

The Role of Iron in the Pathogenesis of Porphyria Cutanea Tarda

AN IN VITRO MODEL

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ABSTRACT Porphyria cutanea tarda (PCT) is characterized biochemically by excessive hepatic synthesis and urinary excretion of uroporphyrin I. Clinical evidence has implicated iron in the pathogenesis of PCT. The synthesis of the normally occurring isomer of uroporphyrin, namely uroporphyrin III, from protoporphobilinogen (PBG) requires two enzymes; uroporphyrinogen I synthetase and uroporphyrinogen III cosynthetase (COSYN). In the absence of COSYN only uroporphyrinogen I is formed.

These experiments were designed to study the effect of iron on porphyrin biosynthesis in porcine and human crude liver extracts and to measure COSYN activity in the presence of iron.

Mitochondria-free crude liver extracts were prepared in 0.25 M sucrose at pH 7.4 by centrifugation at 37,000 *g*. Preparations were incubated with either 0.2 mM aminolevulinic acid (ALA) or 0.1 mM PBG. The addition of ferrous ion (either from ferritin iron [4 µg/ml] and cysteine [6.7 mM] or ferrous ammonium sulfate [0.3 mM Fe] and cysteine) significantly increased the rate of uroporphyrin synthesis from either ALA or PBG. The predominant porphyrin synthesized in the presence of ferrous ion was uroporphyrin I whereas coproporphyrin III predominated in its absence. Orthophenanthroline blocked these effects of ferrous ion.

To investigate the effect of ferrous ion on COSYN, crude liver extracts were incubated with ferrous ammonium sulfate (0.3 mM Fe) and cysteine (6.7 mM) and the COSYN activity of the incubates was assayed directly. In both porcine and human extracts ferrous ion

caused marked inhibition of COSYN activity. Orthophenanthroline blocked the inhibitory effect.

Inactivation of COSYN by heating resulted in marked enhancement of porphyrin synthesis from PBG. The sole product was uroporphyrin I.

Thus, inactivation of COSYN results in accelerated synthesis of uroporphyrin I. This effect of ferrous ion provides a possible biochemical explanation for the excess production and excretion of uroporphyrin I in patients with PCT and the reversal of this defect by phlebotomy.

INTRODUCTION

Porphyria cutanea tarda (PCT)¹ is the most commonly recognized disorder of porphyrin metabolism (1). It is unique among the porphyrias in that it is an acquired illness, usually associated with alcoholic liver disease (2). It is characterized clinically by a photosensitive dermatitis and biochemically by excessive hepatic synthesis and urinary excretion of uroporphyrin I (3, 4).

Iron overload has been implicated in the pathogenesis of PCT on the basis of three observations. First, hepatic cell iron loading is almost invariably found in patients with the disorder (5, 6). Second, removal of iron by phlebotomy (7-10) or the administration of iron-chelating agents (11) induces both clinical and biochemical remissions in such patients. Finally, the administration of iron is followed by relapse in patients in whom a remission has been induced by phlebotomy therapy (12, 13).

Neither the mechanism of the iron effect nor the nature of the defect in porphyrin synthesis has been de-

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Received for publication 17 May 1972 and in revised form 3 August 1972.

¹ *Abbreviations used in this paper:* ALA, δ-aminolevulinic acid; COSYN, uroporphyrinogen III cosynthetase; PBG, protoporphobilinogen; PCT, porphyria cutanea tarda.

fined. Of the two naturally occurring isomers of uroporphyrinogen, only uroporphyrinogen III can be utilized for the formation of protoporphyrin and heme. Uroporphyrinogen I, normally produced and excreted in very small amounts, is synthesized in great excess by patients with PCT and is excreted in the urine where it is usually detected as the colored and fluorescent, oxidized form, uroporphyrin I. The reaction in which uroporphyrinogen III is synthesized from PBG requires two enzymes: uroporphyrinogen I synthetase (PBG deaminase) and uroporphyrinogen III cosynthetase (uroporphyrinogen isomerase) (14). In the absence of the cosynthetase (COSYN) the reaction yields only uroporphyrinogen I. Previous studies of porphyrin synthesis in liver biopsy specimens from patients with PCT have suggested that the total rate of uroporphyrinogen synthesis may be slightly (15) or greatly (16) increased. Biosynthesis of the porphyrin precursor, ALA, was found to be normal in two studies (15, 17) and increased in another (18).

The purpose of the present investigation was to study the effect of iron on hepatic porphyrin synthesis. The results of these studies demonstrated that porphyrin synthesis in human or porcine, mitochondria-free, crude liver extracts is greatly stimulated by ferrous ion and that the porphyrin produced is chiefly uroporphyrinogen I. This effect of ferrous ion occurs because of a direct inhibitory effect on the enzyme COSYN. Thus, ferrous ion produces a biochemical change in liver extracts that appears identical to the *in vivo* defect of PCT.

METHODS

Normal porcine liver was obtained from exsanguinated animals. Human liver was obtained immediately after death from a subject utilized as a kidney donor and, in some instances, at laparotomy from patients undergoing surgery for a variety of benign intra-abdominal disorders. Fresh or frozen liver was homogenized in 10 vol of 0.25 M sucrose in 0.1 M Tris-HCl buffer, pH 7.4. The homogenate was centrifuged at 37,000 *g* for 45 min at 4°C. This procedure yielded an essentially mitochondria-free solution which will be referred to as a "crude liver extract." Protein determinations were performed by the method of Lowry and Rosenbrough (19).

Substrates, either ALA (Sigma Chemical Co. St. Louis, Mo.) or PBG (Protex, Ltd., Montreal, Canada), ferrous ammonium sulfate and cysteine and all other additives studied, were freshly prepared just before use by dissolving in 0.1 M Tris-HCl, pH 7.4. Horse spleen ferritin (Calbiochem, San Diego, Calif.) was dialyzed against Tris-HCl buffer for 48 hr before use.

Incubations were performed in the dark at 37°C in 25 ml Erlenmeyer flasks in a Dubnoff shaker with an air atmosphere. In preliminary experiments, substitution of nitrogen for air made no difference. Each flask contained 5 ml of the enzyme source, 1 ml of the substrate preparation, and appropriate volumes of other additives. The final volume in each flask was brought up to 10.0 ml with Tris buffer. The reactions were stopped by adding 1.2 ml concentrated hydro-

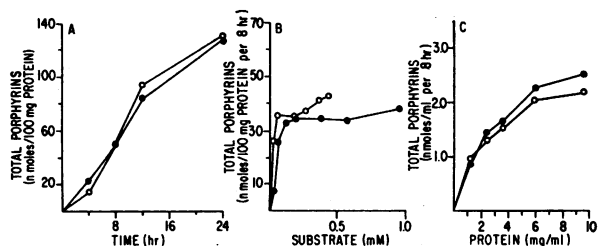


FIGURE 1 The relationship, in a porcine crude liver extract, of total porphyrins synthesized to: (A) Time, (B) Substrate concentration, (C) Protein content of the incubation mixture. ●—● ALA as substrate. ○—○ PBG as substrate.

chloric acid, and the mixture was allowed to stand in the light for 30 min in order to oxidize the synthesized porphyrinogens to porphyrins (20). In some experiments oxidation of porphyrinogens was accomplished by the addition of a drop of 0.2 M iodine in 0.3 M KI (21). A clear supernatant fluid was harvested after centrifuging at 37,000 *g* for ½ hr. Porphyrin concentration was measured spectrophotometrically by scanning the spectrum from 370 to 440 nm with a Cary spectrophotometer (Cary Instruments, Monrovia, Calif.) and applying the correction factors of With (22). The pH of the supernate was adjusted to 3.1 with solid sodium acetate, and the porphyrins were adsorbed on talc and esterified in H₂SO₄-methanol (23). Porphyrins were then identified chromatographically by the method of Cornford and Benson (24), eluted with chloroform, and quantified spectrophotometrically as described above. To confirm isomer identification the synthesized uroporphyrin was decarboxylated to coproporphyrin by the method of Edmonson and Schwartz (25) and the resulting isomers of coproporphyrin were identified by ascending thin-layer chromatography with cellulose M 300 sheets as the supporting medium and 2,6-lutidine-0.7 M NH₄OH (10/7, v/v) as the solvent (26).

Uroporphyrinogen III cosynthetase (COSYN) was assayed by the method of Levin (21). Crude liver extracts were incubated both with and without ferrous ammonium sulfate (0.3 mM Fe) and cysteine (6.7 mM) for 2 hr at 37°C, and samples of the mixtures were then used as the source of COSYN in the assay system.

Tritium-labeled porphobilinogen was prepared enzymatically from aminolevulinic acid-3,5-³H (New England Nuclear, Boston, Mass.) by Dr. Ephraim Y. Levin. Dr. Levin also kindly supplied the uroporphyrinogen I synthetase for the COSYN assay. For chromatographic markers the methyl ester of uroporphyrin I was prepared from the urine of porphyric cows, and the ester of uroporphyrin III was prepared from the feathers of Turacos Hartlaub (27). Methyl esters of coproporphyrins I and III were obtained from Calbiochem. Markers for the thin-layer chromatographic system were prepared by overnight hydrolysis of the coproporphyrin methyl esters in 6 M HCl.

RESULTS

Effect of time, substrate concentration, and volume of liver extract on porphyrin synthesis in vitro. In crude extracts prepared from either human or porcine liver, porphyrin synthesis proceeded linearly in relation to time for 12 hr, after which perceptible slowing of the reaction

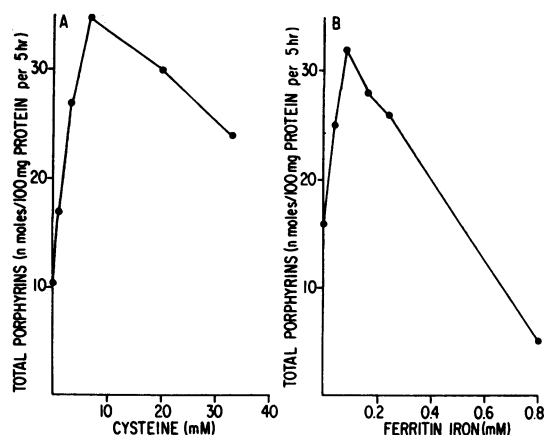


FIGURE 2 (A) The effect of cysteine concentration on total porphyrins synthesized from ALA by a porcine crude liver extract. The ferritin iron concentration was kept constant at 0.08 mM. (B) The effect of ferritin iron concentration on total porphyrins synthesized from ALA by a porcine crude liver extract. The cysteine concentration was kept constant at 6.7 mM.

occurred. The rate of porphyrin synthesis was similar when either ALA or PBG were provided as substrates (Fig. 1 A). With the porcine preparations the substrate activity curve reached a plateau at approximately 0.2 mM ALA and at 0.1 mM PBG (Fig. 1 B). Therefore, these concentrations of substrate were used in subsequent experiments with both porcine and human preparations. The reaction rate with either substrate was related to the amount of liver homogenate present (Fig. 1 C).

On chromatographic analysis (24) over 90% of the porphyrins synthesized by either porcine or human extracts was coproporphyrin. The remainder consisted of small amounts of 7-COOH-porphyrin and uroporphyrin. Analysis of the porphyrins by thin-layer chromatography revealed that all were of isomer type III.

TABLE I
Effect of Ferritin and Cysteine on Porphyrin Synthesis
by a Porcine Crude Liver Extract

Additives	Porphyrin synthesis	
	From ALA*	From PBG†
	n moles/ 5 hr per 100 mg protein	n moles/ 8 hr per 100 mg protein
None	22	35
Cysteine (6.7 mM)	20	
Ferritin (0.08 mM Fe)	18	
Ferritin (0.08 mM Fe) +cysteine (6.7 mM)	30	52

* ALA, aminolevulinic acid.

† PBG, porphobilinogen.

TABLE II
Effect of Ferrous Ammonium Sulfate and Cysteine on Porphyrin
Synthesis by a Porcine Crude Liver Extract

Additives	Porphyrin synthesis	
	From ALA*	From PBG†
	n moles/8 hr per 100 mg protein	
None	47	42
Ferrous ammonium sulfate (0.3 mM Fe) +cysteine (6.7 mM)	68	56

* ALA, aminolevulinic acid.

† PBG, porphobilinogen.

Effect of iron on the amount and isomer types of porphyrins synthesized. Two sources of iron were utilized to measure the effect of iron on porphyrin synthesis. The effect of a biologically-occurring iron compound was studied by adding ferritin to the incubations with cysteine as a reducing agent to liberate ferrous ion. Neither cysteine nor ferritin alone had any significant effect on porphyrin synthesis but the combination of the two accelerated synthesis (Table I). In this system, optimal porphyrin synthesis occurred at a concentration of cysteine of 6.7 mM (Fig. 2 A). When ferritin was added optimal porphyrin synthesis occurred at a concentration of ferritin iron of 0.08 mM (Fig. 2 B).

Porphyrin synthesis increased when ferritin and cysteine were added, with either ALA or PBG as substrate. The results of a typical experiment are illustrated in Table I. In five such experiments with ALA as substrate, the rate of porphyrin synthesis increased from a mean of 18 nmoles/5 hr per 100 mg protein (range 10–23) to 33 nmoles/5 hr per 100 mg protein (range 27–40). The mean increase was 15 nmoles (range 6–21) or about 83%. In four experiments with PBG as substrate the rate of synthesis increased from a mean of 41 nmoles/8 hr per 100 mg protein (range 35–50) to 50 nmoles/8 hr per 100 mg protein (range 41–59). The mean increase was 8 nmoles (range 3–17) or about 20%.

In contrast to control incubations, chromatographic (24) analysis of the porphyrins synthesized in the presence of optimal concentrations of ferritin and cysteine revealed that over 75% of the yield was uroporphyrin I rather than coproporphyrin III. Similar results were obtained when porphyrin isomers were determined by decarboxylation and thin-layer chromatography of the coproporphyrin isomers (25, 26).

With paper chromatography (24), uroporphyrin was detected in incubations containing ferritin and cysteine whereas none was visible in those without these additives. With increasing ferritin concentrations, the uroporphyrin I levels increased and coproporphyrin III

levels decreased, as estimated by the intensity of fluorescence of the spots on chromatograms. At ferritin concentrations associated with maximum rates of porphyrin synthesis, elution, and quantification of porphyrin isomers was performed; more than 75% of the yield was found to be uroporphyrin I. Similar results were obtained when porphyrin isomers were determined by decarboxylation and thin-layer chromatography of the coporphyrin isomers (25, 26).

The effect of an inorganic iron compound was studied by adding ferrous ammonium sulfate to the incubations, again in the presence of cysteine (6.7 mM). Again there was no significant change in synthesis with the addition of the iron alone, but significant acceleration of porphyrin synthesis occurred with the combination of ferrous ammonium sulfate and cysteine (Table II). The optimal concentration of inorganic iron was 0.15–0.3 mM. Increased synthesis with the addition of ferrous ammonium sulfate and cysteine was observed with either PBG or ALA as substrate. The results of a typical experiment are illustrated in Table II. In seven such experiments with ALA as substrate the rate of porphyrin synthesis increased from a mean of 45 nmoles/8 hr per 100 mg protein (range 26–82) to 70 nmoles/8 hr per 100 mg protein (range 48–130). The mean increase was 24 nmoles (range 19–48) or about 55%. In four experiments with PBG as substrate the rate of synthesis increased from a mean of 42 nmoles/8 hr per 100 mg protein (range 38–45) to 61 nmoles/8 hr per 100 mg protein (range 53–64). The mean increase was 19 nmoles (range 12–26) or about 45%.

Isomer analysis of the porphyrins synthesized in the presence of ferrous ammonium sulfate and cysteine by paper chromatography or by decarboxylation and thin-layer chromatography revealed the predominant porphyrin synthesized to be uroporphyrin I, as was the case when ferritin was used as the iron source.

To determine whether the effect of the iron compound and cysteine depended upon the formation of ferrous ion, use was made of 1,10-phenanthroline, three molecules of which form a stable chelate with one molecule of ferrous ion (28). Phenanthroline alone had no effect on porphyrin synthesis in crude liver extracts containing no added iron (Table III); however phenanthroline, when added in increasing concentrations to flasks containing 0.3 mM iron and 6.7 mM cysteine, partially inhibited synthesis and, at a concentration of 0.9 mM, essentially obliterated the iron effect. Phenanthroline also inhibited the effect of iron and cysteine on the porphyrin isomer synthesized.

Effect of other cations on the amount and isomer types of porphyrins synthesized. Four other cations (Mg^{++} , Ca^{++} , K^+ , and Na^+) were added to the liver extracts to

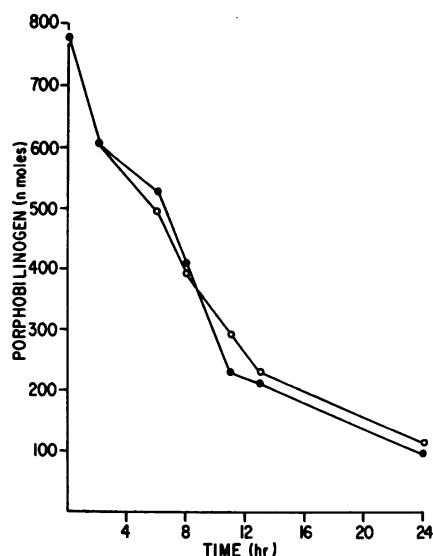


FIGURE 3 Porphobilinogen disappearance as related to time in a porcine crude liver extract. ●—● Incubation without additives. ○—○ Incubation containing ferrous ammonium sulfate (0.3 mM Fe) and cysteine (6.7 mM).

measure their effect on porphyrin synthesis. With each cation, there was an optimal concentration at which porphyrin synthesis was accelerated and the predominant porphyrin synthesized was uroporphyrin I. The optimal concentrations and per cent of increase over extracts with no added cation were as follows: Mg^{++} : 0.05 M, 30%; Ca^{++} : 0.05 M, 10%; Na^+ : 0.2 M, 20%; and K^+ : 0.2 M, 17%. At greater concentrations porphyrin synthesis decreased but uroporphyrin I continued to be the predominant product. None of these cations had any effect on porphyrin synthesis at a concentration of 0.3 mM, the concentration for maximal Fe^{++} effect.

TABLE III
Inhibition of the Ferrous Ion* Effect on Porphyrin
Synthesis by 1,10-Phenanthroline

Additives	Porphyrin synthesis from PBG† n moles/8 hr per 100 mg protein
None	37
Fe^{++} (0.3 mM)	48
Fe^{++} (0.3 mM) + 1,10-phenanthroline (0.15 mM)	46
Fe^{++} (0.3 mM) + 1,10-phenanthroline (0.3 mM)	43
Fe^{++} (0.3 mM) + 1,10-phenanthroline (0.6 mM)	43
Fe^{++} (0.3 mM) + 1,10-phenanthroline (0.9 mM)	39
1,10 phenanthroline (0.9 mM)	38

* In the presence of 6.7 mM cysteine.

† PBG, porphobilinogen.

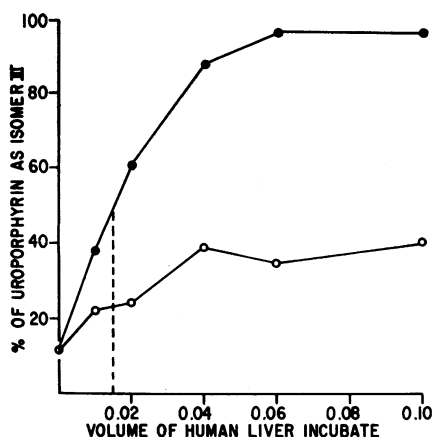


FIGURE 4 Cosynthetase activity of a human liver incubation. ●—● Incubation without additives. ○—○ Incubation containing ferrous ammonium sulfate (0.3 mM Fe) and cysteine (6.7 mM). 0.15 ml of the incubation without additives, when added to uroporphyrinogen I synthetase and PBG, yielded 50% of the uroporphyrin as isomer III.

Effect of iron on the utilization of porphobilinogen. In all incubations, the rate of PBG consumption exceeded that of porphyrin formation in that less than 1 mole of porphyrin was synthesized for every 4 moles of PBG utilized. Thus, the fate of an appreciable fraction of the PBG was unaccounted for. In incubations without iron, this fraction averaged 41% (range 36–47%). Fig. 3 illustrates the essentially identical rate of disappearance of PBG in both control and iron added incubations although porphyrin synthesis in the iron added flasks in the experiment illustrated was 43% greater than the control at 8 hr. Thus, the PBG fraction unaccounted for was smaller (mean, 34%; range 27–41%) in the presence of iron. PBG was determined both by direct reaction with modified Ehrlich's aldehyde reagent and by extraction with column chromatography (29).

Effect of prior heating on porphyrin synthesis by liver extracts. Crude porcine liver extracts were prepared in sucrose at pH 7.4, samples were heated at 65° C for 20 min and precipitated protein was removed by centrifugation, a procedure which will inactivate COSYN without affecting the activity of uroporphyrinogen I synthetase (30). The heated preparations, when incubated with PBG, synthesized 74% more porphyrin than controls. The heated flasks synthesized only uroporphyrin I whereas the controls synthesized predominantly coproporphyrin III. The rate of PBG disappearance, however, was the same in the heated flasks as in the controls.

Effect of iron on COSYN activity. PBG incubated with uroporphyrinogen I synthetase yields uroporphyrin I as the only product. If COSYN is added to such a mixture, a proportion of the yield will be uroporphyrin III, and increasing amounts of COSYN will eventually

result in a yield of nearly all uroporphyrin III. This relation forms the basis for the COSYN assay method of Levin (21). 1 U of COSYN activity is defined as that amount which produces a yield of 50% uroporphyrin III.

To investigate the effect of ferrous ion on COSYN activity, both porcine and human crude liver extracts were incubated with ferrous ammonium sulfate (0.3 mM Fe) and cysteine (6.7 mM) for 2 hr at 37° C. COSYN activity in extracts so treated was compared with COSYN activity in extracts to which no iron or cysteine had been added. When 0.015 ml of a human liver incubation mixture was added to uroporphyrinogen I synthetase and PBG, 50% of the uroporphyrin formed was of isomer type III (Fig. 4). The protein content of the liver extract was 12.0 mg/ml and that of the total incubation mixture was 6.0 mg/ml. Thus, in this human liver the COSYN activity was 10.7 U/mg protein. In the incubations containing iron and cysteine, volumes of up to 0.1 ml failed to yield 50% uroporphyrin III, thus demonstrating marked inhibition of COSYN activity.

The mean value for COSYN activity in seven porcine liver homogenates was 5.7 u/mg protein (range 2.3–9.9). Incubations containing the iron salt and cysteine in samples of up to 0.1 ml failed to yield 50% uroporphyrin III. When 1,10-phenanthroline (0.9 mM) was added to the incubations containing the iron salt and cysteine the COSYN activity was identical to that of the control.

DISCUSSION

These studies demonstrate that when iron is added to mitochondria-free, crude extracts of normal liver, two effects on porphyrin synthesis are observed. First, there is an increase in the total amount of porphyrin synthesized. Second, the predominant porphyrin isolated is uroporphyrin I rather than coproporphyrin III. These effects occurred with both human and porcine liver, and with either ALA or PBG as substrate.

The altered porphyrin synthesis could be induced with either horse-spleen ferritin (Fig. 2) or ferrous ammonium sulfate as the iron source. However, two observations suggest that the phenomenon requires that the iron be converted to or maintained in the ferrous form. First, no effect was observed unless the reducing agent, cysteine, was added (Tables I and II). In the absence of a reducing agent, rapid auto-oxidation of ferrous ion is to be expected under the conditions of these experiments. Cysteine had no effect in the absence of iron. Second, the effects of iron could be blocked by the addition of 1, 10-orthophenanthroline (Table III), a ferrous chelator of high specificity (28). This chelating agent had no effect unless iron was present. The effect of iron could be mimicked by certain other cations, but greater concentrations were required.

The change in isomer type that was observed when iron was added suggested that the mechanism of action was inhibition of the enzyme uroporphyrinogen III co-synthetase (COSYN). This enzyme is required for uroporphyrin III synthesis; in the absence of COSYN, PBG is converted to uroporphyrin I (14). Thus, a predominance of type I porphyrin isomers in a crude tissue extract is evidence that COSYN has been inactivated. The suggestion was confirmed by direct assay; addition of iron resulted in marked inhibition of the enzyme (Fig. 4).

The action of ferrous ion on COSYN activity may be similar to that of ammonium ions and hydroxylamine in that none of these inhibitors of uroporphyrin III synthesis affect the rate of porphobilinogen consumption (31). This would imply that the metabolism of an intermediate compound is being altered. Tait has proposed that COSYN, by virtue of a negatively charged group, possibly a carboxyl group, associates with the positively charged aminomethyl group of a linear polypyrrole, thus orienting the molecule in such a way that uroporphyrin III would be the product of ring closure (32). The inhibitory cations might compete for this negatively charged binding site. The kinetics of the uroporphyrinogen I synthetase-COSYN reaction are compatible with this concept (21).

Several observations suggest that the increased rate of porphyrin synthesis is secondary to COSYN inactivation. First, exposure of the liver extract to heat, a procedure known to inactivate COSYN (30), also resulted in increased synthesis along with the expected change in isomer type. Second, neither iron nor heat resulted in an increased rate of PBG utilization (Fig. 3).

In all experiments, more PBG disappeared than could be accounted for in porphyrins formed. Furthermore, in the presence of iron or other cations or after heating a proportion of the fraction of PBG unaccounted for was diverted to the synthesis of uroporphyrin I. Thus, in this system the total yield of porphyrins could be increased without increasing the turnover of PBG. One possible explanation for these phenomena is that the fraction unaccounted for consists of polypyrrole intermediates (32-34). These may either be converted spontaneously and rapidly to uroporphyrinogen I or serve as a substrate for a slower and more complex reaction catalyzed by COSYN. Thus, inhibition of COSYN would favor the more rapid reaction, leading to an increase in total porphyrin synthesis. An alternate possibility is that PBG is metabolized in crude liver extracts by enzymes other than uroporphyrinogen I synthetase. For example, the existence of an enzyme capable of oxidizing the pyrrole ring has been reported in both plant and animal tissues (35, 36). Iron, cations, and heat might inhibit not only COSYN but one or more of such alternate path-

ways for PBG metabolism, thus making more intermediates available for porphyrin synthesis.

In addition to the inactivation of COSYN, ferrous ion and heating may also affect the activity of uroporphyrinogen decarboxylase, for uroporphyrin I and not coproporphyrin I is the product under these conditions. The decarboxylase is known to be both heat labile and dependent upon sulfhydryl groups for enzymatic activity (37).

The findings reported herein provide a possible explanation for the disturbance of porphyrin synthesis that occurs in PCT. This illness is characterized by *in vivo* biochemical abnormalities similar to those observed in our *in vitro* system; namely, excessive total porphyrin production and a predominance of type I isomers. Iron overload accompanies the disturbance, and measures directed at removal of the iron relieve it (7-13). It is reasonable to conclude that these observations are explained by an inhibitory effect of iron on COSYN. It is important to realize that iron overload alone does not induce PCT, nor does iron content alone induce abnormal porphyrin synthesis in our *in vitro* model. In the latter, conversion to the ferrous form is required. Thus, the *in vivo* development of PCT may depend partly on iron overload and partly on the presence of a system capable of reducing iron. Other abnormalities in the chelation or oxidation of normally formed ferrous ion might also be contributory. Additional possible *in vivo* factors of importance include the cellular and subcellular location of the iron in the liver. Porphyrin synthesis takes place in the hepatocyte and COSYN is a cytoplasmic rather than a mitochondrial enzyme. The proposed mechanism is consistent with the observation that ALA-synthetase activity may not be greatly increased in PCT, in contrast to certain other forms of porphyria (15, 17). Excess porphyrin synthesis occurred *in vitro* when COSYN was inactivated without an increase in PBG utilization. Thus, no net increase in ALA production is required in PCT; rather, a more efficient utilization of PBG for uroporphyrin I synthesis may occur.

Not well explained by the above formulation is the observation that uroporphyrin III may also be excreted in excess in patients with PCT (4). It is difficult to understand how this abnormality could occur as the result of COSYN inhibition. Indeed, excessive accumulation of type III porphyrins was not observed when COSYN was inhibited in our *in vitro* system. However, that type III isomers may be excreted *in vivo* under conditions associated with COSYN inactivation is suggested by observations in congenital erythropoietic porphyria. In this illness, uroporphyrin III may also be excreted in excess, even though reduced COSYN activity appears to be the primary genetic abnormality (26).

ACKNOWLEDGMENTS

The authors are indebted to Dr. Ephraim Y. Levin who kindly supplied both reagents and advice essential to the success of the uroporphyrinogen III cosynthetase assays. The technical assistance of Mrs. Alice Tustison and Mrs. Diana Steinmuller is acknowledged with appreciation.

This investigation was supported by a Veterans Administration Research and Education Award and by a Research Grant (AM04489) and a Graduate Training Grant (AM-5098) from the National Institute of Arthritis and Metabolic Diseases, The National Institutes of Health, Bethesda, Md.

REFERENCES

1. Dean, G. 1963. The Prevalence of the porphyrias. *S. Afr. J. Lab. Clin. Med.* **9**: 145.
2. Schmid, R. 1966. The porphyrias and hyperbilirubinemia. In *The Metabolic Basis of Inherited Disease*. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill Book Company, New York. 813, 871.
3. Nacht, S., L. C. San Martin DeViale, and M. Grinstein. 1970. Human porphyria cutanea tarda. Isolation and properties of the urinary porphyrins. *Clin. Chim. Acta.* **27**: 445.
4. Dowdle, E., P. Goldswain, N. Spong, and L. Eales. 1970. The pattern of porphyrin isomer accumulation and excretion in symptomatic porphyria. *Clin. Sci. (Oxf.)*. **39**: 147.
5. Uys, C. J., and L. E. Eales. 1963. The Histopathology of the liver in acquired (symptomatic) porphyria. *S. Afr. J. Lab. Clin. Med.* **9**: 190.
6. Epstein, J. H., and J. B. Pinski. 1965. Porphyria cutanea tarda-association with abnormal iron metabolism. *Arch. Dermatol.* **92**: 362.
7. Ippen, H. 1961. Allgemeinsymptome der Späten Hautporphyrie (porphyria cutanea tarda) als Hinweise für deren Behandlung. *Dtsch. Med. Wochenschr.* **86**: 127.
8. Epstein, J. H., and A. G. Redeker. 1968. Porphyria cutanea tarda, a study of the effect of phlebotomy. *N. Engl. J. Med.* **279**: 1301.
9. Kalivas, J. T., M. A. Pathak, and T. B. Fitzpatrick. 1969. Phlebotomy and iron-overload in porphyria cutanea tarda. *Lancet*. **1**: 1184.
10. Walsh, J. R., W. C. Lobitz, Jr., D. J. Mahler, and F. A. J. Kingery. 1970. Phlebotomy therapy in cutaneous porphyria. Effect on iron and trace metals. *Arch. Dermatol.* **101**: 167.
11. Thivolet, J., H. Perrot, R. Mallein, and P. Sabater. 1968. Effects cliniques et biologiques des chélateurs dans la porphyrie cutanée tardive. *Presse Med.* **76**: 367.
12. Lundvall, O., and A. Weinfeld. 1968. Studies of the clinical and metabolic effects of phlebotomy treatment in porphyria cutanea tarda. *Acta Med. Scand.* **184**: 191.
13. Felsher, B. F., and A. G. Redeker. 1972. The relation of iron to hepatic uroporphyrin synthesis in porphyria cutanea tarda. *Clin. Res.* **20**: 182. (Abstr.).
14. Bogorad, L. 1958. The Enzymatic synthesis of porphyrins from porphobilinogen. II. Uroporphyrin III. *J. Biol. Chem.* **233**: 510.
15. Strand, L. J., B. F. Felsher, A. G. Redeker, and H. S. Marver. 1970. Heme biosynthesis in intermittent acute porphyria: decreased hepatic conversion of porphobilinogen to porphyrins and increased delta aminolevulinic acid synthetase activity. *Proc. Natl. Acad. Sci. U. S. A.* **67**: 1315.
16. Miyagi, K., R. Cardinal, I. Bossenmaier, and C. J. Watson. 1971. The serum porphobilinogen and hepatic porphobilinogen deaminase in normal and porphyric individuals. *J. Lab. Clin. Med.* **78**: 683.
17. Kaufman, L., and H. S. Marver. 1970. Biochemical defects in two types of human hepatic porphyria. *N. Engl. J. Med.* **283**: 954.
18. Dowdle, E. B., P. Mustard, and L. Eales. 1967. Δ Aminolevulinic acid synthetase activity in normal and porphyric human livers. *S. Afr. Med. J.* **41**: 1093.
19. Lowry, O., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin-phenol reagent. *J. Biol. Chem.* **193**: 265.
20. Sancovich, H. A., A. M. C. Battle, and M. Grinstein. 1969. Porphyrin biosynthesis. VI. Separation and purification of porphobilinogen deaminase and uroporphyrinogen isomerase from cow liver. Porphobilinogenase an allosteric enzyme. *Biochim. Biophys. Acta.* **191**: 130.
21. Levin, E. Y. 1968. Uroporphyrinogen III cosynthetase from mouse spleen. *Biochem. J.* **7**: 3781.
22. With, T. K. 1955. Porphyrin concentrations from ultraviolet extinction. A note on the calculation. *Scand. J. Clin. Lab. Invest.* **7**: 193.
23. Falk, J. E. 1964. Porphyrins and metalloporphyrins. (*Biochim. Biophys. Acta.*) *Libr.* **2**: 125.
24. Cornford, P. A. D., and A. Benson. 1963. A qualitative and quantitative study of the separation of uroporphyrin octamethyl esters I and III by dioxan chromatography. *J. Chromatogr.* **10**: 141.
25. Edmondson, P. R., and S. Schwartz. 1953. Studies of the uroporphyrins. III. An improved method for the decarboxylation of uroporphyrin. *J. Biol. Chem.* **205**: 605.
26. Romeo, G., and E. Y. Levin. 1969. Uroporphyrinogen III cosynthetase in human congenital erythropoietic porphyria. *Proc. Natl. Acad. Sci. U. S. A.* **63**: 856.
27. San Martin De Viale, L. C., A. Viale, S. Nacht, and M. Grinstein. 1970. Experimental porphyria induced in rats by hexachlorobenzene. A study of the porphyrins excreted by urine. *Clin. Chim. Acta.* **28**: 13.
28. Smith, G. F., and F. P. Richter. 1944. Phenanthroline and substituted phenanthroline indicators. The G. Frederick Smith Chemical Co., Columbus, Ohio. 13.
29. Mauzerall, D., and S. Granick. 1956. The occurrence and determination of δ -aminolevulinic acid and porphobilinogen in urine. *J. Biol. Chem.* **219**: 435.
30. Burnham, B. F. 1969. Metabolism of porphyrins and corrinoids. In *Metabolic Pathways*. Amino acids and tetrapyrroles. D. M. Greenberg, editor. Academic Press, Inc., New York. **3**: 429-435.
31. Bogorad, L. 1963. Enzymatic mechanisms in porphyrin synthesis: possible enzymatic blocks in porphyrias. *Ann. N. Y. Acad. Sci.* **104**: 676.
32. Tait, G. H. 1968. General aspects of haem synthesis. In *Porphyrins and Related Compounds*. T. W. Goodwin, editor. Academic Press, Inc., New York. 19-34.

33. Stella, A. M., V. E. Parera, E. B. C. Lambias, and A. M. C. Battle. 1971. Pyrrolymethane intermediates in the synthesis of uroporphyrinogen III by soybean callus porphobilinogenase. *Biochim. Biophys. Acta.* **252**: 481.
34. Pluscec, J., and L. Bogorad. 1970. A dipyrrolymethane intermediate in the enzymatic synthesis of uroporphyrinogen. *Biochem. J.* **9**: 4736.
35. Frydman, R. B., M. L. Tomaro, and B. Frydman, 1971. Pyrrolooxxygenase: a new type of target enzyme, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **15**: 305.
36. Frydman, R. B., M. L. Tomaro, and B. Frydman. 1971. Pyrrolooxxygenases: the biosynthesis of 2-amino acetophenone. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **17**: 273.
37. Mauzerall, D. 1960. The Condensation of porphobilinogen to uroporphyrinogen, *J. Am. Chem. Soc.* **82**: 2605.