrase, this loss of substrate may enhance the effect of a decrease in ALA dehydrase in decreasing heme synthesis. Because the $K_m$ of the dehydrase is so much higher than that of PBG deaminase, leakage of ALA probably affects heme synthesis more than does leakage of PBG.

*Separate actions of iron to decrease ALA dehydrase and to potentiate drug-mediated induction of ALA synthase.* Our studies with iron-dextran and AIA show that treatment with iron-dextran potentiates the induction of ALA synthase produced by AIA as early as 4 h after treatment (Fig. 4a), before the iron-mediated decrease in activity of ALA dehydrase occurred (Fig. 4b). In fact, simultaneous treatment with AIA and iron-dextran virtually abolished the decrease in activity of ALA dehydrase caused by iron-dextran alone (Fig. 4b; 18-h time point). The explanation for this protection is unknown, but may be due to substrate stabilization due to the increased ALA synthase (Fig. 4). It is not due to a decrease in hepatic iron uptake in rats treated with both AIA and iron-dextran since hepatic iron concentrations in such rats are similar to those in rats given iron-dextran alone (results not shown). These results demonstrate that the potentiation of induction of ALA-synthase following administration of iron and AIA, is not mediated by a decrease in ALA-dehydrase.

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