Hepatic Heme Metabolism and Its Control

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This review summarizes heme metabolism and focuses especially upon the control of hepatic heme biosynthesis. Activity of δ-aminolevulinic acid synthetase, the first enzyme of heme biosynthesis, is of primary importance in controlling the overall activity of this biosynthetic pathway. Δ-aminolevulinic acid synthetase is subject to inhibition and repression by heme, and numerous basic and clinical studies support the concept that there exists within hepatocytes a "regulatory" heme pool which controls activity of δ-aminolevulinic acid synthetase. In addition, activity of this enzyme is repressed by feeding, especially by ingestion of carbohydrates (the so-called "glucose effect"). Studies pertaining to the mechanisms underlying this effect are also reviewed. The "glucose effect" appears to be mediated by glucose or perhaps by glucose-6-phosphate or uridine diphosphate glucose, rather than by metabolites further removed from glucose itself. Unlike the situation in *E. coli*, the "glucose effect" in liver of higher organisms is not mediated by alterations in intracellular concentrations of cyclic AMP. Effects of heavy metals, especially iron, on hepatic heme metabolism are also considered. Iron has been found to inhibit formation and utilization of uroporphyrinogen III and to lead to decreased concentrations of microsomal heme and cytochrome P-450. Administration of large amounts of iron is also associated with an increase in activity of heme oxygenase, a property shared by several other metal ions, most notably cobalt. This effect of iron or cobalt administration is similar to the effect of heme administration in increasing heme oxygenase activity; however, we believe it is unlikely that iron, rather than heme itself, is a physiologic regulator of hepatic heme metabolism, although this hypothesis has lately been proposed.

ABBREVIATIONS USED

AIA, allylisopropylacetamide
AIP, acute intermittent porphyria
ALA, δ-aminolevulinic acid
cAMP, 3',5'-cyclic adenosine monophosphate
DDC, 3,5-diethoxycarbonyl-1, 4-dihydrocollidine
HMPS, hexose monophosphate shunt
MDA, malonyldialdehyde
PIA, propylisopropylacetamide
PBG, porphobilinogen
PCT, porphyria cutanea tarda
TCA, tricarboxylic acid
Uro-D, uroporphyrinogen decarboxylase

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INTRODUCTION

Virtually all cells in higher animals are capable of heme synthesis, either continually throughout life or at some stage of their development. The heme thus made serves as the prosthetic group in a variety of proteins concerned with (*i*) the carriage and transfer of oxygen within and among cells (e.g., hemoglobin, myoglobin); (*ii*) the protection of cells against toxic oxidizing species (catalase, peroxidase); (*iii*) the orderly and efficient transfer of electrons with attendant synthesis of ATP (mitochondrial cytochromes); and (*iv*) microsomal metabolism of fatty acids, steroids, and xenobiotics (microsomal cytochromes). Quantitatively, developing erythrocytes account for most of the heme produced by higher animals, but the liver is a second major locus of heme synthesis. Furthermore, both in the bone marrow and in liver an increase in rate of heme synthesis is highly inducible: in bone marrow in response to increased demand for circulating erythrocytes and in liver in response to inducers of microsomal cytochrome P-450. Cytochrome P-450 is the terminal oxidase for metabolism of numerous endogenous and exogenous compounds. This oxidative metabolism produces polar products which are more readily conjugated and excreted than are the parent compounds.

In recent years, however, it has also become clear that cytochrome P-450 and the other components of the microsomal mixed-function oxidase system serve to *activate* certain chemicals, producing proximate carcinogens and hepatotoxins from less noxious precursors.

The primacy of bone marrow and liver as major sites of heme biosynthesis is further exemplified by consideration of *the porphyrias*, a group of diseases in which there exist inborn or acquired defects in heme biosynthesis. In these diseases various heme precursors are over-produced in the bone marrow or the liver. The biochemical bases for these diseases and the theoretical bases for their rational management can be understood only if one understands the factors controlling normal heme biosynthesis.

In this review, we shall focus only upon hepatic heme biosynthesis and its control. In particular, we shall concentrate upon several clinically relevant factors which influence activity of the enzyme δ-*aminolevulinic acid synthetase*, the rate-limiting step of hepatic heme biosynthesis. These factors are (*i*) heme itself, the end-product of the pathway, which can inhibit and repress δ-aminolevulinic acid synthetase; (*ii*) iron which plays a promoting role in certain types of clinical and experimental porphyria; and (*iii*) carbohydrate feeding which can repress the enzyme and ameliorate the porphyric state, an action termed the "glucose effect."

HEPATIC HEME BIOSYNTHESIS AND CATABOLISM

The steps in the pathway are outlined in Fig. 1.

In the first step, succinyl-CoA is condensed with glycine, forming the intermediate α-amino, β-keto adipic acid which is probably decarboxylated to δ-aminolevulinic acid (ALA) before being removed from the enzyme surface [1]. This reaction is catalyzed by the mitochondrial enzyme ALA synthetase (E.C. 2.3.1.37). ALA leaves the mitochondrion and in the cytoplasm, two molecules of ALA are condensed to form porphobilinogen (PBG), the monopyrrole precursor of porphyrins. This reaction (Fig. 1, step 2) is catalyzed by the enzyme ALA dehydratase (E.C. 4.2.1.24).

The intermediates formed from PBG which can give rise to protoporphyrin are not themselves porphyrins but hexahydroporphyrins or porphyrinogens. The next step requires two enzymes acting in concert (Fig. 1, enzymes 3 and 4) to condense four
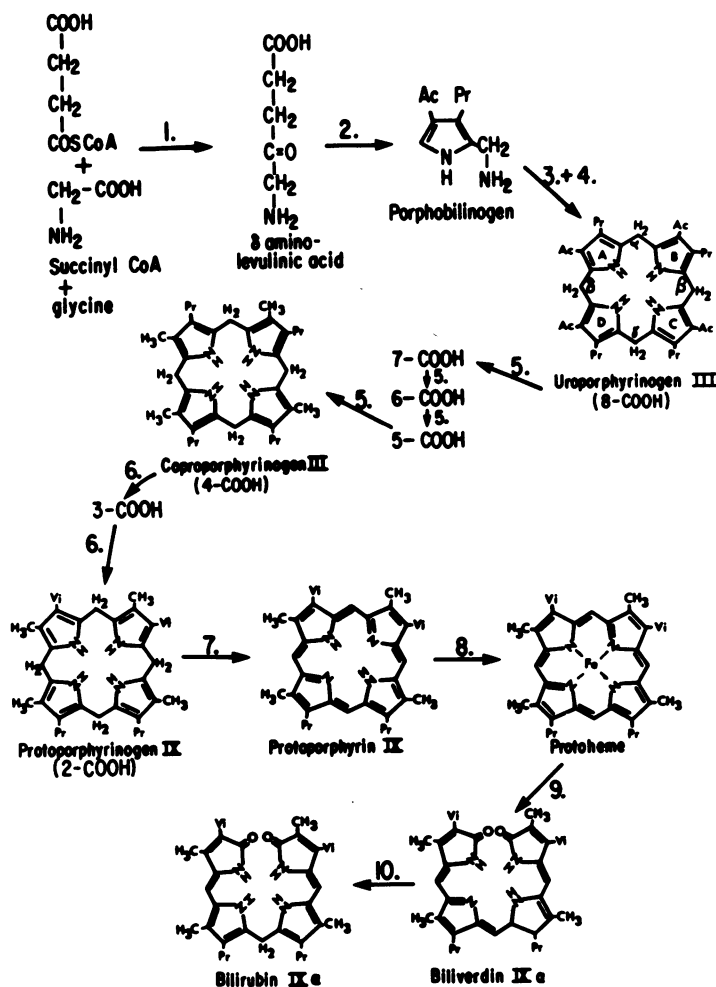
FIG. 1. Biosynthesis and Catabolism of Heme. The enzymes involved are identified by number: 1. δ - aminolevulinic acid synthetase; 2. δ-aminolevulinic acid dehydratase; 3. Uroporphyrinogen synthetase; 4. Uroporphyrinogen III co-synthetase; 5. Uroporphyrinogen decarboxylase; 6. Coproporphyrinogen oxidase; 7. Protoporphyrinogen oxidase; 8. Heme synthetase; 9. Heme oxygenase; 10. Biliverdin reductase. Abbreviations: Ac, acetyl; Pr, propionyl; Vi, vinyl.

molecules of PBG into one of uroporphyrinogen III. These two enzymes are uroporphyrinogen synthetase (E.C. 4.3.1.8), also called PBG deaminase, and uroporphyrinogen III cosynthetase. The former enzyme acting alone catalyzes a head-to-tail polymerization of four PBG rings to form uroporphyrinogen I, in which the "D" ring has not been "flipped round" as it has been in uroporphyrinogen III. The cosynthetase is a heat labile protein, which readily associates with the deaminase to form a stable complex [2,3]. Recently it has been suggested that this complex first catalyzes the formation of a dipyrrole, joined head-to-head, followed by two head-to-tail additions, to form the physiologic heme intermediate uroporphyrinogen III [4]. However, the precise mechanism of this complicated reaction is not yet settled. Activity of uroporphyrinogen synthetase is decreased in hemolysates [5–7], skin fibroblasts [8,9] and livers [10,11] of patients with acute intermittent porphyria. This genetically determined decrease is believed to be the primary defect in heme

biosynthesis in this disease. A relative deficiency in uroporphyrinogen III cosynthetase, compared to uroporphyrinogen I synthetase, occurs in congenital erythropoietic uroporphyria [12]. The molecular basis for this imbalance is unknown.

The acetic acid side-chains of both the I and III isomers of uroporphyrinogen may be sequentially decarboxylated, eventually to form the corresponding 4-carboxyl coproporphyrinogen. These reactions are catalyzed by another cytoplasmic enzyme, uroporphyrinogen decarboxylase (Fig. 1, enzyme 5) (E.C. 4.1.1.37). Activity of this enzyme has been reported to be decreased in livers of patients with porphyria cutanea tarda [13,14].

Coproporphyrinogen III then enters the mitochondrion where the enzyme coproporphyrinogen oxidase (E.C. 1.3.3.3) catalyzes oxidative decarboxylation of the propionate side chains of rings A and B to vinyl groups, the product being protoporphyrinogen IX (Fig. 1, enzyme 6). This enzyme has an absolute requirement for molecular oxygen in animals [15] but not bacteria [16]. It cannot act on the I isomer of coproporphyrinogen; thus in certain porphyric states uroporphyrinogen I and coproporphyrinogen I, but never protoporphyrinogen I, are overproduced and overexcreted. Activity of coproporphyrinogen oxidase has been found to be deficient in leukocytes [17,18] and fibroblasts [19] of patients with hereditary coproporphyria. This is believed to be the primary defect in this disease.

Protoporphyrinogen formerly was believed to be oxidized non-enzymatically to protoporphyrin, a reaction which is rapid in the presence of oxygen. However, preliminary studies suggested that this reaction is enzyme-catalyzed in mammalian liver mitochondria [20,21] and chicken erythrocyte hemolysates [22]. More recently firm evidence for an oxygen-dependent protoporphyrinogen oxidase (Fig. 1, enzyme 7) has been found in mitochondrial extracts from *Saccharomyces cerevesiae*[23] and rat liver [24]. It seems likely that activity of this enzyme is deficient in the human disease, porphyria variegata.

In a similar vein, although Fe^{+2} can be inserted into protoporphyrin non-enzymatically at appreciable rates [25], the insertion is catalyzed enzymatically in biological systems [26–28] by the enzyme heme synthetase or ferrochelatase (E.C. 4.99.1.1). Activity of this enzyme is deficient in liver [28], skin fibroblasts [28], and erythroblasts [29] of patients with protoporphyria. The enzyme will also utilize Co^{+2} and Zn^{+2} and perhaps other divalent cations instead of Fe^{+2} as metal substrate.

Some of the heme synthesized by the liver undergoes rapid catabolism, contributing to "early labelled" bilirubin [29a], but most normally is incorporated in hepatic hemoproteins, of which microsomal cytochrome P-450 quantitatively is most important.

Hepatic heme may be catabolized in several ways. The major route normally involves the microsomal enzyme heme oxygenase (Fig. 1, enzyme 9) (E.C. 1.14.99.3) which requires molecular oxygen and NADPH-cytochrome P-450 reductase for activity [30,31]. The final products of the reaction are iron, carbon monoxide (the carbon deriving exclusively from the α-methene bridge of heme), and biliverdin-IXα. Heme oxygenase can be induced by heme [32] and certain other substances [33–35].

The location and source of the heme which serves as substrate for heme oxygenase is uncertain. Indirect evidence is compatible with the model proposed in Fig. 2.

In mammals but not birds, biliverdin IX is reduced to bilirubin IX α by a cytoplasmic enzyme biliverdin reductase (E.C. 1.3.1.24). This enzyme can use either NADPH or NADH as cofactor; only the IXα isomer is reduced, not the β, γ, or δ isomers. Most of the bilirubin formed is then conjugated, the major products in most

species studied being glucuronides formed by ester-linkages with the propionic acid side chains of bilirubin [36].

CONTROL OF HEME BIOSYNTHESIS:
IMPORTANCE OF ALA SYNTHETASE

From the above summary of the heme biosynthetic pathway, it can be appreciated that this process is quite complex. Likewise the regulation of heme synthesis is complicated, and undoubtedly much remains to be learned about this regulation. A detailed consideration of this regulation is beyond the scope of this paper. Instead we shall focus on certain selected aspects of the problem in which we have been particularly interested.

A major site for control of the hepatic heme biosynthetic pathway is at the level of ALA formation [36–38]. And, despite the fact that ALA in mammalian liver may give rise to [39] or arise from [40] γ, δ-dioxovaleric acid [39,40], most of the ALA formed in animals, bacteria, and yeast undoubtedly is synthesized by ALA synthetase. Our previous inability to detect ALA formation from α-ketoglutarate in rat liver homogenate via direct reduction of α-ketoglutarate to γ, δ-dioxovalerate [41] is in accordance with the notion that ALA synthetase is required. This is also consonant with the general biochemical principle that the first enzyme in an unbranched biosynthetic pathway exerts a key "gating function" in controlling the flux of substrate down the pathway. ALA synthetase may be considered the rate-controlling enzyme of heme biosynthesis in most circumstances. However, if activity of ALA synthetase is markedly increased, as occurs in certain types of clinical or experimental porphyria, then other enzymes in the pathway may become rate-limiting, and accumulation of intermediates may occur proximal to these. Indeed in the hereditary hepatic porphyrias, exactly these phenomena appear to occur. As summarized above, the decreased activity of various enzymes of the pathway is genetically determined in these diseases. Under such conditions, the pattern of heme precursor accumulation will vary, depending upon the kinetic parameters (K_M, V_{max}) of the enzymes and their substrate concentrations. (See Tschudy and Bonkowsky [42] for a mathematical model of a simplified multi-step pathway of this type.)

Regulatory Heme and Control of ALA Formation

One theory which has proved very useful in considering the control of hepatic heme biosynthesis is that the activity of ALA synthetase, and hence of the entire pathway, is under negative-feedback, end-product control, as shown in Fig. 2. It is hypothesized that there exists a "regulatory" or "repressor" heme pool which tightly controls ALA synthetase activity [36–38,43]. Furthermore, as shown in Fig. 2, this pool may be envisioned as exchangeable (and in dynamic equilibrium) with heme in the liver cells' numerous hemoproteins.

Lascelles and her co-workers, studying *Rhodopseudomonas spheroides*, first obtained evidence that heme could both inhibit the activity and repress the synthesis of ALA synthetase [26].

Regulatory Heme and Experimental Porphyria

Major further advances were made possible by the discovery that certain porphyrinogenic chemicals increased markedly the activity of ALA synthetase, apparently by inducing new enzyme synthesis [44]. Evidence from studies with chick embryo liver cells [43] and rat liver [45–57] indicated that inducing drugs stimulated new
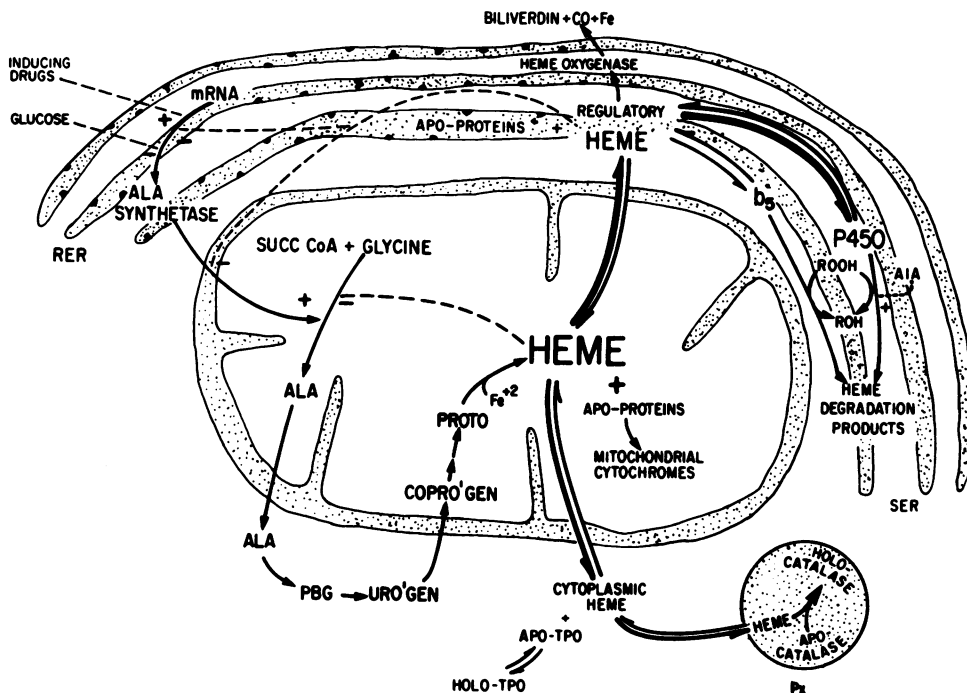
FIG. 2. Summary of Control of Hepatic Heme Metabolism and Compartmentalization of Hemoproteins. A mitochondrion is shown in the center of the figure, draped with protein-synthesizing rough endoplasmic reticulum (RER) to the left and smooth endoplasmic reticulum (SER) to the right. The heaviness of the arrows from mitochondrial heme to other pools roughly indicates the importance of the pathway in rat liver.

Minus signs indicate repression or inhibition; plus signs, induction or activation. Other abbreviations: AIA, allylisopropylacetamide; ALA, δ-aminolevulinic acid; b_5, cytochrome b_5; CO, carbon monoxide; Coprogen, coproporphyrinogen III; P-450, cytochrome P-450; PBG, porphobilinogen; Proto, protoporphyrin IXα; Px, peroxisome; ROH, hydroxy fatty acids, final products of lipid peroxidation; ROOH, lipid hydroperoxides; Succ CoA, succinyl-CoA; TPO, tryptophan pyrrolase; Uro'gen, uroporphyrinogen III.

enzyme formation, a point now firmly established for chick cells, by use of immunological techniques [48].

Heme was found to prevent or reverse these inducing effects [48]; it is unclear whether heme acts at the level of translation [49] or transcription [50]. In the intact rat, intravenous heme has rapidly decreased ALA synthetase activity [51].

Further experimental evidence for the existence of a regulatory heme pool is that a number of potent, porphyrinogenic compounds, which upset the normal control of heme biosynthesis and induce excess production of ALA, PBG, and porphyrins, may be envisioned to reduce the size of the regulatory heme pool [36,52]. For example, allylisopropylacetamide (AIA) degrades liver heme to porphyrin-like "green pigments" (not biliverdin), rapidly decreasing total hepatic heme and microsomal heme. It produces marked increases in ALA synthetase activity and heme precursor accumulation. In contrast, its congener propylisopropylacetamide (PIA), which does not accelerate heme degradation, while a potent inducer of cytochrome P-450, is only a weak inducer of ALA synthetase [53].

The porphyrinogenic compounds 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) and griseofulvin have been found to inhibit heme synthetase, particularly in mice [53]. It has been proposed that this inhibition is sufficient to deplete the

regulatory heme pool and increase ALA formation. Thus the protoporphyrin accumulation characteristically seen in such mice may be due both to a decrease in the efficiency of its utilization and a secondary increase in synthesis. Other actions of the drug remain quite possible, however, since Anderson [53a], Rifkin [53b], and Sinclair et al. (unpublished observations) have found in the chick embryo, the organism in which ALA synthetase is most inducible by DDC, that (i) even 95 percent inhibition of heme synthetase by low doses of DDC does not lead to ALA synthetase induction and (ii) there is no decrease in cytochrome P-450 following DDC administration in amounts which produce marked protoporphyrin accumulation. These results raise questions about the notion that the DDC effect in inducing ALA synthetase relates solely to inhibition of heme synthesis. On the basis of this and certain other evidence, we favor the view that ALA synthetase induction occurs by other mechanisms as well.

A final point of interest is that when two compounds which affect heme metabolism in different ways are given together, a "synergistic" effect is observed [53-55]. That is, the induction of ALA synthetase is much greater than with either alone and is also greater than simply the additive effects of the two separately. An apparent example of this kind of effect is discussed below in the section on Iron and the Regulation of Hepatic Heme Metabolism.

Regulatory Heme and Clinical Porphyria

The human disease, acute intermittent porphyria (AIP), provides a clinical parallel to these ideas. As mentioned above, in this disease there is a genetically determined decrease in activity of uroporphyrinogen synthetase (Fig. 1, enzyme 3). Some persons with this defect do not overproduce ALA or PBG, whereas others do. All, however, are probably uniquely sensitive to drugs which induce cytochrome P-450. Drugs such as phenobarbital or phenytoin are well known to exacerbate or first make manifest the biochemical and clinical features of AIP [37,38]. The increase in ALA production may result from a synergism between the genetically determined partial deficiency in heme production and the drug-induced increase in heme utilization for cytochrome P-450. These two effects, acting synergistically, may be envisioned to deplete the regulatory heme pool and secondarily to increase ALA formation.

We first obtained convincing evidence for the existence of heme regulation of human ALA formation when we demonstrated that heme, given intravenously to a patient with AIP, promptly and dramatically diminished her ALA and PBG overproduction [56, Fig. 3]. This phenomenon has since been reproduced by others [57–60] in nearly all patients thus far treated. In some patients, heme therapy has also been of striking clinical benefit, and in none has it made patients worse, although a large dose led to transient oliguria in one [58]. Furthermore, heme therapy has also been effective in apparently reversing the heme precursor overproduction in variegate porphyria [58], congenital erythropoietic uroporphyria [58], and protoporphyria [51]. In the latter two conditions, porphyrin overproduction arises from erythroid cells, suggesting that the heme effect is not limited to the liver, although a different interpretation was offered by the authors of the abstract on protoporphyria. These results support the concept of heme regulation of ALA synthesis and negative-feedback control of heme synthesis by the end-product, in man as in lower animals and bacteria. They also establish a new and possibly life-saving treatment for porphyria.

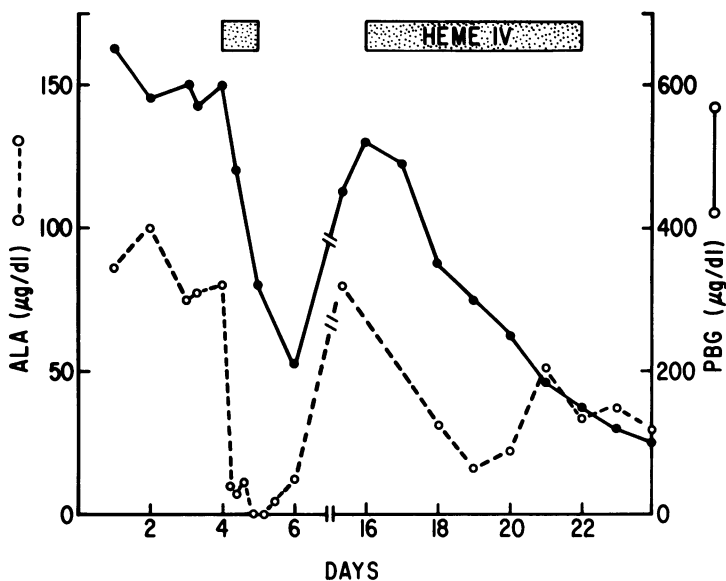However, further pharmaceutical development and pharmacological testing

FIG. 3. First Demonstration of Heme Repression of Hepatic ALA Synthetase Activity in Man. Effect of Intravenous Heme on Serum Concentrations of δ-aminolevulinic acid (ALA) and porphobilinogen (PBG) in a patient with acute intermittent porphyria. (Redrawn from [56]).

should be done. For example, the minimal effective heme dose and the optimal therapeutic regimen have not been established. An oral heme preparation, which can be absorbed by the gut and enter hepatocytes and other cells, would be highly desirable. Unfortunately, thus far we have been unable to interest pharmaceutical companies in producing heme or in trying to develop such an oral preparation. Heme itself is not patentable, and perhaps special formulations would not be profitable. Regardless of this, we believe that heme for human use should be produced as a service to those patients whose well-being or very lives may depend upon it.

In summary, there is now overwhelming clinical and experimental evidence that the heme biosynthetic pathway is subject to end-product negative feedback control, operating primarily at the level of ALA synthetase. The major effect of relatively low endogenous heme concentrations in decreasing activity of the enzyme is probably by *repression* of enzyme synthesis. The purified enzyme activity is also *inhibited* by sómewhat higher concentrations [62,62a]. Since, as shown in Fig. 2, heme is synthesized in the mitochondrion which is the sub-cellular location of ALA synthetase, the "local" concentration of heme may be sufficient to inhibit the enzyme activity directly under physiological conditions.

IRON AND THE REGULATION OF HEPATIC HEME METABOLISM

Iron Lack

As mentioned in the first section, in animals, ferrous iron is the physiological metal substrate for the final enzyme of the heme biosynthetic pathway. Therefore an adequate supply of iron is necessary for normal heme synthesis. Anemia is often due to iron lack, and in such states erythrocyte protoporphyrin levels are increased, as expected. In contrast, although extensive studies have not been done, we are unaware of evidence that hepatic heme synthetase activity is deficient in animals with iron

lack. Indeed, such animals have increased levels of oxidative demethylation of aminopyrine and hexobarbital [63], activities dependent upon the hemoprotein cytochrome P-450. Cytochrome P-450 levels were not affected by iron lack [63,64].

This difference in sensitivity of bone marrow and liver to iron lack probably relates to the fact that the liver's iron requirement is considerably less than the bone marrow's. Also hepatocytes recycle their iron efficiently, since their hemoproteins never leave these cells, whereas erythrocytes are constantly leaving the bone marrow, eventually to be destroyed largely at distant sites of reticulo-endothelial activity.

Iron Overload and Porphyria Cutanea Tarda

In contrast, iron excess does affect hepatic heme metabolism. An early observation in this regard was that patients with porphyria cutanea tarda (PCT) commonly had hepatic siderosis and that the porphyric state was ameliorated by iron removal [37,38,65,66]. Iron overload may be considered a promoting or potentiating factor in pathogenesis of this type of porphyria. Other factors, however, are clearly important in pathogenesis since, on the one hand, most patients with severe hemosiderosis or hemochromatosis do not have overt porphyria; and on the other a toxic porphyria resembling PCT may be produced in animals or man without hepatic siderosis.

Hepatic Iron and ALA Synthetase

It has long been recognized that many patients with PCT have hepatic fibrosis or cirrhosis, as well as an increase in hepatic iron deposition. Many of these patients also imbibe ethanol heavily. In addition, cirrhotic patients without overt PCT commonly exhibit excess porphyrinuria, excreting increased amounts of uro- and coproporphyrins, particularly the former [37,67,68]. Cirrhotics, especially those with large porto-systemic shunts, also tend to have hemosiderosis [69]. The question therefore arose whether disturbances of porphyrin metabolism in cirrhotic subjects resulted from hepatic iron deposition or from other causes.

To answer this question and to learn more about control of human hepatic ALA synthetase activity, we measured ALA synthetase activities in twelve cirrhotic subjects and in 16 controls who had a similar degree of hepatic fat and inflammation, but without cirrhosis [68]. Activity of the enzyme, when assayed optimally, in the presence of an exogenous system for succinyl-CoA generation, was significantly ($p <$ 0.005) increased in homogenates of cirrhotic livers (1297 ± 664 pmol ALA h^{-1} mg^{-1} protein) vs. the controls (548 ± 252 [mean ± 1 SD]).

In the controls in this study, as shown in Fig. 4, activity of hepatic ALA synthetase was correlated positively ($P = 0.04$), albeit weakly, with hepatic iron concentration. In homogenates from cirrhotic livers, the activity of ALA synthetase also tended to be higher in subjects with more liver iron, but the correlation was even less close, suggesting that other factors are more important in this group [68]. (It is of interest that ethanol ingestion could not be incriminated as such a factor, despite the fact that larger doses of ethanol modestly and briefly induce rat liver ALA synthetase [70].)

These studies suggest that, even in subjects with no overt abnormality in porphyrin metabolism, hepatic iron status affects the heme synthetic pathway. They are corroborated by the recent report that activity of hepatic ALA synthetase is increased in rats loaded over six weeks with iron-dextran [71].

The effects of acute, high-dose iron administration on rat liver ALA synthetase have been studied more extensively. Stein et al. first demonstrated a striking "synergism" or potentiation between AIA and ferric citrate on induction of hepatic ALA synthetase in rats [72,73]. The increase was not due to enzyme activation nor to
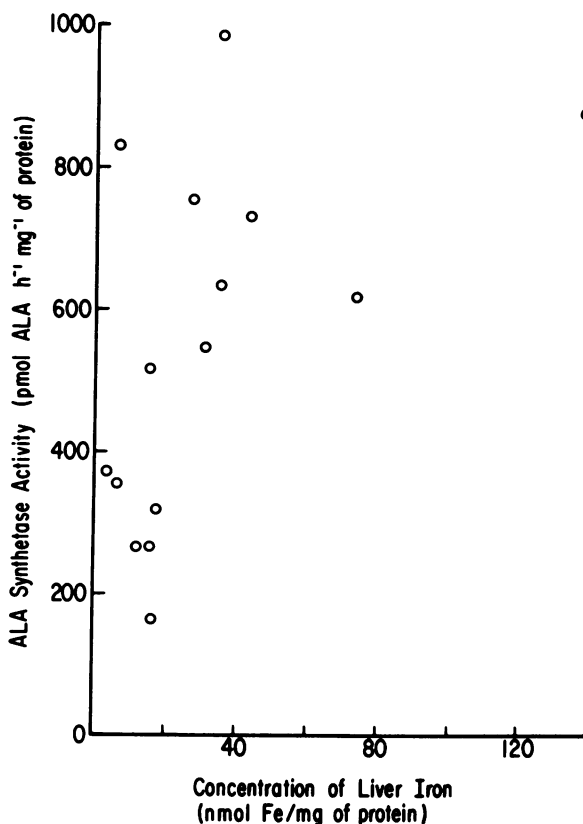
FIG. 4.   Human Hepatic ALA Synthetase vs. Hepatic Iron Concentration in Subjects Without Cirrhosis. Redrawn with permission from [68].
The regression equation is $Y = 3.87\ x + 426$, $r = 0.53$, $P = 0.04$.

alteration in enzyme half-life, and indirect evidence was obtained for increased mRNA synthesis in mediating the induction [73]. We have confirmed this synergistic effect in rats (Fig. 5) and, in preliminary studies with chick embryo liver cells in culture, a similar modest effect has been observed.

While the molecular basis for this effect is unknown, recent studies have suggested several possible explanations. In considering these, it is convenient to refer to Fig. 6, which summarizes known and possible iron effects on hepatic heme metabolism. Site 1 represents the role of iron as substrate for heme synthetase.

Site 3 represents a role for iron in inhibiting uroporphyrinogen decarboxylase (Uro-D, cf. Fig. 1, step 5), first demonstrated in cytoplasmic extracts of pig liver by Kushner et al. [74]. Incubation for 2h at 37°C with 0.7 mM ferrous iron decreased Uro-D by up to 40 percent. They concluded that these studies were relevant to the pathogenesis of PCT, in view of the iron excess common in this disease and in view of the fact that porphyrins containing five to eight carboxyl groups are the major products over-produced and over-excreted in PCT.

In other studies of similar design, iron inhibition of uroporphyrinogen III cosynthetase had also been observed [75]. Nevertheless, it was unclear that these studies in vitro with inorganic iron salts provided an entirely adequate model for
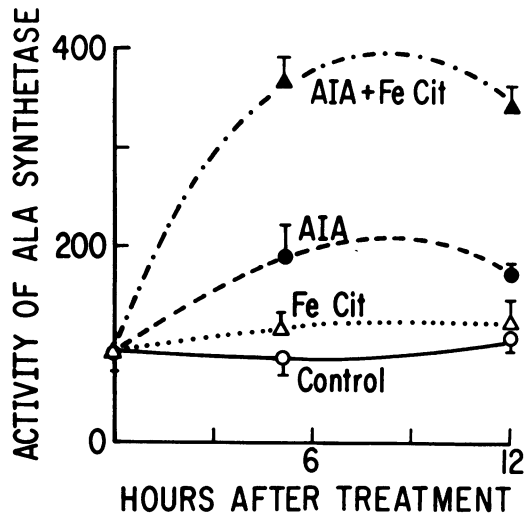
FIG. 5. Iron Potentiation of ALA Synthetase Induction by Allylisopropylacetamide. Female Bullalo rats weighing 130–180 g were fasted for 24 h and then treated with allylisopropylacetamide (AIA, 400 mg/kg SC) and ferric citrate (700 mg/kg po) as shown. ALA synthetase activity for each data point was determined three times in homogenates of pooled liver from 3–5 rats. Results are mean ± SEM, expressed as activity relative to 0 time control.

PCT in which the hepatic iron, chronically accumulated, is presumably stored in ferritin and hemosiderin.

Subsequently, activity of Uro-D was found to be decreased in liver extracts and hemolysates of patients with PCT [13,76]. However, activity in the liver did not
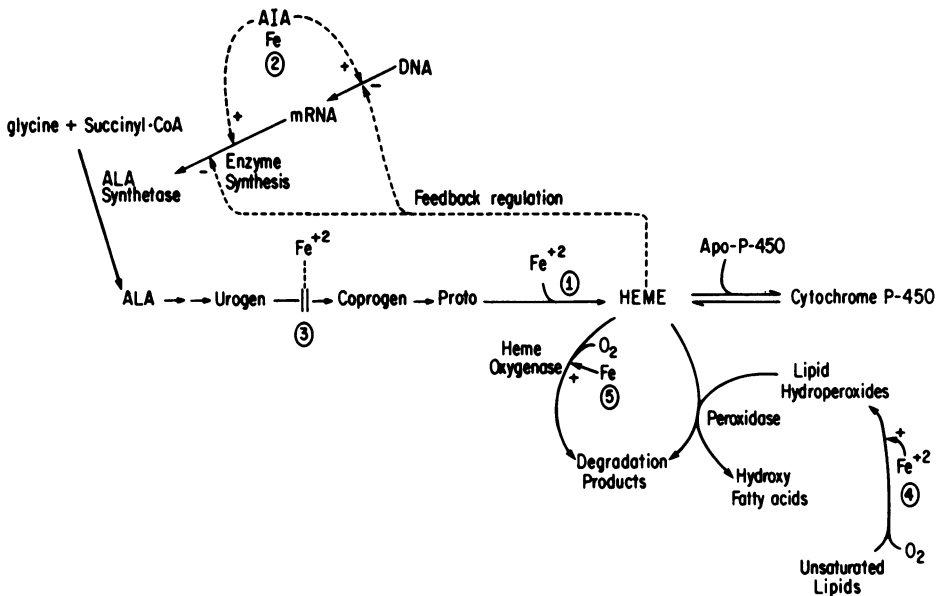

FIG. 6. Possible Sites of Iron Effect on Hepatic Heme Metabolism.

increase after iron removal and clinical remission of disease [13]. This failure to observe an increase in enzyme activity may relate to the fact that the cytosolic liver extract used as the enzyme source is diluted 25-fold for the assay. The dilution may decrease the iron concentration too low to be inhibitory in the assay. If this explanation is correct, then the finding of decreased Uro-D activity in extracts from PCT patients, compared with controls, is evidence for a genetically determined decrease in enzyme activity (see below). It also suggests that addition of iron to the assay mixture might be expected to inhibit activity of the enzyme both from normal human and PCT liver.

Kushner et al. have also found that Uro-D activity is decreased in hemolysates from relatives of PCT patients [13] who clinically are unaffected and who presumably have no iron overload. However, this finding of decreased erythrocyte enzyme activity in PCT patients or their relatives has not been confirmed by Elder, studying other kindreds with a different Uro-D assay [14]. Currently, it is unclear whether these differences are attributable to the different assays or to the different patients studied; an answer to this question should not be difficult to achieve, requiring only that both assays be run on the same hemolysates. The differing results can be reconciled by the existence of two forms of PCT, a genetic and an acquired sporadic form. Kushner et al. appear to have studied kindreds with the former, whereas Elder et al. studied patients with the latter.

In any case, while direct iron inhibition of Uro-D may contribute to the pathogenesis of PCT, the possibility that iron acts in other ways in this disease should not be discarded [77].

Iron and Stimulation of Lipid Peroxidation and Heme Oxygenase

An alternative site for iron action in heme metabolism results from the ability of iron to stimulate lipid peroxidation (Fig. 6, Site 4). This effect was originally described *in vitro* in mitochondrial preparations, incubated with ferrous salts (0.05–0.3 mM) and ascorbate (0.3–2.0 mM) [78,79]. It was found that mitochondrial swelling, dysfunction, and eventual disruption occurred [79]. Later, iron was found to stimulate NADPH- and enzyme-dependent microsomal lipid peroxidation *in vitro*, with accompanying losses of heme and cytochrome P-450 [80–83]. Hrycay and O'Brien have proposed that this loss of cytochrome P-450 heme is due to its action as a lipid peroxidase, during which the heme is degraded [84], as suggested in Fig. 6. Experiments of this type have led to the suggestion that toxic effects of iron overload *in vivo* are secondary to enhancement of lipid peroxidation; however, evidence for this has proved elusive.

Nevertheless, a large single dose of iron-dextran (100 mg Fe/kg) was found 24 hours later to decrease microsomal cytochrome P-450 concentration in rat liver by 35 percent and to increase ALA synthetase activity 3.4-fold [82]. Lipid peroxidation *in vivo* was not examined in these experiments.

Recently a single parenteral injection of a large dose of iron [34,34a] or certain other heavy metals [34] has been observed to lead to an increase in rat hepatic heme oxygenase activity (Fig. 6, Site 5). It is interesting that this activity has several similarities to the heme degradation reaction associated with lipid peroxidation: both utilize NADPH, O_2, and native microsomal protein ("enzyme"); both are activated by fasting; and both produce carbon monoxide and green heme degradation products, biliverdin $IX\alpha$ in the case of heme oxygenase and uncharacterized products in the case of the reaction associated with lipid peroxidation. These latter products may include a mixture of biliverdin isomers.
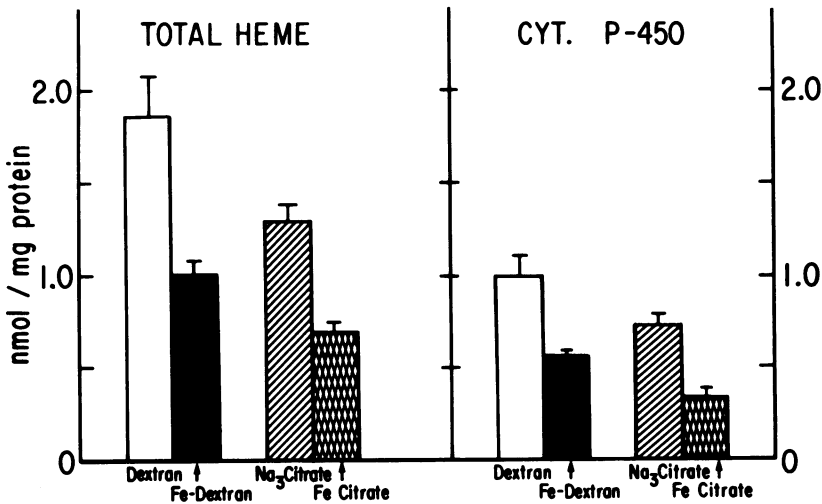
FIG. 7. Chronic Iron Overload and Hepatic Microsomal Heme and Hemoprotein Concentrations in the Rat. 250–350 g male Wistar rats received three 1 ml doses of iron-dextran (50 mg/iron/rat, IP) on alternate days and were killed on the eighth day. Other rats received repeated 1 ml doses of ferric citrate (85–120 μmol IP), five times per week for 9 weeks. Controls received appropriate amounts of dextran-40 or sodium citrate, as shown. Other controls for the iron-dextran experiment received three 1 ml doses of 0.15 M NaCl; microsomes from these saline-treated controls had total heme concentration = 1.7 ± 0.27 and cytochrome P-450 concentration = 0.8 ± 0.09 nmol/mg protein. The latter value is significantly less than that for microsomes from rats receiving dextran-40 ($p < .05$). (Data redrawn by permission from [128].)

As shown in Fig. 7, we have found that decreases in rat hepatic microsomal cytochrome P-450 and heme were even greater with repeated administration of iron dextran than with a single dose. Cytochrome b_5 concentrations also fell, although to a lesser degree. Furthermore, as shown, similar effects were observed when comparable iron overload was achieved by chronic injections of ferric citrate. It is uncertain whether these changes occur in relation to increased lipid peroxidation as well as increased activity of heme oxygenase[71]. Whether these changes are associated with decreased rates of mixed function oxidase activity *in vivo* and whether similar changes occur in patients with hemosiderosis or hemochromatosis are other important unanswered questions. It is also unknown whether these decreases in microsomal heme and hemoproteins are associated with decreased rates of hepatic heme synthesis (e.g., secondarily to a block in uroporphyrinogen III production or utilization as discussed above).

Regardless of whether hepatic iron excess stimulates heme catabolism due to increased lipid peroxidation or heme oxygenase, the net effect on hepatic heme homeostasis and ALA synthetase would be similar. But these effects on heme degradation are different from those due to iron inhibition of heme synthesis.

Studies on Acute Iron Potentiation of ALA Synthetase Induction

Currently, we are studying the mechanism whereby a single dose of ferric citrate, enterally administered, exerts a synergistic effect on AIA-mediated induction of rat hepatic ALA synthetase. To our surprise, despite the results just described for chronic iron overload, in our initial studies of acute ferric citrate administration, we failed to obtain evidence either that iron inhibited Uro-D or decreased microsomal cytochrome P-450 concentrations.

In these experiments 125–150 g female Sprague-Dawley rats were fasted for 24 h

TABLE 1

Activity of Uroporphyrinogen Decarboxylase (Uro-D) and Concentrations of Cytochrome P-450 in
Livers of Rats Treated with Ferric Citrate and AIA

Treatment		Uro-D	Microsomal Cytochrome P-450
Gavage	SC	(Units/mg protein)	(nmol/mg protein)
Sodium Citrate (Control)	+ NaCl	1.86 ± 0.08 range 1.65 – 2.02 (n = 4)	0.99 ± 0.003
Ferric Citrate (Iron only)	+ NaCl	—	1.00 ± 0.007
Sodium Citrate (AIA only)	+ AIA	2.1 , 2.2	0.72 ± 0.008
Fe Citrate (Combination)	+ AIA	1.8 , 1.7	0.71 ± 0.005

Female Sprague-Dawley rats (125–150g), fasted for 24 h, were treated as shown and sacrificed 17 h later.
Uro-D assays were kindly performed by Dr. Jas. Kushner, using his standard assay [75]. Microsomal Cytochrome
P-450 determinations were performed by the method of Omura and Sato [125] and proteins by the method of Lowry
et al. [126], modified in that microsomes were incubated 10 min with deoxycholate (0.5%) prior to assay.

and then treated with ferric citrate (700 mg/kg by gavage), AIA (400 mg/kg S.C.
dissolved in 0.15 M NaCl), or the combination. Controls received sodium citrate and
0.15 M NaCl. As listed in Table 1, ferric citrate treatment alone had no effect on
cytochrome P-450 concentrations nor on Uro-D activity. AIA treatment had slight, if
any, effect on Uro-D, although it led to a significant decrease in microsomal
cytochrome P-450 concentrations as expected. Effect of the combination treatment
on these parameters was not different from AIA alone.

Furthermore, no iron effect on lipid peroxidation *in vivo* was found. Lipid
peroxidation was assessed by measurement of malonyldialdehyde (MDA). Treat-
ment with ferric citrate or AIA alone did not increase endogenous hepatic MDA
concentrations (Fig. 8). However MDA concentrations were moderately increased in
livers of rats treated with AIA and ferric citrate 17 h before sacrifice.

In these experiments, hepatic iron concentrations in rats given ferric citrate were
increased by 40–50 percent above controls and iron potentiation of ALA synthetase
(cf. Fig. 5) induction by AIA was demonstrated. Therefore, our failure to observe an
effect of ferric citrate alone, either upon liver MDA or cytochrome P-450 concentra-
tion, cannot be attributed to the iron not entering the liver or not exerting a
metabolic effect.

These preliminary results do suggest that 17 h after administration of ferric citrate
and AIA to rats, their livers contained increased amounts of MDA, a product of lipid
peroxidation. However, the concentration of microsomal cytochrome P-450 was not
lower than in livers of rats given AIA alone.

In interpreting these results, several points should be kept in mind:

1. Our measurements *in vitro* may not provide an adequate reflection of *in vivo*
events. For example, measurements of Uro-D were done with diluted cytoplasmic
extract; and iron, which may have been reversibly inhibitory *in vivo*, could have been
diluted and no longer active *in vitro*. Also, concentrations of cytochrome P-450 and
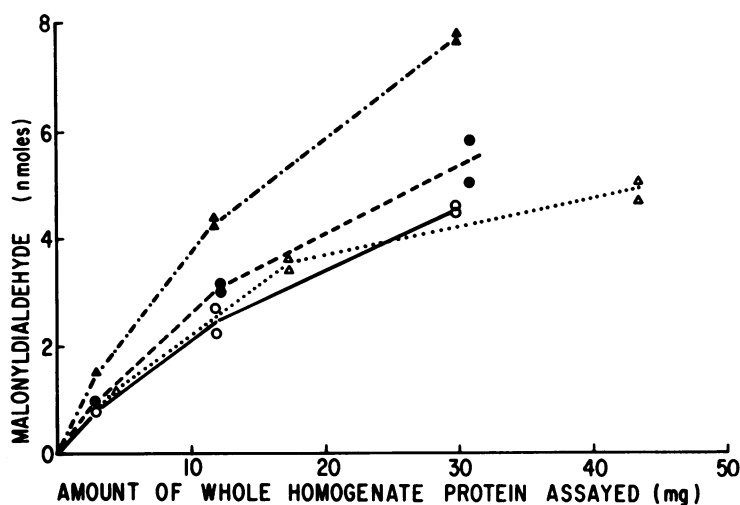MDA may not indicate actual rates of hepatic microsomal heme synthesis or

FIG. 8. Content of Malonyldialdehyde, a Product of Lipid Peroxidation in Homogenates of Livers from Rats Treated with Allylisopropylacetamide (AIA) and Ferric Citrate. Female Sprague-Dawley rats (125–150 g) were fasted for 24 h and then given AIA (400 mg/kg SC), ferric citrate (700 mg/kg, po) or the combination. They were killed 17 h later. Livers were perfused exhaustively with cold 0.15 M KCl and a 25% homogenate of pooled livers of five animals was prepared. Portions of these homogenates were assayed for malonyldialdehyde by the method of Ottolenghi [78].

catabolism since both P-450 and MDA were being formed and degraded continuously. Because of these problems, we have recently undertaken the study of hepatic heme synthesis and degradation in intact rats *in vivo* (see below).

2. Iron may inhibit some other step in heme biosynthesis besides Uro-D. Studies of the overall rate of heme synthesis from ALA *in vivo* should uncover such a block also.

3. Finally, iron may potentiate the drug-mediated induction of ALA synthetase by a mechanism unrelated to the regulatory heme pool or heme biosynthesis. The effect could even be extra-hepatic, although our preliminary studies with chick embryo liver cells in culture do suggest an iron effect in these isolated hepatocytes. Perhaps, therefore, iron administration has an effect on ALA synthetase transcription or translation not mediated by regulatory heme, as suggested in Fig. 6, Site 2. Such an effect would be analogous to the effect of iron on stimulation of apo-ferritin synthesis [85].

Comments on the Hypothesis that Iron and Other Metals Act as Physiological Regulators of Hepatic Heme Metabolism

It has recently been proposed that iron itself rather than the iron-protoporphyrin chelate is the "proximate" physiological regulator of heme metabolism [86]. In this view, the chelation of iron by protoporphyrin as Fe-heme is considered an efficient means for getting iron across various membranes. The effects of heme on ALA synthetase and heme oxygenase activities are envisioned to be due to the iron itself, not the protoporphyrin chelate of iron.

Much of the evidence offered in support of this suggestion has been obtained from studies, primarily carried out in rats, on effects of divalent cobalt on heme metabolism. Certain other divalent metallic ions (Zn, Ni, Cu, Pt, Cd, Hg, Pb, Mn) and Fe^{+2} and Fe^{+3} have also been used, albeit less extensively [34,87]. It is suggested that

effects of cobalt and other non-ferrous metals are due to the same mechanism as for iron.

Administration of $CoCl_2$ to intact rats [88–90] or to chick embryo liver cells in culture [91] is followed by a rapid decrease in ALA synthetase activity. This has been interpreted to be due principally to a direct repression by the metal of the synthesis of the enzyme [86]. However, others ave recently shown that the decrease in ALA synthetase activity is probably due to the protoporphyrin chelate of cobalt (Co-protoporphyrin) and not the metal itself [88a,88b]. The decrease may well be due to inhibition of activity as well as inhibition of synthesis [89]. These data, by analogy, support the more traditional view that heme or Fe-protoporphyrin (like Co-protoporphyrin), not "free" ionic iron, regulates the activity of ALA synthetase.

The proposal that administration of non-chelated ionic iron and cobalt act in the same way on ALA synthetase raises other problems since ferric citrate administration potentiates the drug-mediated induction of ALA synthetase (Fig. 5, [73,74]) during the same interval in which cobalt administration leads to a decrease in induced enzyme activity [88,89].

A second site of action of heavy metals on heme metabolism is their ability, at high toxic doses, to induce hepatic heme oxygenase [33,34,86,87,90,91]. It has been proposed that all these metals act to produce this induction and to decrease activity of ALA synthetase by similar mechanisms [86]. However, much lower doses of $CoCl_2 \bullet 6H_2O$ are required to maximally inhibit ALAS (3 mg/kg) than to maximally induce heme oxygenase (60 mg/kg) in adult [92] and fetal [92a] rats. Similar marked differences in dose response have also been observed for cobalt in cultured chick embryo hepatocytes [91] and for endotoxin in rats [35]. The response of hepatocytes to increase activity of heme oxygenase is associated with toxic (near LD_{50}) doses of metals and endotoxin, and it seems unlikely that this is a physiological regulatory mechanism for heme degradation.

The mechanism underlying increase of heme oxygenase activity by metals, endotoxin or heme itself is not understood. Some consider it due to induction by heme itself in a "regulatory" pool derived from increased degradation of cytochrome P-450 [35] or from newly synthesized heme normally destined for cytochrome P-450 ([93], see Fig. 2), whereas others believe the metals directly [86] or through metalloporphyrins [93a] induce synthesis of heme oxygenase.

Further work suggesting iron as the key regulator of heme metabolism has recently been presented [94] using neonatal rats in which erythrocyte hemolysis may increase the heme, and therefore iron, available to the liver. Normally, these neonates are resistant to inducers of ALA synthetase. Following repeated injections of Na_2 EDTA, however, a response of ALA synthetase induction to AIA administration occurred. These data were interpreted as indicating that iron represses ALA synthetase, but many other possibilities exist.

Two final considerations are worthy of note: 1. As mentioned above (Fig. 4), in man, activity of hepatic ALA synthetase is directly correlated with liver iron concentration, whereas the new hypothesis [86] would suggest the opposite. 2. If excess hepatic iron repressed induction of ALA synthetase, iron might be useful as a treatment for acute hepatic porphyrias. However, ferric citrate potentiation of ALA synthetase induction in rats (Fig. 5) makes this theoretically risky. Also, an AIP patient with a severe fatal attack has been described who also was found to have a considerable degree of hepatic siderosis [95]. The excess iron was suggested to have exacerbated rather than to have mitigated the biochemical and clinical abnormalities.

DIET AND HORMONAL CONTROL OF HEPATIC ALA SYNTHETASE

The Glucose Effect in Experimental and Clinical Porphyria

An effect of diet on experimental porphyria was identified before the biochemical basis for the porphyria had been discovered [96], when it was found that carbohydrate or protein could prevent AIA-induced porphyria in rats. Soon after the discovery by Granick and Urata [44] that ALA synthetase was inducible by a porphyrinogenic compound, it was shown that induction of this enzyme in mammalian liver could be blunted or prevented by high dietary intake of glucose [97] (see Fig. 9). The effect of glucose in diminishing induction of hepatic ALA synthetase provided an explanation for its ability to prevent production of experimental porphyria. It also provided a rationale for the observation, repeatedly made, that fasted animals responded to AIA and other porphyrinogenic compounds with more induction of the enzyme and overproduction of heme precursors than did non-fasted animals. A similar "glucose effect," in which glucose administration repressed the synthesis of other enzymes, had previously been described in microorganisms [98–100] and rat liver [101,102].
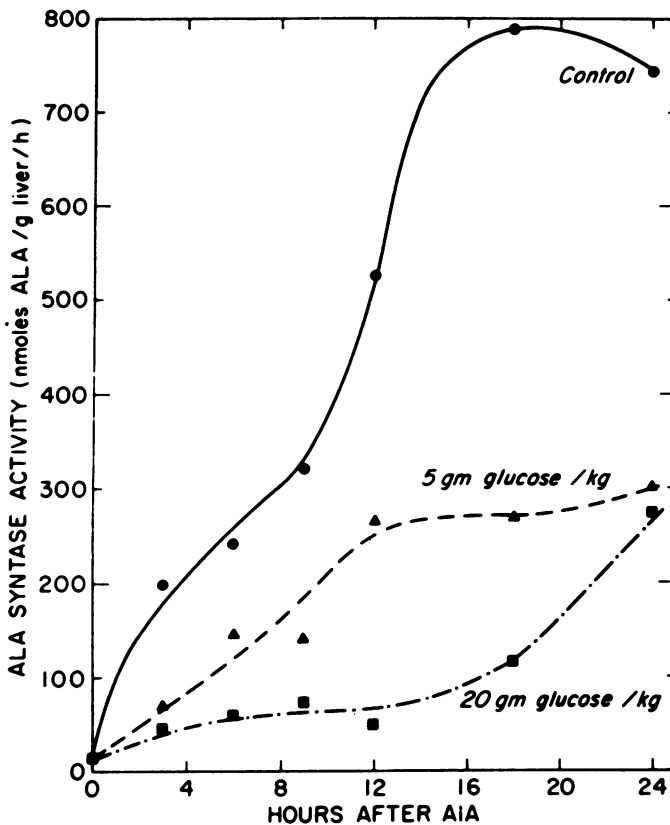

FIG. 9. The glucose effect on induction of hepatic δ-aminolevulinic acid synthetase by allylisopropylacetamide. Female Sprague-Dawley rats, weighing 125–175 g, were fasted 48–72 h prior to sacrifice. Some were given oral glucose at two different dose levels as shown and 15–20 min later received 400 mg/kg allylisopropylacetamide subcutaneously. Control rats received distilled water followed by the same dose of drug. Each point represents activity in pooled homogenate from four rats. Reprinted by permission from [41].

Shortly thereafter these findings in experimental porphyria were put to clinical use; Welland et al. [103], showed that ALA and PBG excretions were reciprocally related to carbohydrate and/or protein intake in acute intermittent porphyria (AIP). These observations have been confirmed repeatedly by others [104–106]. We have also shown that glycerol in man [106] and glycerol and fructose in the rat [41] are as potent as glucose itself. Others have recently demonstrated an effect of fructose in man also [109].

A glucose effect in clinical states of porphyrin overproduction is not limited to AIP: it has also been demonstrated in variegate porphyria [107] and in protoporphyria [108]. Therefore, a high intake of glucose is a cornerstone of therapy, particularly in the hereditary hepatic porphyrias [37,38]. It is often assumed that a glucose effect does not occur in the erythropoietic porphyrias. However, several pieces of evidence suggest otherwise:

1. A high levulose (fructose) intake was followed by increased erythrocyte uroporphyrinogen synthetase and decarboxylase activities in hemolysates from AIP patients, activities suggesting a possible carbohydrate effect on the erythroid apparatus [109]. Unfortunately, this report did not include the duration of levulose therapy and the daily IV dose purportedly given (372 mol or 67 kg) seems impossibly high. Nor was it clear whether the sugar was being administered at the time that bloods were drawn for the enzyme assays and whether levulose directly activated these enzymes. Unless therapy had been prolonged (1–3 weeks) prior to assay, any measured increases in hemolysate enzyme activities presumably would be due to activation of enzymes already present in circulating erythrocytes. The only alternative possibility is that the treatment somehow stimulated release of young erythrocytes and reticulocytes into the peripheral circulation, cells known to have higher activities of uroporphyrinogen synthetase.

In this same paper [109], peripheral blood leukocyte ALA synthetase was significantly decreased (-35 to -72%, $p < 0.001$) following levulose therapy, further suggesting that a glucose effect may occur in the bone marrow in human porphyria.

2. As mentioned above, a high carbohydrate intake has been found to decrease fecal protoporphyrin excretion in protoporphyria [108]. The mechanism for this effect is unknown, and may not be due to alterations in the rate of protoporphyrin production *per se*, but rather to alterations in its secretion into bile or of its enterohepatic circulation. Furthermore, developing erythrocytes may not be the sole source of the excess protoporphyrin in this disease [110], although two research groups [111,112] have recently made this proposal. Since decreased activity of heme synthetase has been found in homogenates of liver and cultured skin fibroblasts [28], as well as in erythroblasts [29], from patients with protoporphyria, the possibility of extra-erythrocytic sources of protoporphyrin production in this disease cannot yet be discarded entirely. Despite these lingering uncertainties, the existence of a glucose effect in protoporphyria raises the possibility that glucose can repress overproduction of heme precursors in the bone marrow as well as in the liver.

3. As mentioned before, intravenous heme infusions decrease porphyrin overproduction in congenital erythropoietic porphyria [58], a disease in which the bone marrow is acknowledged to be the source of such overproduction. It is therefore clear that at least one repressor of ALA synthetase (heme) can act both in the liver and the bone marrow in human porphyrias, and it suggests that others (carbohydrates) may do the same.

Unfortunately, the glucose effect is not uniform in all the animals of any one experiment (Bonkowsky, HL, unpublished observations) or in patients [56,105,106],

for unknown reasons. Patients who have failed to respond biochemically to high carbohydrate intakes have responded to heme infusions[56,59], indicating that heme should be used in those patients who fail to respond sstisfactorily to more conservative therapy.

Biochemical Basis for the Glucose Effect

In microorganisms, the earlier hypothesis of "catabolite repression" [100] as the explanation for the glucose effect has given way to the well-supported conclusion that this effect is mediated by 3'5'-cyclic-AMP (cAMP) [113,114]. Glucose inhibits the synthesis of cAMP in *E. coli* with a resultant decrease of intracellular concentrations to one-tenth of that which occurs in the absence of glucose. cAMP, by binding to a specific protein called "CAP," enhances transcription of many (but not all) bacterial genes [113], including those subject to the glucose effect. Thus glucose acts to decrease transcription by decreasing cAMP concentrations. Interestingly, this appears to be an effect mediated by glucose itself, not a metabolite.

In rat liver, several enzymes induced by cAMP (tyrosine aminotransferase, serine dehydrase, and phosphoenolpyruvate carboxykinase) are repressed by glucose feeding, which suggested that the glucose effect was mediated by cAMP in mammals as well as in bacteria. However, this notion has proved not to be correct [113,115].

With respect to cAMP and hepatic ALA synthetase, we found that even large doses of cAMP (400 mg/kg) or dibutyryl cAMP (200 mg/kg), given 7 and 3 h before sacrifice decreased enzyme activity by 20–34 percent, rather than inducing it [41]. These compounds also decreased activity after AIA treatment (400 mg AIA/kg, 5 h before sacrifice). These effects may be due to enzyme inhibition since both compounds inhibited the formation of ALA as shown in Table 2.

Theophylline, an inhibitor of the phosphodiesterase which hydrolyzes cAMP, decreased ALA synthetase activity when given alone or with dibutyryl cAMP, the combination acting in an additive fashion [41]. Dibutyryl cAMP did not materially influence the glucose effect on ALA synthetase [41]. The findings of others in intact rats [116,117] were similar to ours just described.

However, in suspensions of isolated rat liver cells, dibutyryl cAMP (50 μM) or AIA (1.2 mM), while producing no induction alone, together led to a 4–5 fold increase of ALA synthetase activity after 6 h of exposure [118]. In this system, the cyclic nucleotide thus appears to exert a "permissive effect" on enzyme induction, similar to that described *in vivo* for hydrocortisone [119]. A similar role for cAMP has recently been described in chick embryo liver cells in culture [120]. It is also of

TABLE 2
Inhibition of Rat Liver ALA Synthetase by Cyclic AMP

Cyclic Nucleotide	Final Concn. (mM)	Relative Activity (%)
None	0	100
Cyclic AMP	5	71
	0.1	78
	0.01	79
N[6], O[2']-dibutyryl	5	43
Cyclic AMP	0.1	86

Assays were performed by the method of Marver et al. [127] on homogenate of the pooled livers of ten 48 h fasted female Sprague-Dawley rats (100–150g), given AIA (400 mg/kg SC) 18 h before sacrifice. Control activity of ALA synthetase was 630 nmol ALA/g liver/h.

interest that glucose did not prevent induction produced by AIA and dibutyryl cAMP in isolated rat liver cells [118], a finding which parallels Granick's earlier failure to observe a glucose effect on the enzyme in isolated chick embryo liver cells [43].

In contrast, a modest glucose effect (25–30 percent lowering of induction) has been described in 17-day-old chick embryo liver *in ovo*, and in this system, unlike the rat, dibutyryl cAMP enhanced by 10–15 percent the AIA-mediated induction of ALA synthetase [121]. As in the rat, however, theophylline markedly repressed the induction of chick embryo enzyme mediated by AIA, phenobarbital, and steroids [122]. This inhibitory effect in the chick embryo could not be reversed by administration of dibutyryl cAMP [121].

In summary, current evidence favors the following conclusions:

1.   cAMP is not a primary inducer of hepatic ALA synthetase, as it is for several other enzymes which, like ALA synthetase, are repressed by glucose.

2.   The glucose effect in rat liver, for all enzymes studied, is not mediated by a lowering of cAMP levels.

Therefore, other explanations of the glucose effect must be sought. We did several experiments to study this problem further [41]. First we tested the hypothesis that it was not glucose *per se* but some metabolite of glucose which mediated the effect. We administered large doses of several carbohydrates, fatty acids, and acidic glycolytic and TCA cycle intermediates to rats who also received AIA to induce ALA synthetase. To our surprise, we found that glucose or those carbohydrates which are efficiently converted to hepatic glucose (fructose, glycerol) were most effective, repressing induction of the enzyme by 50–90 percent. Furthermore, 2-deoxyglucose, a potent inhibitor of glucose-6-phosphate isomerase and thus of glycolysis did not prevent the glucose effect. A role for glycogen deposition, previously suggested as a possible mediator of the glucose effect [123], was not supported since diazoxide, a potent glycogenolytic agent, did not prevent carbohydrate repression.

These results suggested that glucose, glucose-6-phosphate, or perhaps an intermediate in the hexose monophosphate shunt (HMPS) or uronic acid pathway were candidates as mediators of the glucose effect on rat hepatic ALA synthetase. This conclusion is also supported by the recent finding that uridine diphosphate glucose feeding decreased enzyme induction [124].

Hormonal Influences on ALA Synthetase

Furthermore, we found no evidence for mediation of the glucose effect by hormonal changes induced by glucose feeding. For example, although insulin secretion is enhanced by glucose feeding in rats, insulin injections did not inhibit ALA synthetase induction, and insulin lack, produced by prior alloxan administration, did not lead to enhanced but rather to decreased enzyme induction [41]. Some liver enzymes susceptible to a glucose effect are induced by glucocorticoids and this hormonal action is blocked by glucose feeding [113]. In the case of ALA synthetase, this does not occur. Although, as mentioned above, glucocorticoids are required for induction to occur [119], in large doses they do not induce the enzyme nor enhance induction by AIA [41].

Available data therefore suggest that glucose itself, glucose-6-phosphate, or a distal metabolite of glucose in the HMPS pathway, but not in the glycolytic pathway, mediates the glucose effect on ALA synthetase. Despite numerous studies, however, the precise mechanism of this fascinating and clinically important effect is yet to be established.

CONCLUSION

There have been many recent advances in our understanding of hepatic heme metabolism and its control, but we still have much to learn. This area of research therefore remains important to biochemists, to clinicians caring for patients with disorders of porphyrin metabolism, and to clinical investigators seeking to provide a bridge between basic and clinical disciplines for the mutual benefit of both and especially for the eventual benefit of generations of patients yet unborn.

ACKNOWLEDGEMENTS

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