Liver Porphyrias

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I. HISTORICAL ASPECTS OF PORPHYRIAS

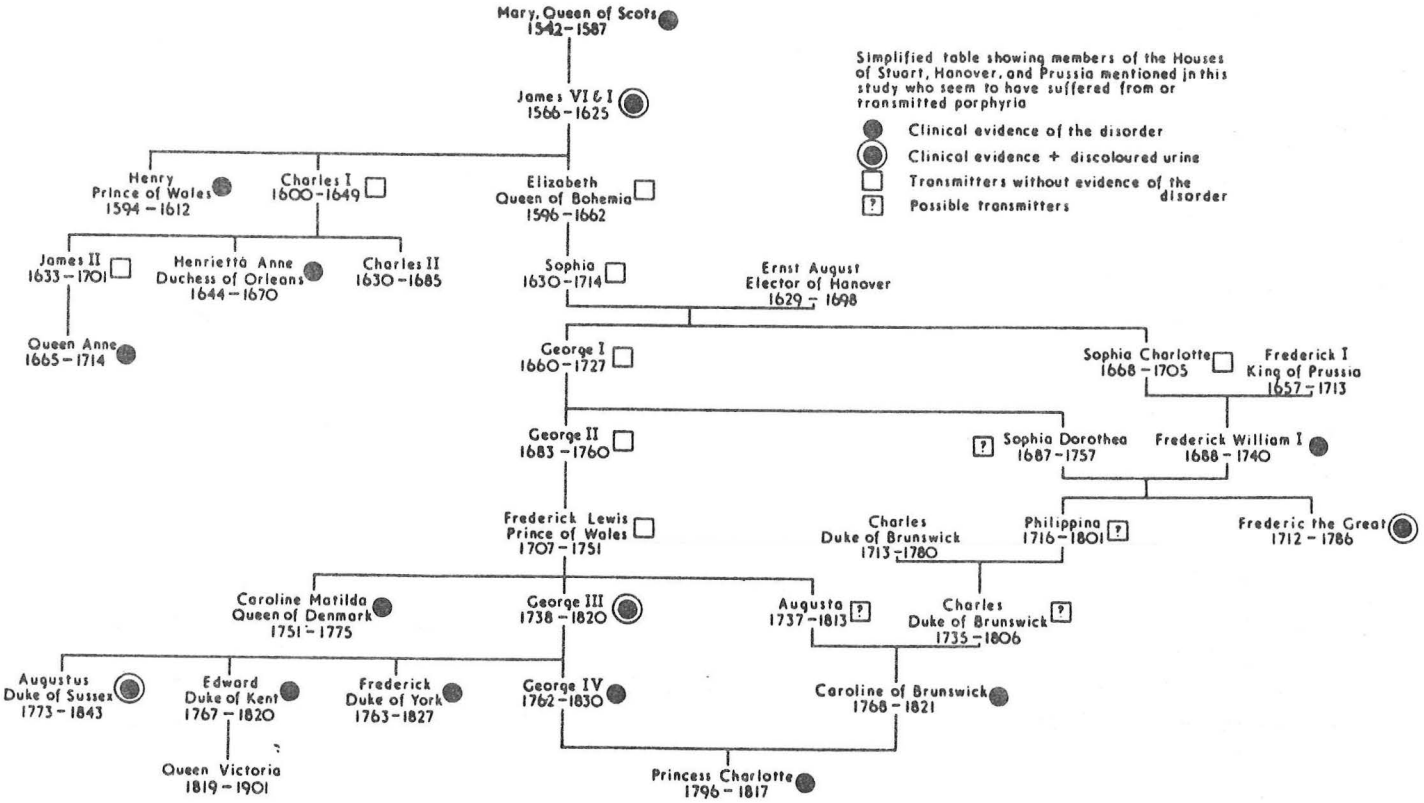
The cover of this Grand Rounds protocol depicts George III (1738-1820) who was king of England from 1760 to 1820. Soon after having ascended to the throne he set about, together with the Earl of Bute, reforming the wicked ways of English politics. For the first ten years, George appointed and dismissed members of the "Cabinet Council" at a bewildering rate, especially ministers responsible for colonial affairs. Consequently, English colonial policy in this crucial decade was inconsistent and incoherent, not to say capricious (1).

Many sometimes irrational decisions by the king and his council governed the colonial policy during the era of the American Revolution (1763-1783) which was highlighted by the Declaration of Independence on July 4, 1776, and ended by the peace treaty signed with Britain on September 3, 1783.

The decision-making ability of George III, which so severely affected the early history of the United States, may have been impaired by a disease called the "royal malady" and has now been identified as a form of hepatic porphyria. Such historical perspective gives the acute porphyrias an important place in medical as well as American history.

Figure 1

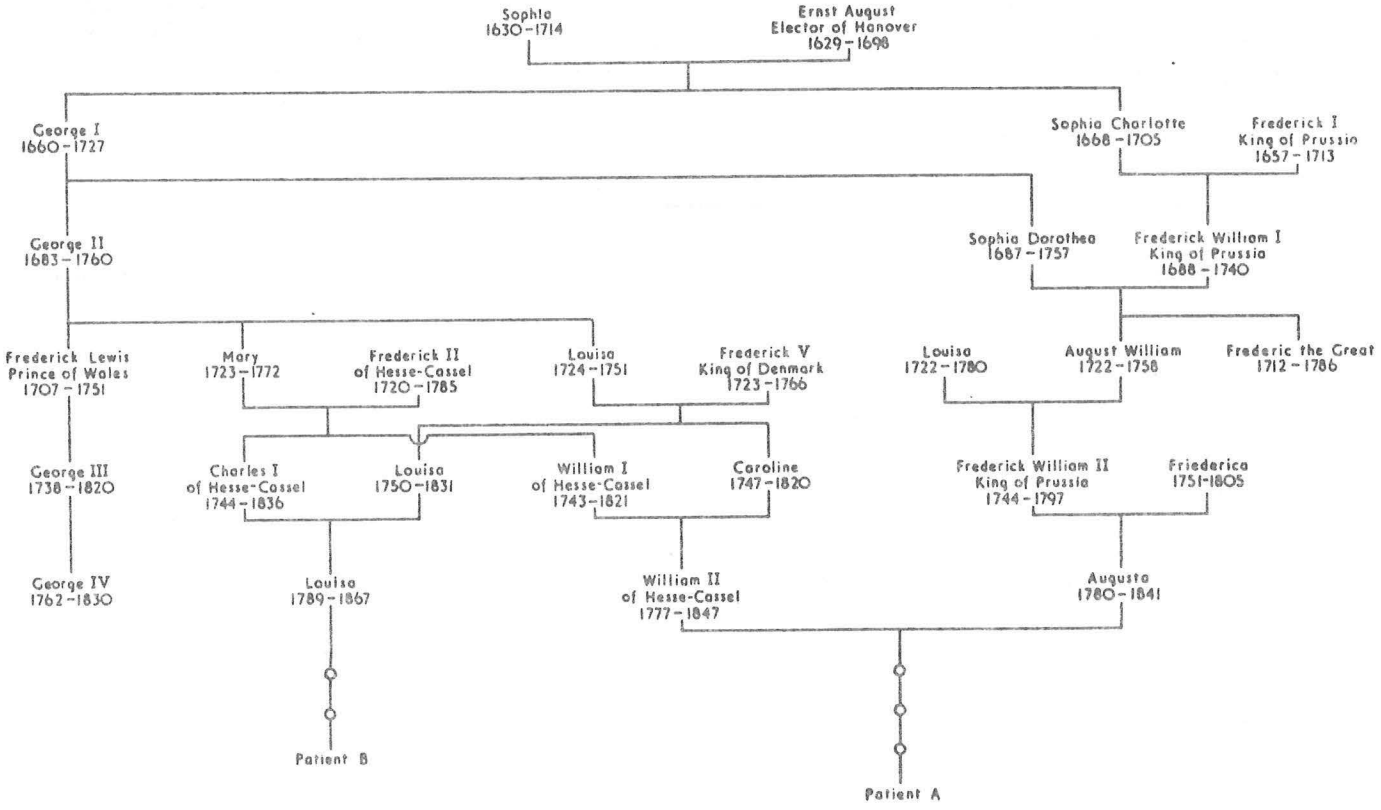
Members of the Houses of Stuart, Hanover, and Prussia who seem to have suffered from or transmitted porphyria.



In their two medical-historical papers, Macalpine and Hunter (2) and Macalpine et al. (3) traced back the medical history of King George III. The medical recordings of the kings physicians which the authors utilized identified the "royal malady" as acute porphyria, most likely variegate porphyria. This disease affected members of the houses of Stuart, Hanover and Prussia whose family trees can be traced back to Mary, Queen of Scots (1542-1587; Figure 1). Additionally, more recent studies of two descendents (Patients A and B, Figure 2) strongly supported the above diagnosis. George III's illness spawned years of great political significance for Great Britain (4). In addition to his inept handling of political problems concerning the American colonies, the Regency Crisis as well as the death of Princess Charlotte (probably due to acute porphyria) and her infant during childbirth in 1817 also contributed to the political turmoil.

Figure 2

Showing descent of Patients A and B.



George III's illness took the form of five major and several minor attacks. His urine was described as "bilious, bluish and bloody" and his physical and mental symptoms included the following: abdominal pain, tachycardia, constipation, weakness and stiffness of the limbs, hoarseness, hyperesthesia, hypoesthesia, tremor, agitation, confusion and hallucinations.

The puzzled physicians at the time tried to explain the illness on an organic basis (2, 3):

> "The cause to which they all agree to ascribe it, is the force of a humor which was beginning to show itself in the legs, when the king's imprudence drove it from thence into the bowels; and the medicines which they were there obliged to use for the preservation of his life, have repelled it upon his brain. The physicians are now endeavoring to bring it down again into his legs, which nature had originally pointed out as the best mode of discharge".

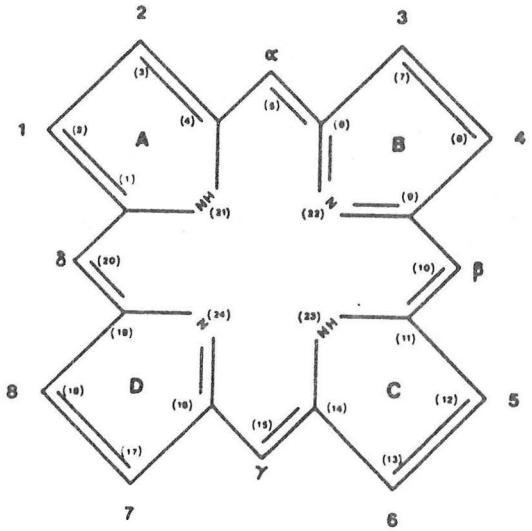
Despite the lack of specific treatments such as propranolol, hematin or glucose infusions, George III died at the old age of 82 years in 1820.

Since the days of inadequate medical knowledge where the diagnostic evaluation was often centered on the appearance of body excretions, the porphyrias have emerged today as one of the most impressive demonstrations of modern biomedical research. The fairly recent clarification of the biochemistry of heme synthesis greatly contributed to the understanding of the genetic diseases called porphyrias. In addition, the porphyrias are a classical example of a latent genetic disease, the clinical expression of which is triggered by exogenous environmental factors such as toxins or common clinical drugs. A thorough understanding of the heme synthetic pathway is therefore required to delineate a logical and rather simple classification of the clinical porphyrias.

II. PORPHYRIN CHEMISTRY

Porphyrin (Figure 3) is the parent structure of all biologically important hemoproteins. The carbon atoms of this ring structure have been identified by the Fischer numeration system (outer numerals and symbols) and by the IUPAC system (inner numerals); IUPAC stands for International Union of Pure and Applied Chemists (5). Free porphyrins are very soluble in strong acids by virtue of the basicity of the ring nitrogens. Metal complexes of porphyrins have no basic nitrogen function and hence are insoluble in aqueous acids. They are soluble in alkali, pyri-

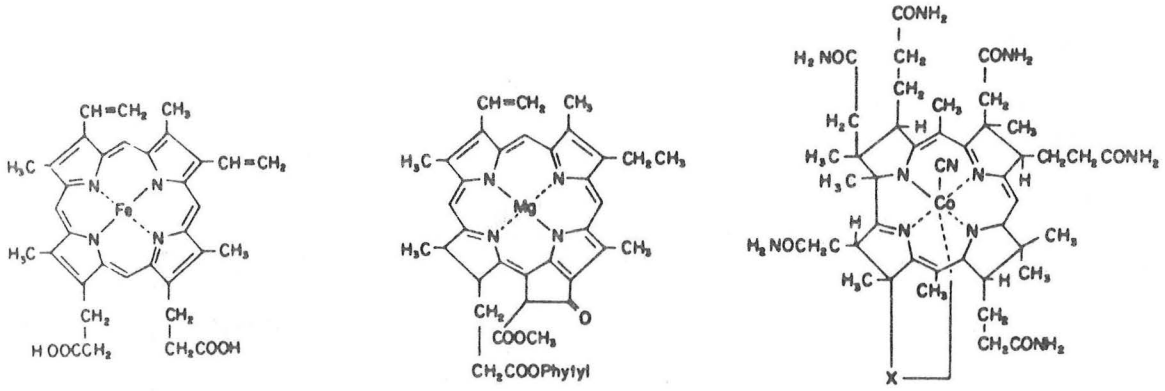
Figure 3 (Ref. 5)



PORPHYRIN

dine, dioxane and less so in alcohol. The planar molecules, composed of four pyrrole rings joined by carbon bridges, readily bind transition metals such as iron, magnesium or cobalt (6). These metalloporphyrins act as the prosthetic group for molecules essential to oxidative respiration (heme), photosynthesis (chlorophyll) and are also found as Vitamin B_{12} (Figure 4). The biological important porphyrins have similar absorption spectra as exemplified by the absorption spectrum of the methyl

Figure 4 (Ref. 6)



Ferriprotoporphyrin IX (heme)

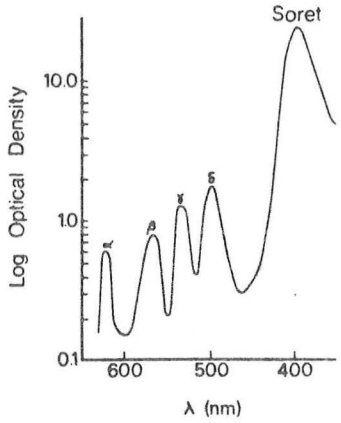
Chlorophyll a

Vitamin B_{12}

Biologically important metalloporphyrins.

ester of coproporphyrin in chloroform (Figure 5). The most striking feature is the prominence of the Soret band in the vicinity of 400 nm, which exceeds the intensity of the visible spectra by a factor of 10-20 (5). This is the band that is usually used in the quantitative spectroscopic determination of porphyrins. In addition, the strong absorption at this wavelength region is responsible for the severe skin lesions expressed in porphyrias with photosensitivity (see below).

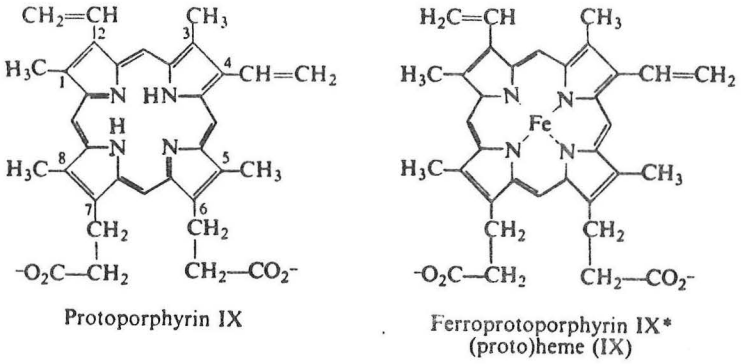
Figure 5 (Ref. 5)



The typical appearance of an etio type of spectrum of the porphyrin methyl esters as exemplified by the Copro methyl ester spectrum. Note the prominence of the Soret band and the regular increase in spectral intensity of the visible spectral bands α, β, γ, and δ, which are also commonly referred to as bands I, II, III, and IV.

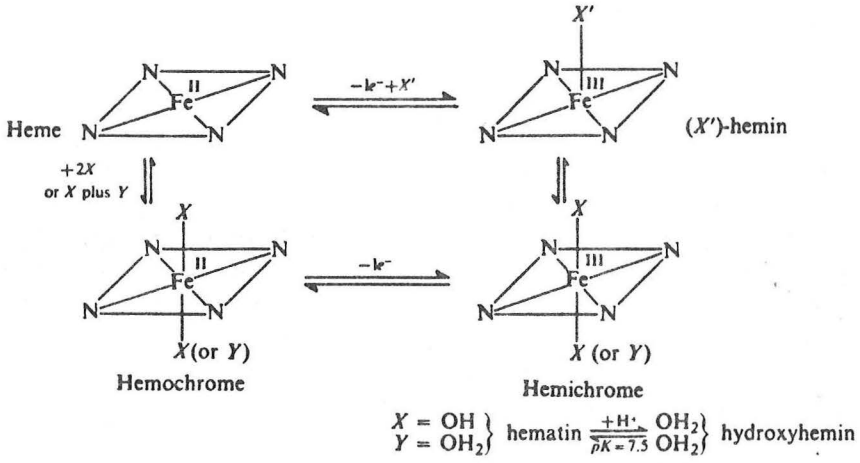
The endproduct of the heme synthetic pathway, before iron insertion occurs, is protoporphyrin IX, the only one of 15 possible protoporphyrin isomers found in nature (Figure 6). Protoporphyrin IX, when associated with Fe^{2+} (ferrous iron), forms ferroprotoporphyrin IX, which serves as the prosthetic group for various hemoproteins such as hemoglobin, myoglobin, erythrocruorin, tryptophan pyrrolase, catalase, peroxidase, mitochondrial cytochromes, microsomal cytochrome b_5 and microsomal cytochrome P-450 (7). The transition metal iron, located in the center of the porphyrin molecule, is held by four coordination bonds. Such a chelate complex of protoporphyrin with Fe(II) (equal to Fe^{2+}) is called <u>protoheme</u> or simply <u>heme</u>; a similar complex with Fe(III) (equal to Fe^{3+}) is called <u>hemin</u> or <u>hematin</u> (Figure 7). The latter

Figure 6 (Ref. 7)



metalloporphyrin (hematin) is the compound utilized for the treatment of acute porphyrias (see below). In heme, the four ligand groups of the porphyrin form a square-planar complex with the iron; the remaining fifth and sixth coordination positions of iron are perpendicular to the plane of the porphyrin ring. When the fifth and sixth position of iron are occupied, the resulting structure is a <u>hemochrome</u> or <u>hemochromogen</u> (Figure 7).

Figure 7 (Ref. 7)



Probably all mammalian cells can synthesize prophyrins although the erythropoietic cells form nearly 80% of the body heme and almost all the rest is synthesized by the liver parenchymal cells (8). Most of the erythropoietic heme is incorporated into hemoglobin (stable for ~120 days in erythrocytes). In contrast, the liver synthesizes a great number of hemoproteins which all have rather rapid turnover rates (Table I; Ref. 9). Since exact data for the human liver are not available, the relative concentrations and turnover rates of hemoproteins in rat liver are assumed to be representative. As we will discuss in more detail under the section "Regulation of Heme Synthesis", the extremely high utilization of heme by cytochrome P-450 plays a major role in the drug induced hepatic porphyrias. After the mitochondrial cytochromes, the microsomal cytochrome P-450 represents quantitatively the major hepatic hemoprotein.

Table I (Ref. 9)

Relative concentrations and turnover rates of hemoproteins in rat liver

Hemoprotein	*Concentration, nmol/g liver*	$t_{1/2}$, *h*	*Heme utilized, nmol/(g liver · h)*
Catalase	5.3	29.0	0.44
Tryptophan oxygenase	0.1	2.2	0.04
Mitochondrial cytochromes	30.0	132.0	0.16
Microsomal cytochrome b_5	12.0	45.0	0.18
Microsomal cytochrome P450	18.0	7-10	1.68
(biphasic)	4.5	24-48	0.09

SOURCE: The data for these calculations are based on the references cited in Table 45-2 of the third edition of *The Metabolic Basis of Inherited Disease* (1972).

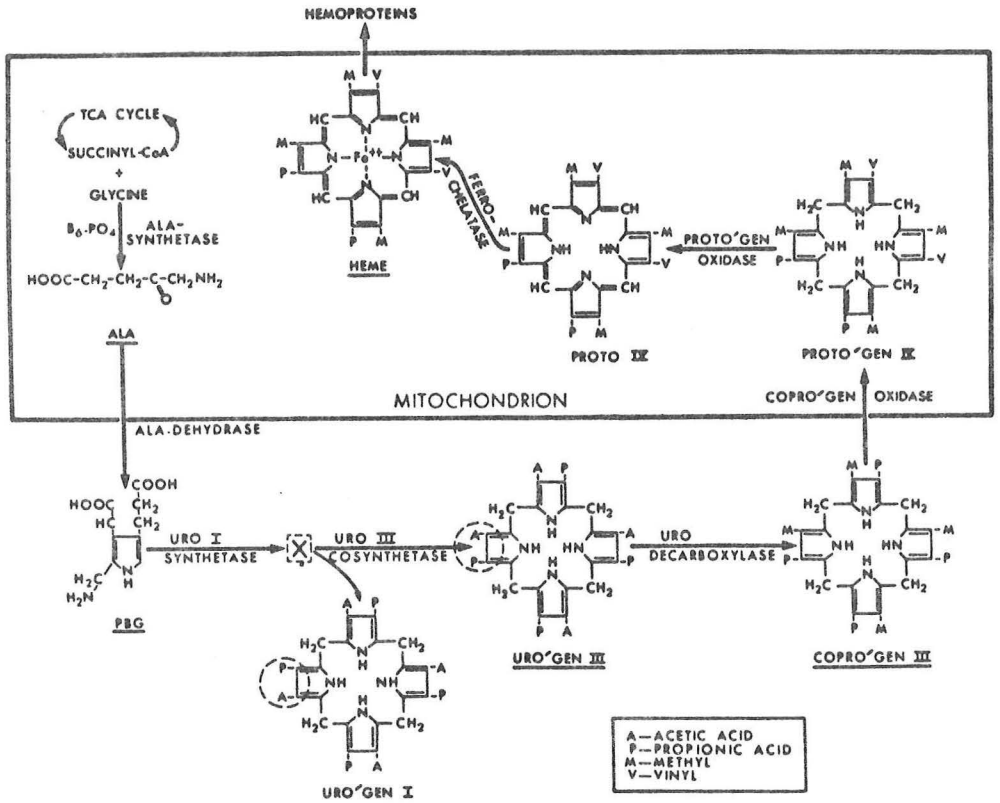
III. HEME BIOSYNTHESIS

The synthesis of heme involves eight enzymes, four of which are localized in the cytoplasmic compartment (aminolevulinate dehydratase, uroporphyrinogen I synthetase, uroporphyrinogen III cosynthetase and uroporphyrinogen decarboxylase) and four in the mitochondrial compartment (coproporphyrinogen oxidase, protoporphyrinogen oxidase, heme synthetase (or ferrochelatase) and aminolevulinate synthetase). In Figure 8, the complete heme synthetic pathway is summarized.

1. Aminolevulinate Synthetase (EC 2.3.1.37)

Aminolevulinate synthetase (ALA-S), the first enzyme of the heme synthetic pathway, catalyzes the formation of one mol of δ-aminolevulinic acid (ALA) by combining one mol of glycine and one mol of succinyl CoA in the presence of pyridoxal phosphate (B_6-PO_4). Glycine reacts with enzyme-bound B_6-PO_4 to form a stable carbanion; glycine then reacts with succinyl CoA to form ALA with loss of CoA. The enzyme requires sulfhydryl-groups for activity. The mammalian enzyme is normally exclusively found in mitochondria, either free in the mitomatrix or loosely bound to the inner mitochondrial membrane (8). In livers and kidneys of animals treated with porphyrinogenic drugs (ALA-S is induced), a significant proportion of the total activity is present in cytoplasm. Dependent on the animal species, the molecular weight (MW) of both enzyme species is either equal (mouse) or much higher in cytoplasm (rat). The MW of the rat cytoplasmic enzyme has been estimated to be 178,000 and that of the mitochondrial enzyme 77,000. When ALA-S is

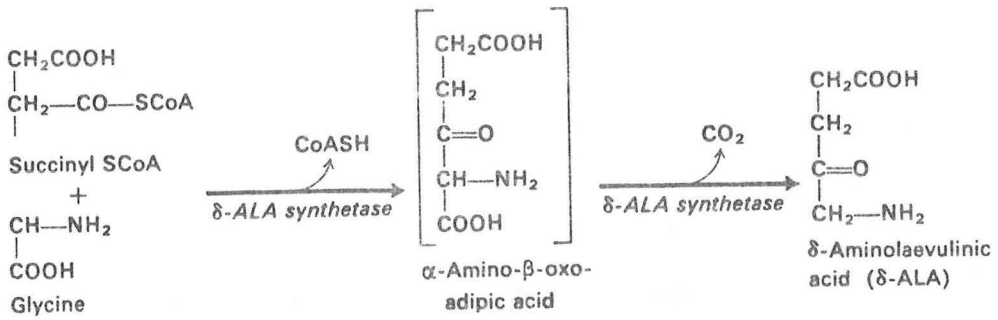
Figure 8 (Ref. 9)



Outline of heme biosynthesis. ALA, δ-aminolevulinic acid; PBG, porphobilinogen; URO, UROgen, uroporphyrinogen; COPROgen, coproporphyrinogen; PROTOgen, protoporphyrinogen; PROTO, protoporphyrin.

assayed in liver homogenates (mitochondria intact), the enzyme activity is usually very low (10% of the enzyme activity induced with porphyrinogenic drugs). But after sonication of the normal homogenate (mitochondria broken), the activity rises ten-fold and equals the activity of the induced enzyme (measured un-sonicated). Therefore, an endogenous inhibitor (hemin?) may mask normal total enzyme activity. After sonication, the inhibitor may be diluted out and the inhibition of ALA-S will be released. The $T_{\frac{1}{2}}$ of the rat cytoplasmic enzyme has been estimated as 20 minutes (120 minutes in the presence of hemin) and that of the mitochondrial enzyme as 70 minutes. The cytoplasmic enzyme is presumably the precursor of the mitochondrial enzyme. The human liver ALA-S activity has been estimated between 10-100 nmol ALA formed/hour/g liver. The rat liver ALA-S is specific for succinyl CoA and glycine; it does not act on other acyl CoA esters or on other amino acids. The mammalian K_m values for glycine are 5-19 mM, for succinyl CoA 60-200μM and for B_6-PO_4 1-10μM. Fifty percent inhibition of ALA-S can be achieved by 50μM hemin (hematin). The detailed two-step catalytic reaction of ALA synthesis is shown in Figure 9.

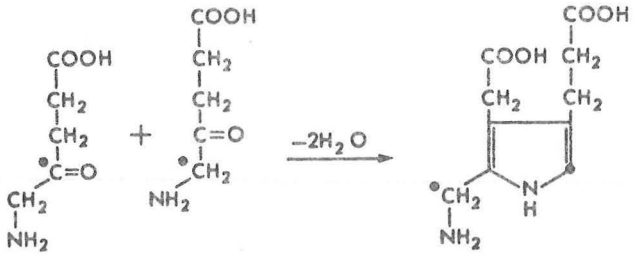
Figure 9 (Ref. 10)



2. Aminolevulinate Dehydratase (EC 4.2.1.24)

Aminolevulinate dehydratase (ALA-D) catalyzes the second reaction of the heme synthetic pathway, namely the condensation of 2 molecules of ALA to form the pyrrole porphobilinogen (PBG) with loss of 2 molecules of water (Figure 10). This enzyme reaction is the first one of a sequence of enzymatic steps of heme synthesis occurring in the cytoplasmic compartment. After ALA is transported out of mitochondria to cytosol, the ALA molecules bind as Schiff base to the enzyme. Part of the ALA molecules contribute the acetic acid side chains of the PBG. The enzyme is sensitive to sulfhydryl-inhibitors, has a pH optimum of 6.3-7.0 and its K_m for ALA is 0.1-0.4 mM. Various heavy metal ions including lead (Pb^{2+}) are non-competitive inhibitors with ALA. Such inhibition can be reversed with thiol compounds. Although the rat liver enzyme has been shown to be inhibited by Fe^{3+}, hemin, protoporphyrin and coproporphyrinogen III, the physiological significance of this "feedback" inhibition remains

Figure 10 (Ref. 9)



Condensation of 2 moles of δ-aminolevulinic acid to form 1 mole porphobilinogen. The labeled carbon atoms are the original α-carbon atoms of glycine.

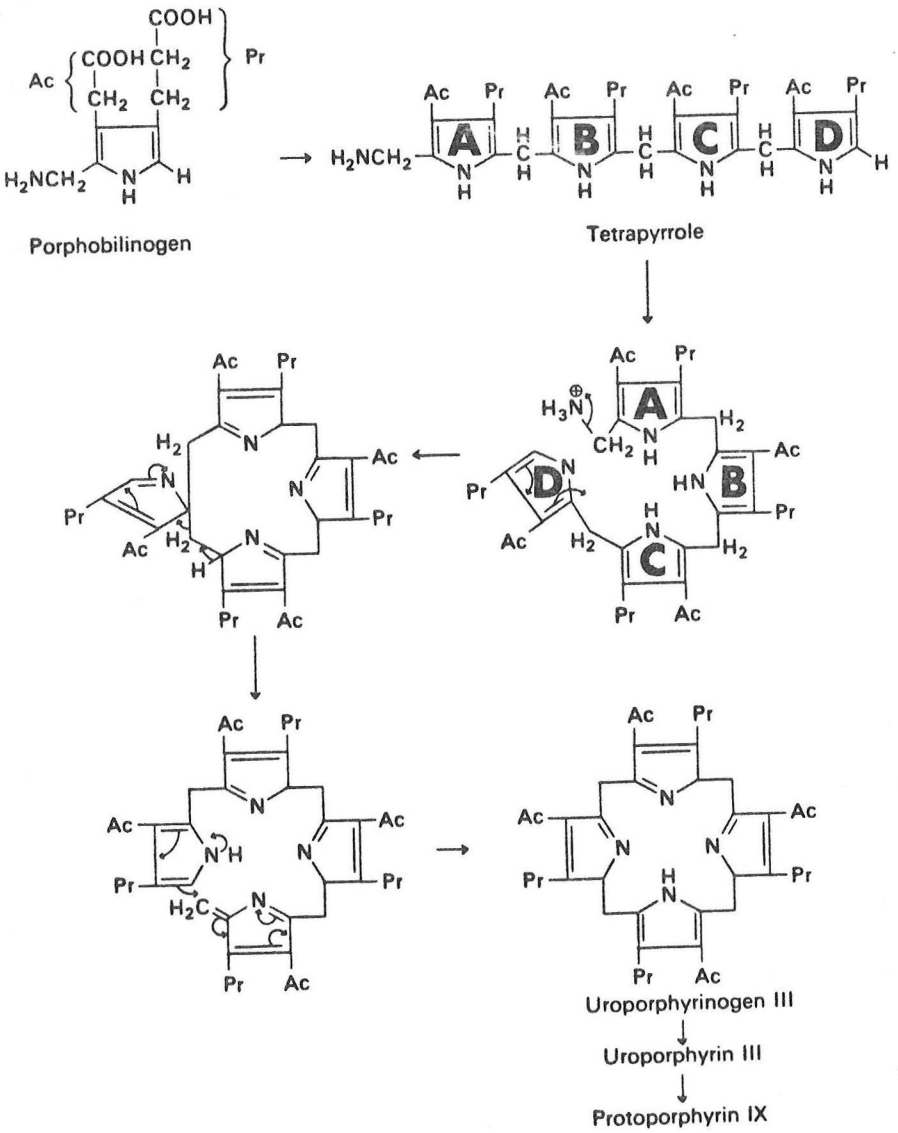
to be determined. The enzyme is a multisubunit protein complex (8-14 subunits) with a MW between 260,000-289,000. Recent evidence suggests that zinc (5-6 g atoms Zn^{2+}/mol enzyme) may be essential for enzyme activity. In all tissues studied, the V_{max} of ALA dehydratase vastly exceeds the V_{max} of ALA-S (by 50-100 times). The $T_{\frac{1}{2}}$ of the enzyme protein

has been determined for mice between 5-6 days. ALA as well as porphobilinogen are soluble in aqueous solution at physiological pH, are colorless and non-fluorescent.

3. Uroporphyrinogen I Synthetase (EC 4.3.1.8) and Uroporphyrinogen III Cosynthetase

The uroporphyrinogen I synthetase (UROgen I-S; also called porphobilinogen deaminase) catalyzes the condensation of four molecules of PBG to form the symmetrical cyclic tetrapyrrole uroporphyrinogen I (UROgen I) and four molecules of ammonia (8). The four condensation steps take place on the enzyme surface and intermediates are firmly bound to the enzyme. Formation of uroporphyrinogen III (UROgen III) from PBG, where one PBG (D) is inserted the opposite way (Figure 11; Ref. 11)) occurs under combined action of UROgen I-S and a second protein, uroporphyrinogen III cosynthetase (also called "specifier protein"). Uroporphyrinogen III cosynthetase does not by itself react with PBG or with UROgen I.

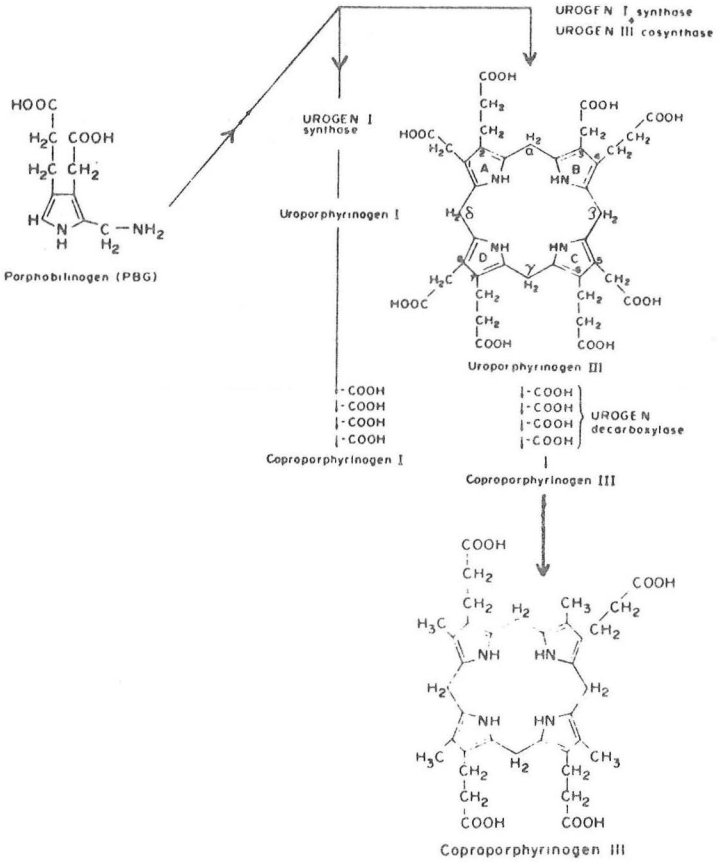
Figure 11 (Ref. 11)



A proposed mechanism for the formation of Uroporphyrin III, the most abundant natural isomer.

Normal human erythrocytes and all mouse tissues tested have a much higher activity of uroporphyrinogen III cosynthetase than uroporphyrinogen I synthetase. This activity relationship insures that in vivo, under normal conditions, only the biologically useful III isomer is formed. The molecular weight of the uroporphyrinogen I synthetase is approximately 25,000 and that of the uroporphyrinogen III cosynthetase is in the range of 280,000. The activity of the uroporphyrinogen I synthetase in mouse liver and erythrocytes is between 11-60 nmol uroporphyrinogen I formed/hour/g wet tissue.

Figure 12 (Ref. 12)

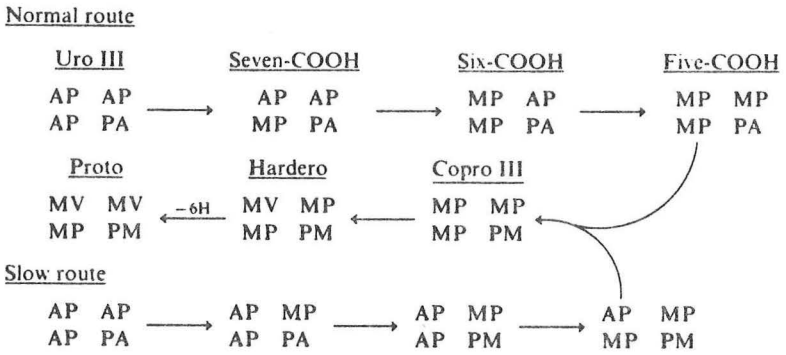


4. Uroporphyrinogen Decarboxylase (EC 4.1.1.37)

This fourth and last cytoplasmic enzyme of the heme synthetic pathway catalyzes the formation of coproporphyrinogen III from uroporphyrinogen III (this enzyme catalyzes also the I-isomer transformation). The enzymatic reaction involves a stepwise decarboxylation of the four acetic acid side chains of the porphyrin ring to methyl groups (Figure 12, 13). It has been demonstrated (8) that the decarboxylation of the acetic acid side chains starts with the pyrrole ring D (see Figure 3 for nomenclature) and proceeds in clockwise fashion, finishing with ring C. Decarboxylation intermediates with varying numbers of carboxyl groups have been discovered in urine of porphyric patients (see below). Whether the four sequential decarboxylation

steps are performed by a single or by several closely related enzymes is unknown. This cytoplasmic enzyme has a pH-optimum of about 7.0, a K_m value for uroporphyrinogen III of 1-5μM and does not seem to require any particular cofactors such as B_6-PO_4. The enzyme is inhibited by compounds such as Hg^{2+}, Cu^{2+}, iodoacetamide, p-chloromercuribenzoate and Mn^{2+}. Reversal of this inhibition can be achieved by reduced glutathione (GSH).

Figure 13 (Ref. 5)



The steps involved in the decarboxylation of Urogen to Coprogen, according to Jackson *et al.*[36] Note that decarboxylation proceeds clockwise in both systems commencing in ring D in the normal pathway and in ring A in the slow alternate route (A, acetic acid; P, propionic acid; M, methyl; V, vinyl).

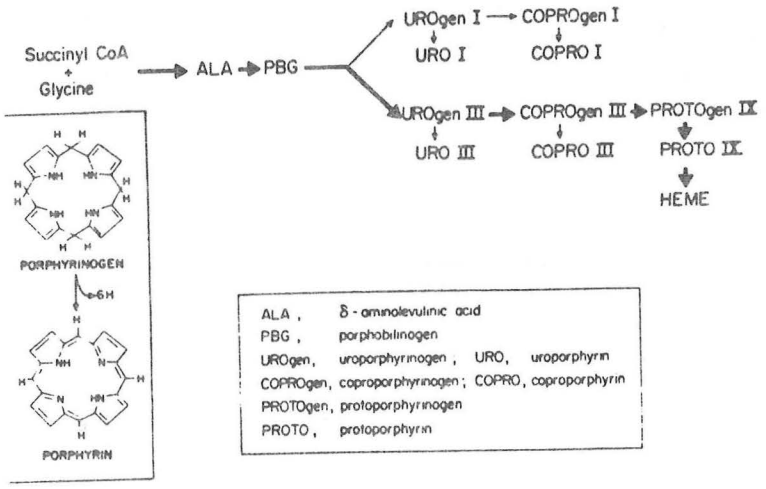
In addition to the stepwise decarboxylation of uroporphyrinogen to coproporphyrinogen, an alternate albeit minor pathway has recently been discovered which leads to the formation of so-called isocoproporphyrins (9); these include isocoproporphyrin, diethylisocoproporphyrin, dihydroisocoproporphyrin, and hydroxyisocoproporphyrin. Their source presumably is a pentacarboxylic precursor of coproporphyrinogen III. Significant quantities of isocoproporphyrins are excreted in porphyria cutanea tarda (PCT) and in rats with experimental porphyria induced by hexachlorobenzene or other polychlorinated hydrocarbons.

Nonenzymatic oxidation of uroporphyrinogen to uroporphyrin can take place by photocatalytic autoxidation, a process that is activated by the product, uroporphyrin (Figure 14).

5. Coproporphyrinogen Oxidase (EC 1.3.3.3)

This mitochondrial enzyme catalyzes the oxidative decarboxylation of the propionic acid side chains on the pyrrole rings A and B to vinyl groups. The propionate side chains of rings C and D are not modified. The sequential decarboxylation starts with ring A and concludes with ring B. The enzyme requires molecular oxygen (O_2). This enzyme is specific for coproporphyrinogen III and forms protoporphyrinogen IX as result of its catalytic reaction (Figure 15). The K_m value for coproporphyrinogen III is approximately 30μM.

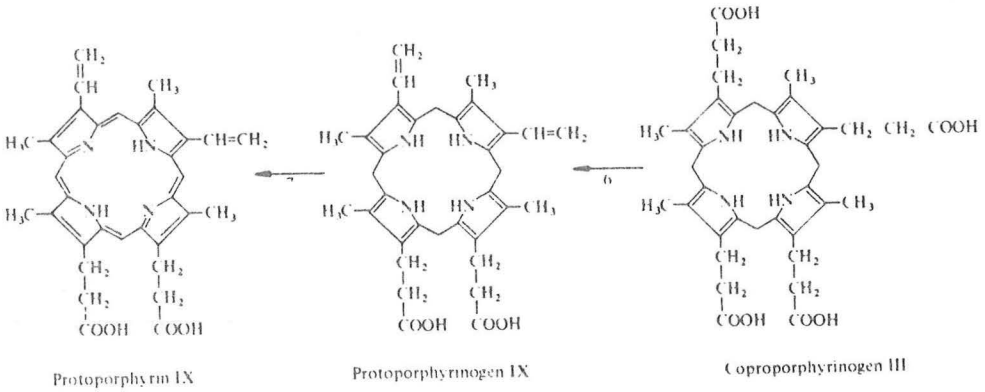
Figure 14 (Ref. 6)



The relationship of porphyrinogens and porphyrins to the haem synthetic pathway.

Although no known cofactors have been associated with this enzyme, it is suspected to also be a metallo-enzyme. Its pH-optimum is pH 7.5 and the MW has been assessed as 80,000. The highest activity measured in guinea pig and rabbit liver is 150 nmol protoporphyrinogen IX formed/hour/g wet weight tissue.

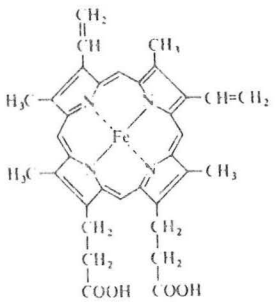
Figure 15 (Ref. 8)



Protoporphyrin IX

Protoporphyrinogen IX

Coproporphyrinogen III



Protoheme IX

Enzyme Reactions:

(6) Coproporphyrinogen Oxidase
(7) Protoporphyrinogen Oxidase
(8) Heme Synthetase

6. Protoporphyrinogen Oxidase

This mitochondrial enzyme oxidizes protoporphyrinogen IX to protoporphyrin IX by removing 6 hydrogen atoms and creating 3 new double bonds within the porphyrin molecule (Figure 15). The pH-optimum of this enzyme is approximately pH 7.5. Its MW has been assessed as 180,000 and its K_m for protoporphyrinogen IX is 4.8μM. The enzyme is most active in the presence of GSH or dithiothreitol (DTT), both reducing agents of sulfhydryl groups. Inhibitor studies revealed that the enzyme is non-competitively inhibited by heme and by hemin; 50% inhibition was observed in the presence of 20μM heme and of 50μM hemin.

7. Heme Synthetase (EC 4.99.1.1)

This mitochondrial enzyme is also frequently called "Ferrochelatase" and it catalyzes the incorporation of Fe^{2+} (ferrous iron) into protoporphyrin IX to form protoheme IX (Figure 15). The enzyme, which has not yet been obtained in soluble form (8), is located at the inner side of the inner mitochondrial membrane. At this point, the exact MW remains to be determined and relatively little is known about its properties. Protoporphyrin is formed enzymatically in mitochondria and Fe^{3+} (ferric iron) is reduced to Fe^{2+} (ferrous iron) inside the inner mitochondrial membrane in proximity to the heme synthetase (ferrochelatase). Fe^{3+} is not a substrate for the enzyme but CO^{2+} as well as Zn^{2+} can be utilized for incorporation.

Heme synthetase of human bone marrow has an optimum activity at pH 7.4, a K_m for Fe^{2+} of 17μM and a K_m for protoporphyrin of 1.8μM. The rat liver enzyme has a pH-optimum of 8.2, but K_m values for both substrates are similar to the human bone marrow. Protoheme IX, the product of this reaction, can inhibit the enzyme by 50% at 16μM and 100μM, respectively, using either the rat liver or the human bone marrow enzyme.

The heme synthetase activity (n mol cobalt deuteroporphyrin formed/min/mg mitochondrial protein) measured in various tissues is 0.71, 0.40, 0.34 and 0.33 in rat liver, brain, kidney and heart, respectively.

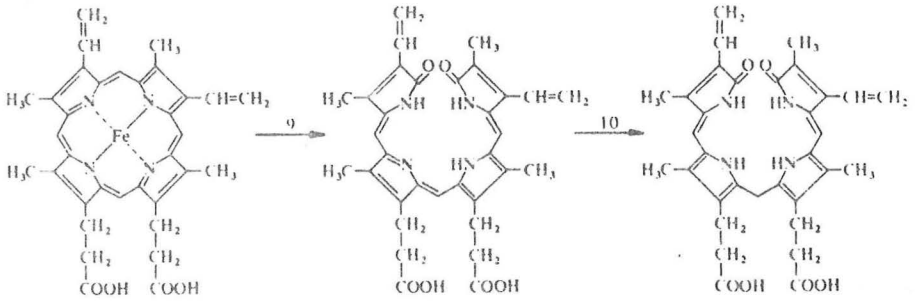
The completed heme is now available to interact with the apoproteins of mitochondrial, peroxisomal and microsomal hemoproteins. More recently (8) it was found that ligandin, a cytoplasmic protein of liver, binds protoheme IX with an apparent affinity constant of 10^8 M^{-1}. It has been suggested that this protein may play a role in the removal of heme from mitochondria.

IV. HEME DEGRADATION

1. Heme Oxygenase (EC 1.14.99.3)

Heme oxygenase is a microsomal enzyme which requires NADPH and oxygen; it catalyzes the conversion of hemin as well as the heme of a number of hemoproteins to equimolar amounts of biliverdin IXα and carbon monoxide (Figure 16). Porphyrin molecules without iron are not subject to degradation by this enzyme.

Figure 16 (Ref. 8)



Heme Degradation

(9) Heme Oxygenase
(10) Biliverdin Reductase

2. Biliverdin Reductase (EC 1.3.1.24)

This enzyme converts biliverdin to bilirubin. Enzyme activity has been described in liver, kidney, brain (gray matter) and spleen. The subsequent metabolism and excretion of bilirubin has been previously reviewed for Grand Rounds by Dr. Burton Combes.

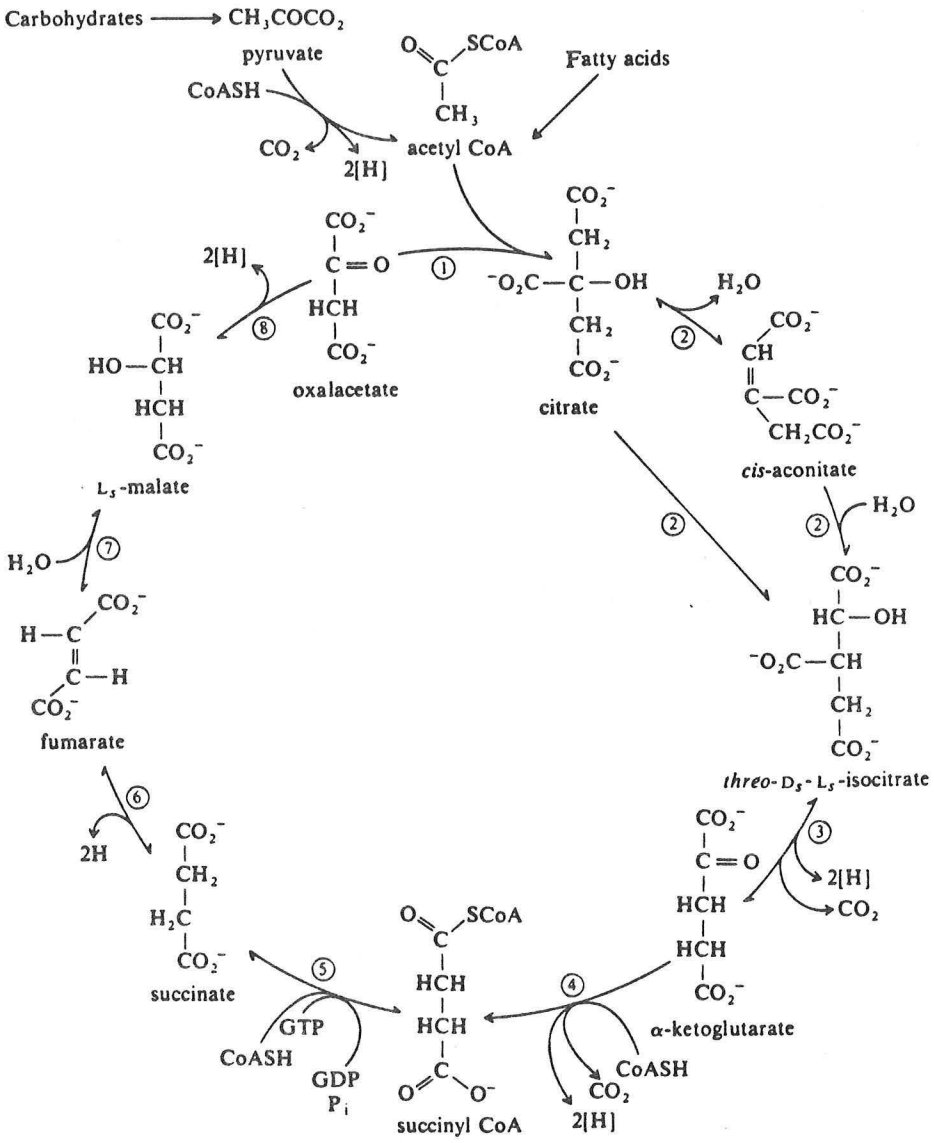
V. REGULATION OF HEME AND HEMOPROTEIN BIOSYNTHESIS

1. Substrate Availability for ALA-Synthetase

As described above, two substrates (succinyl CoA, glycine) and one co-factor (pyridoxal phosphate) are required for the synthesis of δ-aminolevulinic acid (ALA). Succinyl CoA is a key intermediate of the tricarboxylic acid cycle (Figure 17) and is formed from α-ketoglutaric acid by the multienzyme complex of the α-ketoglutaric acid dehydrogenase (Figure 18). In addition to the Krebs cycle, succinyl CoA is also provided through the metabolism of propionic acid (Figure 19) as well as by a few other metabolic pathways involving valine and isoleucine degradation. Heme biosynthesis, by way of one of his first substrates, is therefore intimately linked to the intermediary metabolism of carbohydrates, proteins and lipids. Nevertheless, at present there is no evidence that under physiological conditions the provision of succinyl CoA could become rate limiting for heme synthesis.

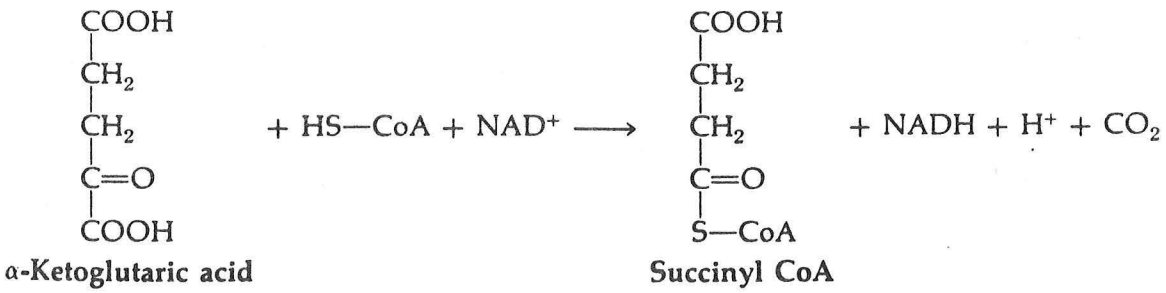
The second substrate of ALA-S, glycine, also participates in a great number of enzymatic reactions of intermediary as well as drug metabolism. Such linkage of glycine to various functional pathways could subject heme biosynthesis, at least theoretically, to various modes of controls resulting in the fluctuation of substrate supply for ALA-S. Glycine is closely linked to serine and folic acid metabolism as well as to the regulation of the active "C^1" carbon pool (Figure 20). This pool supplies the substrate for methylation reactions

Figure 17



The Citric Acid (Tricarboxylic Acid) Cycle.

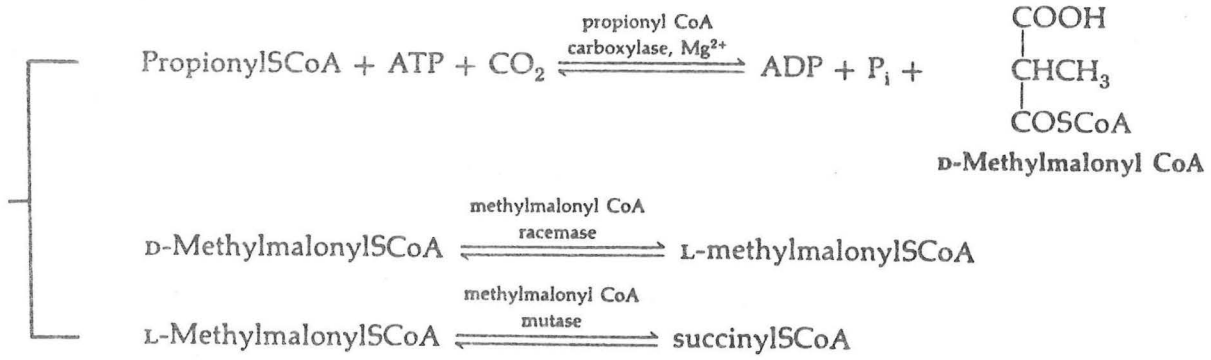
Figure 18



α-Ketoglutaric acid

Succinyl CoA

Figure 19



in catecholamine metabolism and other pathways. It is of interest to note that glycine and serine were recently implicated (13) in the induction of acute psychosis in four patients of whom two were proven to suffer from porphyria. Glycine, by action of glycine oxidase (D-amino acid oxidase), is also a precursor of glyoxylic acid (Figure 21). During this reaction, ammonia and H_2O_2 are produced. Another reversible reaction between glycine and glyoxylic acid is the transamination of glyoxylic acid to glycine by glyoxylate aminotransferase (not shown).

Figure 20

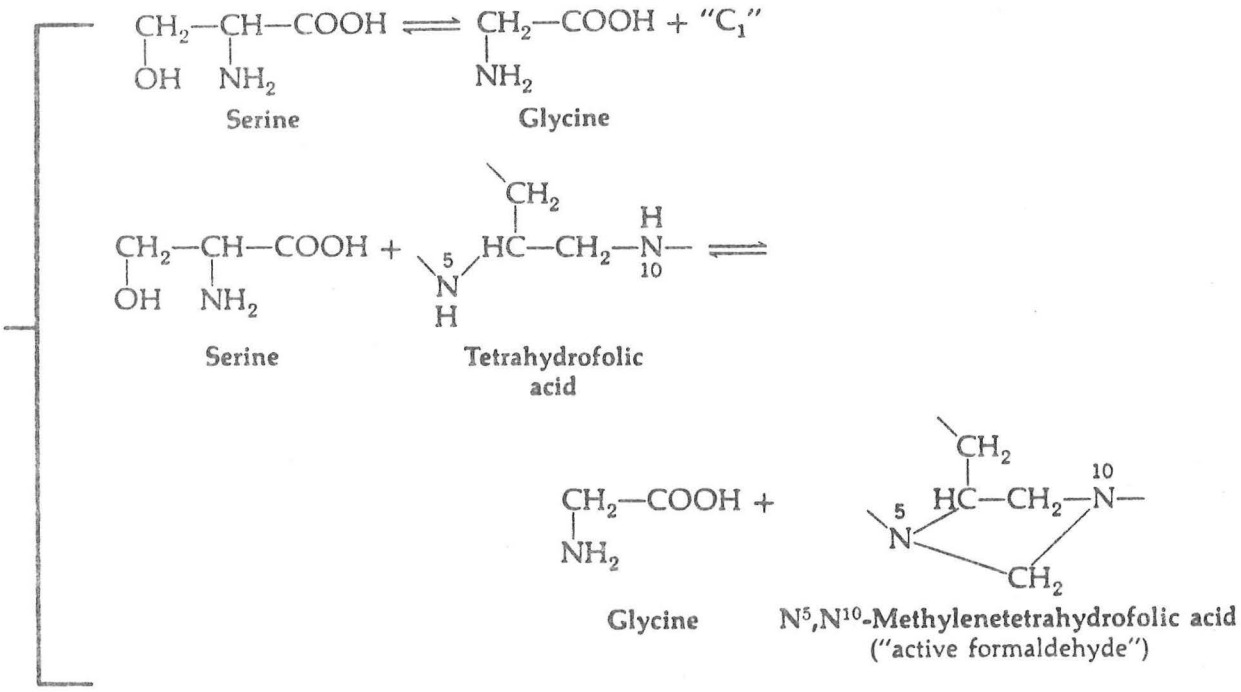
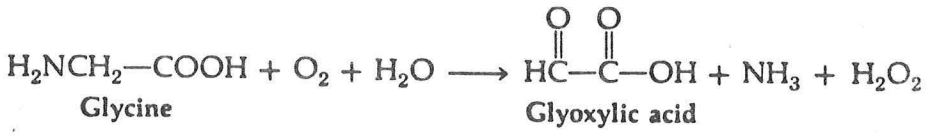
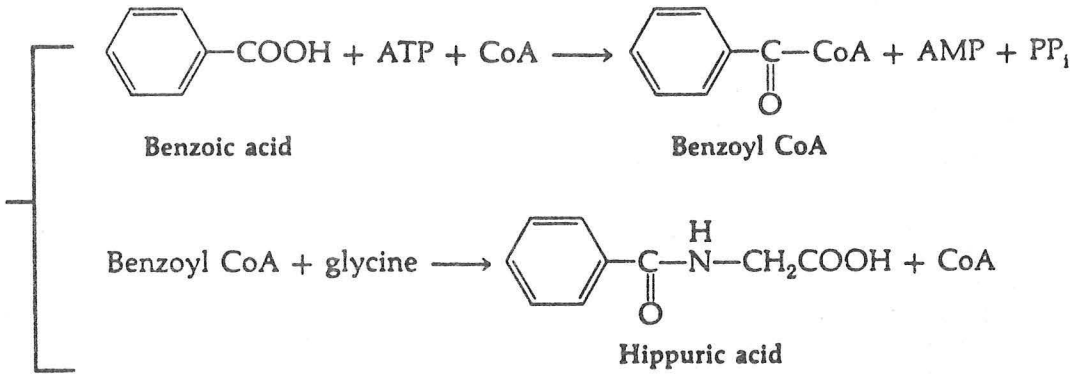


Figure 21



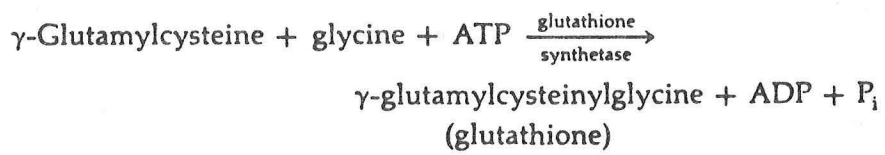
A very important reaction in which glycine participates (and therefore is subject to elimination) is shown in Figure 22 concerning the classical removal and detoxification of benzoic acid by conjugation with glycine. The mitochondrial enzyme acyl-CoA-glycine transferase catalyzes the elimination of foreign compounds by amino acid conjugation after they have been converted to the CoA derivatives of carboxylic acids. Therefore, under particular conditions, glycine could be subject to depletion by such reactions.

Figure 22



And finally, glycine is intimately linked to glutathione metabolism by being the third amino acid utilized for the last step of glutathione synthesis, catalyzed by glutathione synthetase (Figure 23). It is conceivable (but not proven) that in acute porphyria, when large amounts of glycine are consumed by the highly induced ALA-S, glutathione metabolism may be impaired. This could result in decreased elimination of toxic metabolites of drugs or environmental toxins, thereby perpetuating their effect on the heme biosynthetic pathway.

Figure 23



Although pyridoxal-5-phosphate is a required cofactor of the ALA-S, under physiological conditions and even in experimental porphyria it does not appear to assume an important role (9).

2. Regulation of ALA Synthetase Activity

Extensive studies in animals as well as in vitro have firmly established that in normal liver the primary regulatory control of heme synthesis is exercised at the level of ALA synthetase (6,9). ALA-S has been shown (see above) to be the rate limiting step in heme synthesis.

Studies conducted in recent years have provided convincing evidence that heme is involved in the negative feedback control of hepatic ALA synthetase. In addition to the biochemical evidence of such regulation it should be noted that the physical closeness of ALA synthetase to heme synthetase (the first and last enzyme of the heme synthetic pathway) within the inner mitochondrial membrane provides an almost perfect system for feedback regulation of ALA-S by heme, the product of heme synthetase.

Three major modes of feedback inhibition of ALA-S have been proposed (9): (1) feedback inhibition of ALA-S activity by heme; (2) feedback repression of the synthesis of ALA-S by heme; and (3) heme inhibition of the transfer of soluble cytosolic ALA synthetase into mitochondria.

Any loss of such feedback inhibition will result in overproduction of porphyrins or porphyrin precursors, resulting in the clinical syndromes of porphyria in patients who are genetically predisposed.

Hypothesis (1): Direct inhibition of ALA synthetase activity by heme has been demonstrated with partially purified enzyme preparations and with concentrations of heme between 10^{-4} M and 10^{-5} M (14, 15, 16). However, direct proof of such regulation in vivo has not been obtained.

Hypothesis (2): Studies in primary avian hepatocyte cultures as well as in intact animals strongly suggest that the principal mode of negative feedback regulation consists of repression of the synthesis of ALA-S by heme (17). However, the precise mechanism of heme repression of ALA-S still remains unclear. But in any case, the short biological half-life of ALA synthetase and of its messenger RNA (70-300 min) as shown in rat liver and chick embryo make a primary regulation of this enzyme at the level of its synthesis an attractive assumption. This feedback repression could be achieved at heme concentrations as low as 10^{-7} to 10^{-8} M (18).

Hypothesis (3): Although there is some experimental evidence that heme may interfere with the transfer of newly synthesized ALA-S from cytoplasm to mitochondria, there remain significant doubts about the potential biological significance of this mechanism for heme mediated regulation of ALA synthetase.

It can be concluded that, whatever the mechanism may be, ALA-S activity can definitely be regulated by heme.

3. ALA Synthetase Induction by Chemicals and Drugs

The first demonstration that certain drugs could induce ALA-S was provided by Granick and Urata (19). That indeed the synthesis of enzyme-protein occurs as result of such induction has been quantitatively proven by specific antibody precipitation of the purified enzyme (20).

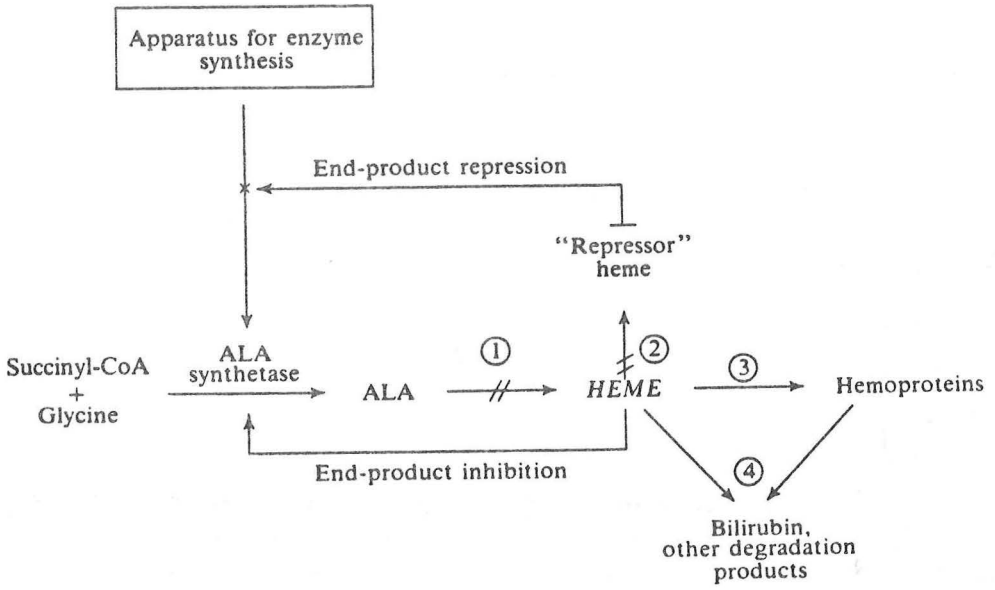
In view of the feedback regulation of ALA-S by heme it was postulated that inducing substances would affect the size or kinetics of a postulated intracellular heme pool ("regulatory heme") involved in the control of ALA-S. Such "regulatory heme" would, under normal circumstances, repress the synthesis of ALA-S to a degree that this enzyme remains rate limiting for heme synthesis but nevertheless meets the "physiological" demand. If such "heme pool" becomes reduced, the enzyme synthesis of ALA-S is de-repressed and a high rate of synthesis of the enzyme-protein results. Following this induction, great quantities of porphyrin precursors (ALA, PBG) virtually flood the heme synthetic pathway and are excreted prior to reaching the final product (protoheme) in urine or feces if along the pathway a genetic enzyme deficiency exists (see below for details).

Several mechanisms could lead to a reduction of the "regulatory heme" pool (Figure 24).

a) increased heme demand (utilization)
b) increased heme breakdown
c) impaired heme synthesis

Hemoproteins of mitochondria, cytosol and microsomes consistently compete for newly synthesized heme. Of all hemoproteins in liver, cytochrome P-450 (probably more than twenty species) accounts for the major part of the total hepatic heme synthesized (see Table I; Ref. 9). Since cytochrome P-450 is known in liver to increase in response to the application of various lipophilic chemicals, the observed parallel induction of ALA-S was not unexpected. A rise in ALA-S following administration of P-450 inducing compounds

Figure 24 (Ref. 21)



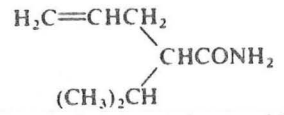
Four possible mechanisms by which a drug may reduce the feedback control exercised by heme at the level of ALA-S: *1*) inhibition of heme synthesis; *2*) inhibition of the formation of "repressor" heme (GRANICK, 1966); *3*) increased rate of heme utilization; *4*) increased rate of heme degradation.

can be postulated to be the result of an increased heme demand to accommodate the increasing level of cytochrome P-450 apoproteins. Heme itself does not stimulate the synthesis of the protein moiety of hemoproteins. It appears from many experimental data that the rate of synthesis of individual apoproteins change in response to environmental affectors; the rate of heme synthesis is correlated to the availability of apoprotein.

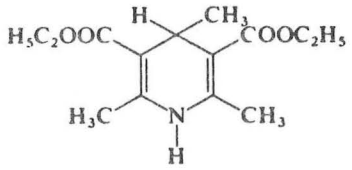
Accumulation of apo-cytochrome P-450 is magnified by partial inhibition of heme synthesis with heavy metals and other chemicals; under these circumstances, there is also massive induction of hepatic ALA-S.

Different from the sequence: induction of P-450 apoprotein by drugs or chemicals - depletion of "regulatory heme" for binding to the apoprotein - depression of ALA-S, a specific group of chemicals causes massive induction of ALA-S and produces in rodents a disorder mimicking human hepatic porphyria (Figure 25).

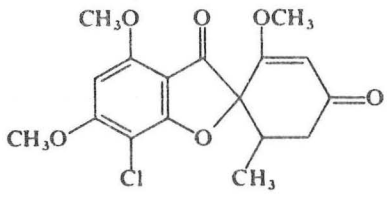
Figure 25 (Ref. 21)



2-Allyl-2-isopropylacetamide
(AIA)



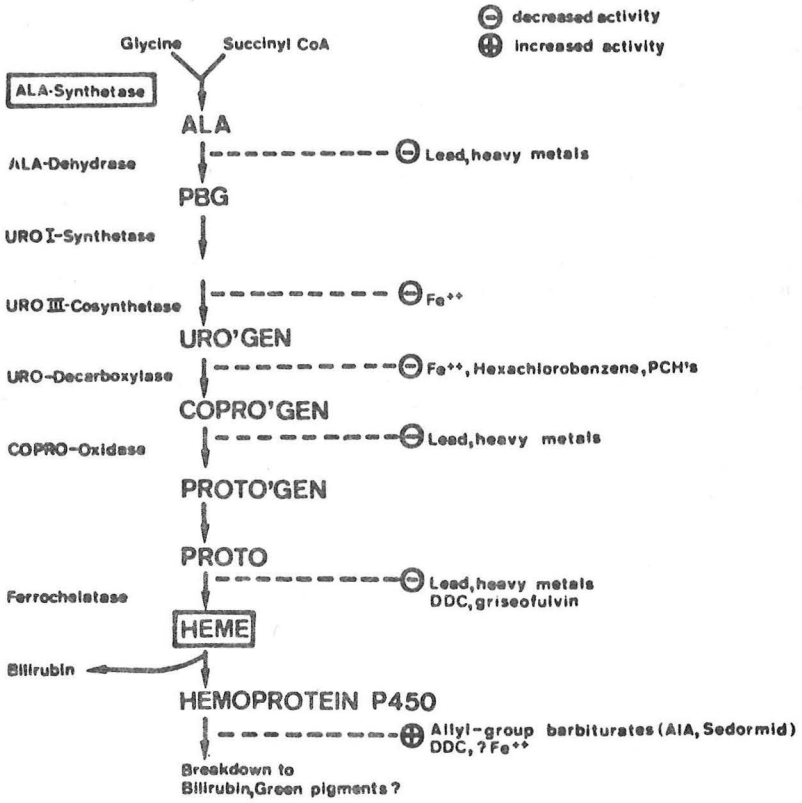
3,5-Diethoxycarbonyl-1,4-dihydrocollidine
(DDC)



Griseofulvin

Allylisopropylacetamide (AIA), dicarbethoxydihydrocollidine (DDC) and griseofulvin are such chemicals which precipitate depletion of the intracellular heme pool either by inhibiting enzymatic steps in heme biosynthesis (DDC, griseofulvin; inhibition of heme synthetase (ferrochelatase)) and/or by direct heme destruction (AIA, DDC) (Figure 26). The result is in all cases massive induction of ALA-S.

Figure 26 (Ref. 9)



Toxic and experimental porphyria. Defects at individual sites of hepatic heme biosynthesis and breakdown produced by various chemicals. PCH's, polychlorinated hydrocarbons; DDC, dicarbethoxydihydrocollidine; AIA, allylisopropylacetamide.

In Table II (Reference 21), the special properties of cytochrome P-450 are listed which make this hemoprotein a preferential target for the toxic action of chemicals.

The time course of cytochrome P-450 concentrations in liver after injection of AIA is shown in Figure 27. With decreasing levels of cytochrome P-450 one can observe a striking induction of ALA-S.

In summary, the effect of various inducers of cytochrome P-450 as well as of toxic chemicals can all be traced to alterations (decrease) of the so-called "regulatory heme" pool (Figure 28). This "regulatory heme" is subject to direct interaction with exogenously administered heme, which has recently been shown by Correia et al. (22).

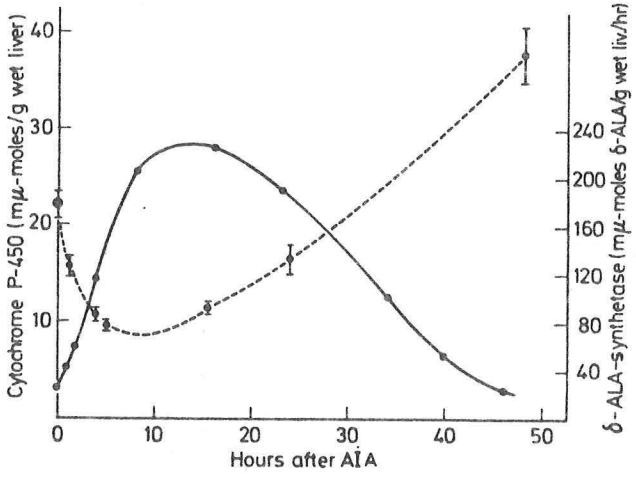
And finally, Table III presents a tabulation of cytochrome P-450 inducing drugs which are therefore potentially able to precipitate ALA-S induction and clinical porphyrias in patients who carry the particular genetic enzyme defects of the heme synthetic pathway (23).

Table II (Ref. 21)

Several properties of cytochrome P-450 which make it a preferential target for the toxic action of several chemicals

Property of cytochrome P-450	Type of lesion	Example of chemicals involved
Terminal oxidase and binding site for the activation of foreign chemicals	Damage to either heme or apoprotein moieties by reactive metabolites	AIA CS_2
Organisation of hemoprotein within the hydrophobic environment of the membrane of endoplasmic reticulum	Conversion of P-450 to P-420 and destruction of the heme moiety during lipid peroxidation	CCl_4 ? Fe ? Pb
Presence of a SH ligand from the apoprotein to the Fe of heme. Other SH groupings in the apoprotein and in the membrane	Blockage of SH groupings; conversion of P-450 to P-420, followed by loss of heme	Heavy metals and other SH reagents
Heme moiety may serve as a substrate for heme oxygenase	Increased breakdown of heme moiety	Co, ? Heme ? Fe, ? Pb and other metals

Figure 27 (Ref. 21)



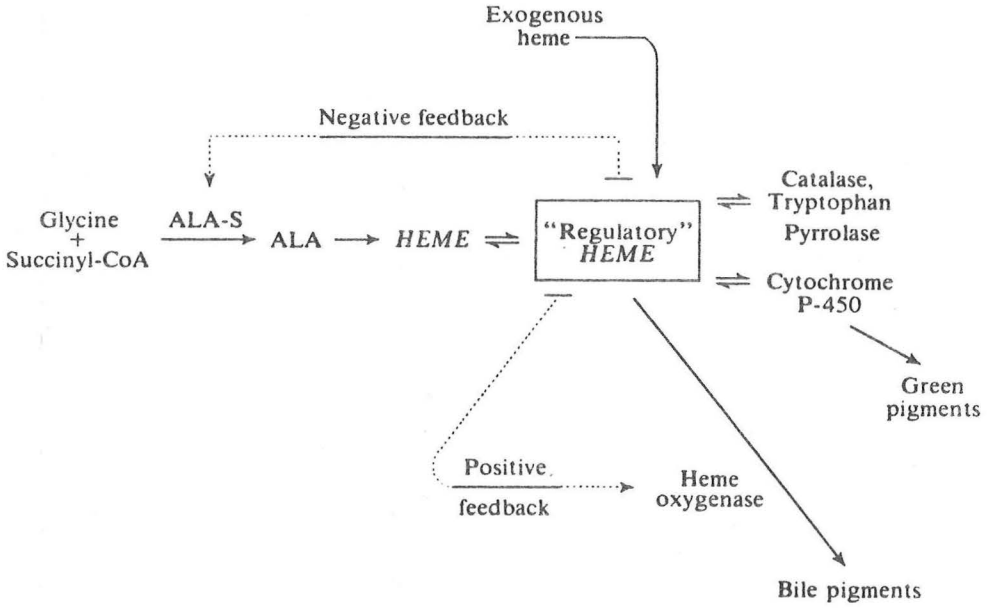
The effect of a single dose of 2-allyl-2-isopropylacetamide on microsomal cytochrome P-450 levels and ALA-S activity of the liver homogenates. P-450 levels (●---●) are given as averages (± S.E.M.) of the values observed in four animals. The data on ALA-S activity (●——●) are from MARVER et al. (1966). (Reproduced from DE MATTEIS (1970), with permission) mission)

Table III (Ref. 23)

Compounds inducing cytochrome(s) P-450 and cytochrome P-450 dependent monooxygenase(s)

Pharmacologic action	Compound
Hypnotics and sedatives	Barbiturates (Phenobarbital)
	Gluthethimide
	Chloral hydrate
Anticonvulsants	Diphenylhydantoin
Tranquilizer	Meprobamate
	Chlordiazepoxide
Antipsychotics	Chlorpromazine
	Imipramine
Central nervous system stimulants	Nikethamide
Anti-inflammatory agents	Phenylbutazone
	Aminopyrine
Hypoglycemic agents	Tolbutamide
	Carbutamide
Antihistaminics	Orphenadrine
	Diphenhydramine
	Chlorcyclizine
Antibiotics	Rifampicin
	Griseofulvin
Fungicides	Clotrimazole
Steroids	Spironolactone
	Pregnenolone-16-carbonitrile
Insecticides	DDT
	Chlordane
	Dieldrin
	Aldrin
	Hexachlorocyclohexane (HCH)
	Hexachlorobenzene
Carcinogenic polycyclic hydrocarbons	3-Methylcholanthrene
	3,4-Benzpyrene

Figure 28 (Ref. 21)



A model of regulation of liver heme metabolism centered on the "regulatory" heme

4. Other Modifying Factors of ALA Synthetase Activity

a) "Glucose effect"

It has been demonstrated that glucose as well as its metabolites glucose-6-phosphate and uridine diphosphate glucose can block ALA-S induction, whereas fasting enhances induction of this enzyme. However, the "glucose effect" is not demonstrable in isolated hepatocytes. The mechanism of the "glucose effect" remains unclear. Application of tryptophan has been shown to oppose any "glucose effect". It has been hypothesized that the observed increase of heme-binding to the apoenzyme of tryptophan pyrrolase in the presence of tryptophan leads to a decrease of the "regulatory heme" pool and to subsequent induction of ALA-S (25). The effect of fasting on ALA-S induction could possibly be explained on the basis of the known observation that the synthesis of cytochrome P-450 significantly increases during fasting. That should lead to an enhanced heme demand, a decrease of the "regulatory heme" pool and to induction of ALA-S.

b) Effect of Iron

Administration of iron dextran resulted in loss of microsomal heme and in a threefold increase of ALA synthetase. Additional experimental evidence suggests that the effect of iron on ALA-S may be the result of enhanced heme degradation leading to de-repression of ALA-S. Furthermore, inhibitory effects of iron on URO III cosynthetase and URO decarboxylase have been observed (9). The combination of the above findings may be relevant to the pathogenesis of porphyria cutanea tarda (PCT) since hepatic iron overload states appear to be related to the clinical expression of this disease.

c) Effects of Steroids

The most active ALA-S inducing steroids are C-19 and C-21 metabolites of parent hormones such as testosterone, progesterone or of intermediates in the steroid hormone biosynthetic pathway. The structural prerequisite appears to be the 5β-H-configuration. Highly active inducers are etiocholanolone, etiocholandiol, etiocholandione, pregnandiol, pregnanolone, pregnandione and pregnantriol.

VI. THE CLINICAL PORPHYRIAS

1. Classification (Table IV)

According to the two major sites of heme synthesis where the error of metabolism is biochemically expressed, the porphyrias are divided into two main groups, namely erythropoietic and hepatic.

Table IV (Ref. 26)

Characteristics of the porphyrias

	Erythropoietic porphyria	*Hepatic porphyrias*				*Erythrohepatic porphyria*
	Congenital erythropoietic porphyria (CEP)	*Intermittent acute porphyria (IAP)*	*Hereditary coproporphyria (HCP)*	*Variegate porphyria (VP)*	*Porphyria cutanea tarda (PCT)*	*Protoporphyria (PP)*
Enzyme deficiency	Uroporphyrinogen I synthetase and/or uroporphyrinogen III cosynthetase (?)	Uroporphyrinogen I synthetase	Coproporphyrinogen oxidase	Protoporphyrinogen oxidase or ferrochelatase (?)	Uroporphyrinogen decarboxylase	Ferrochelatase
Inheritance	Autosomal recessive	Autosomal dominant	Autosomal dominant	Autosomal dominant	Autosomal dominant	Autosomal dominant
Metabolic expression	Erythroid cells	Liver	Liver	Liver	Liver	Erythroid cells and liver
Signs and symptoms:						
Photosensitive cutaneous lesions	Yes	No	Infrequent	Yes	Yes	Yes
Attacks of abdominal pain, neuropsychiatric syndrome	No	Yes	Yes	Yes	No	No
Laboratory abnormalities:						
Red blood cells:						
Uroporphyrin	+++	N	N	N	N	N
Coproporphyrin	++	N	N	N	N	+
Protoporphyrin	(+)	N	N	N	N	+++
Urine:						
δ-Aminolevulinic acid	N	(+++)	(+++)	(+++)	N	N
Porphobilinogen	N	(+++)	(+++)	(+++)	N	N
Uroporphyrin	+++	++	+	+	+++	N
Coproporphyrin	++	N	++	++	+	(+)
Feces:						
Coproporphyrin	+	N	+++	+	(+)	(+)
Protoporphyrin	+	N	+	+++	N	++

NOTE: *N, normal; +, increased levels or excretion; ++, moderately increased; +++, markedly increased; (+), increased in some patients only; (+++), frequently increased only during acute attacks.*

Protoporphyria (PP) which was previously considered purely erythropoietic, is now classified as erythrohepatic prophyria, since porphyrins accumulate in both tissues.

The congenital erythropoietic porphyria (CEP) is the only pure erythropoietic form of porphyria. It is extremely rare and will be subject to only limited consideration in this review.

Autosomal dominant inherited enzyme deficiencies govern the clinical syndromes of the hepatic porphyrias: Intermittent acute porphyria (IAP), hereditary coproporphyria (HCP) and variegate porphyria (VP). Another hepatic porphyria, porphyria cutanea tarda (PCT), was previously classified as an acquired form of porphyria. However, an autosomal dominant inherited deficiency of uroporphyrinogen decarboxylase has now been demonstrated in this syndrome.

Toxic acquired porphyrias, which may resemble PCT, occur in patients exposed to polychlorinated hydrocarbons (Figure 26) and in association with tumors. Also lead poisoning induces characteristic abnormalities of heme metabolism.

2. Normal Porphyrin Formation and Excretion

The amount of heme daily synthesized for hepatic hemoproteins approaches 50-100 mg, whereas the bone marrow requires up to 300 mg heme for hemoglobin synthesis (9). In relation to the total rate of heme synthesis, the excretion of porphyrins and porphyrin precursors in urine and bile is very small. The upper limit of normal values of porphyrin and porphyrin precursors are demonstrated in Table V.

Table V (Ref. 9)

Excretion of porphyrins and porphyrin precursors: upper limit of normal values

	Urine, μg/24 h	*Feces, μg/g dry wt*	*Erythrocytes, μg/100 ml cells*
ALA	4000*		
PBG	1500		
Uroporphyrin†	50	5	Trace
Coproporphyrin†	300	50	3
Protoporphyrin†		120	80
"Ether-insoluble porphyrins"‡		20‡	

*This value may include aminoacetone, which is not distinguished in all procedures.

†Most methods for the quantitative determination of porphyrins in urine, feces, and tissues depend on solvent extraction and subsequent fractionation. In these methods, porphyrins are divided into fractions according to their solubility properties, but are not strictly identified. Fractions, which generally are designated "uroporphyrin," "coproporphyrin," or "protoporphyrin," may contain other porphyrins, which are identifiable only with more laborious methods such as thin-layer chromatography [321], electrophoresis [333], or, most effectively, high pressure liquid chromatography [334, 334*a–d*].

‡From Elder et al. [326] and Eales et al. [327].

Urinary coproporphyrin (up to 300µg/24 hours) appears as both type I and type III isomers, but the ratio is subject to significant variation. A slight increase of urinary coproporphyrin excretion has been observed in hemolytic anemia, liver disease and lead poisoning.

In rats, injected coproporphyrin III is almost entirely eliminated in bile unless the bile ducts are occluded or the liver is damaged by toxins. In addition to the porphyrins listed in Table V, also traces (<3µg/day) of porphyrins with seven, six, five and three carboxyl groups are excreted.

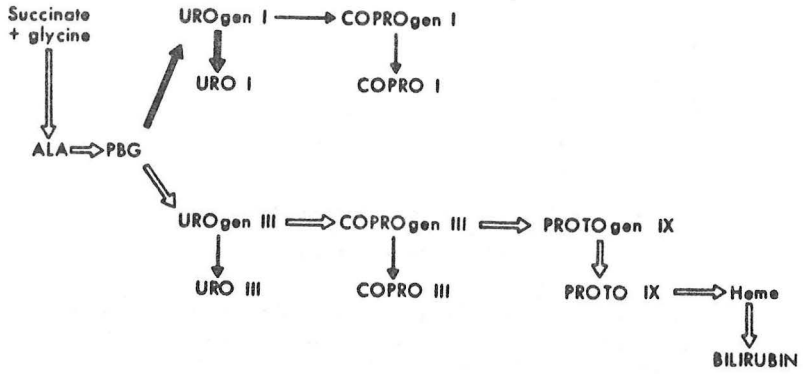
As a rule of thumb, ALA, PBG and uroporphyrin are mainly excreted in the urine. Coproporphyrin is preferentially and protoporphyrin exclusively eliminated through the bile.

Most porphyrins in urine are probably excreted as PBG, porphyrinogens or even ALA, which all, depending on pH, can be converted by exposure to light and air to porphyrins.

3. Congenital Erythropoietic Porphyria (CEP)

(a) Congenital erythropoietic porphyria (CEP) (also called Günther's disease) is a rare (less than 100 cases in the world literature), autosomal recessive, inherited disorder. Its major clinical manifestations include severe porphyrinuria, chronic photodermatitis and frequently hemolysis. The disease and its biochemical expression become apparent during infancy. The genetic defect (Figure 29) consists of a functional imbalance of UROgen I synthetase and UROgen III cosynthetase. This abnormality results in overproduction and accumulation of uroporphyrin I in erythroid cells. Uroporphyrin I, coproporphyrinogen I and coproporphyrin accumulate in tissues and are excreted excessively in urine and feces. Urine assumes a pink or red color shortly after birth.

Figure 29 (Ref. 9)

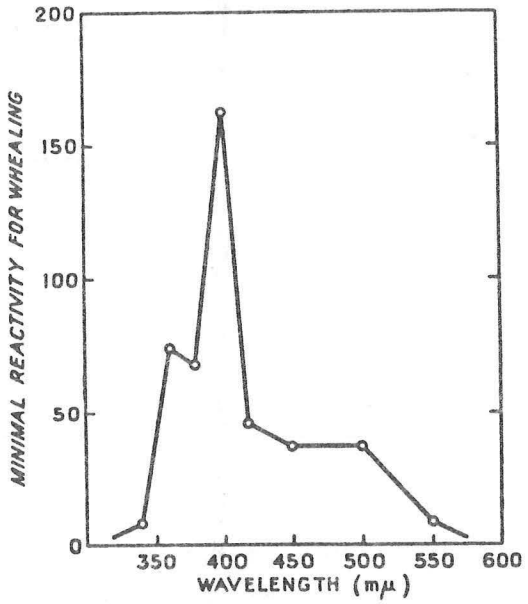


Pathway of porphyrin and heme biosynthesis in congenital erythropoietic porphyria. Heavy arrows indicate overproduction of uroporphyrin I.

Clinical signs include red discoloration of teeth and bones as well as hypertrichosis. Severe cutaneous photosensitivity results in hemolysis and mutilating skin lesions. Treatment demands avoidance of sun light and the use of hematin and oral beta-carotene remains experimental. Occasionally, splenectomy has eliminated hemolytic anemia, porphyrin excretion and photosensitivity.

(b) Photosensitivity. With the exception of IAP and the majority of HCP, all other porphyrias present with photosensitivity. As already discussed previously (Figure 5), the strong light absorption at the 400 nm wavelength region (Soret band) of porphyrins leads to skin injury. The current hypothesis of such injurious event is as follows: Absorption of electromagnetic radiation in the 400 nm band raises the energy state of the porphyrin molecule; energy transfer to molecular oxygen produces "singlet oxygen" (a high energy oxygen species) which interacts with the lysosomal membrane inducing release of histamine and proteolytic enzymes. The result is bullous eruptions followed by ulcerations, infections, scarring and deformity. The therapeutic use, with some success, of β-carotene is based on the powerful singlet oxygen quenching ability of this compound The dependency of photosensitivity skin lesions on the wavelength range is shown in Figure 30. This pattern is not only applicable for protoporphyria as shown but generally holds true for all photosensitive porphyrias.

Figure 30 (Ref. 9)



Action spectrum for cutaneous edema (whealing) in protoporphyria. (The ordinate scale—minimal reactivity for whealing—is the reciprocal of the minimal dose required to produce whealing. This dose of radiation is expressed in arbitrary units—i.e., t [time in seconds] $\times$ I [intensity].) Intensity was measured with a galvanometer and linear vacuum thermopile with quartz window.

VII. HEPATIC PORPHYRIAS

Although porphyria cutanea tarda and erythrohepatic porphyria (protoporphyria, PP) are included in the overall classification of hepatic porphyrias, only the three remaining syndromes (IAP, HCP, VP) are almost indistinguishable in terms of their main clinical features. Despite the distinct genetic differences in enzyme deficiencies, these three porphyrias all present with attacks of abdominal pain, neuropsychiatric symptoms and elevated prophyrin precursors in urine. The porphyrin and porphyrin precursor excretion pattern for the hepatic porphyrias is demonstrated in Figure 31, 32.

Figure 31 (Ref. 9)

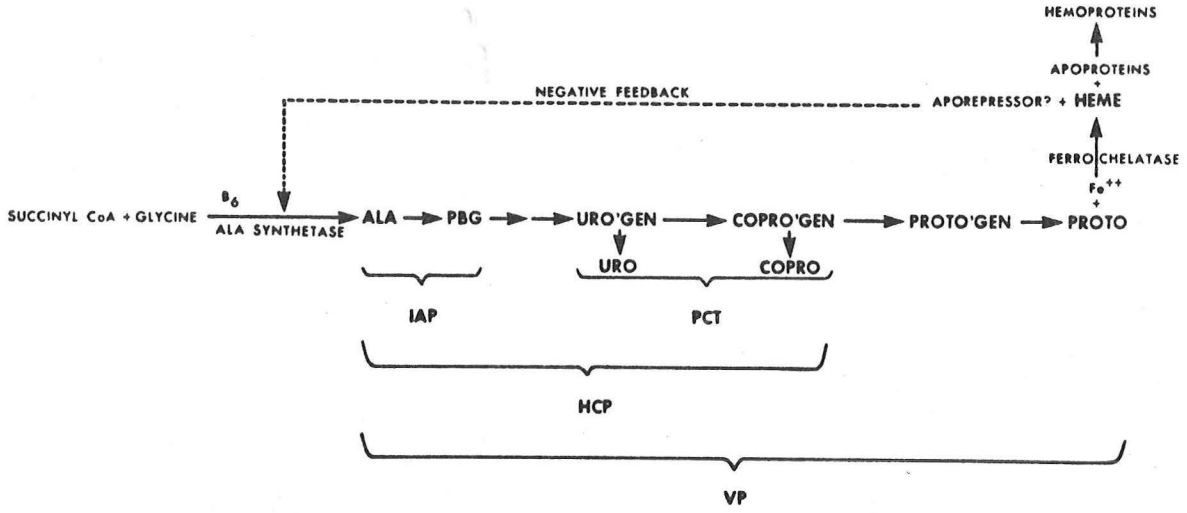
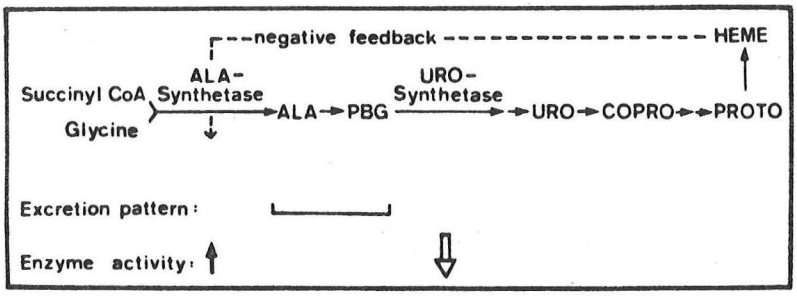


Figure 32 (Ref. 9)



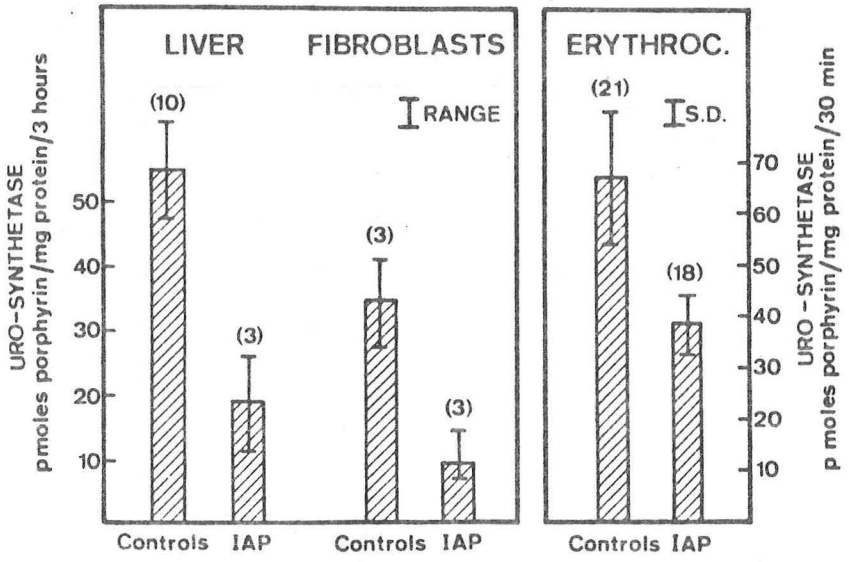
Correlation of the enzymatic abnormalities with the excretory pattern in intermittent acute porphyria (IAP).

A. Intermittent Acute Porphyria (IAP)

(a) Definition

Intermittent acute porphyria (IAP) is characterized by an autosomal dominant inherited deficiency of uroporphyrinogen I synthetase (50%). Such decrease in enzyme activity has been demonstrated in liver, erythrocytes, cultured fibroblasts (Figure 33), lymphocytes and amniotic cells. Its clinical expression includes acute abdominal pain, neuropsychiatric abnormalities and no photosensitivity. The latent disease is often precipitated by a whole variety of drugs (especially barbiturates). The incidence of the abnormal gene is 1:10000 to 1:50000, dependent on the region. The male to female ratio is 2:3. The deficiency of the enzyme does not necessarily lead to a porphyric attack but usually requires a precipitating factor. Such factors include a great number of different drugs (see Appendix) such as barbiturates, anticonvulsants, estrogens, contraceptives and alcohol; in addition, fasting, infections and delivery may precipitate attacks. In women, the exacerbation of IAP may be correlated to the menstrual cycle.

Figure 33 (Ref. 9)



Uroporphyrinogen I synthetase (URO synthetase) activity in liver, cultured fibroblasts, and erythrocytes of patients with intermittent acute porphyria (IAP). The numbers in parentheses refer to the numbers of subjects studied.

(b) Clinical Presentation and Diagnosis

The most obvious symptoms and signs of a porphyric attack include abdominal pain, vomiting, constipation, pain in limbs and back, tachycardia, hypertension, hyponatremia, motorneuropathy and various other neuropsychiatric abnormalities (Table VI). These findings are similar in IAP, HCP and VP.

Table VI (Ref. 6)

Clinical Presentation of Acute Attacks of Haem-deficient Porphyria.

Percentage of patients presenting with symptom or sign			
Symptoms		Physical findings	
Abdominal pain	90	Tachycardia	83
Vomiting	80	Hypertension	55
Constipation	80	Motor neuropathy	53
Pain in limbs	51	Pyrexia	38
Pain in back	50	Leucocytosis (12 000)	20
Confused state	32	Bulbar involvement	18
Urinary frequency	30	Sensory loss	15
Dysuria	28	Cranial nerve involvement	9
Abnormal behaviour	23	Proteinuria	8
Seizures	12	Hepatomegaly	5
Diarrhoea	8		
Stupor	7		
Coma	6		

The data are taken from Eales,[27] a study of 80 patients with variegate porphyria. The presentation of acute intermittent porphyria[77,83] and of hereditary coproporphyria[18] is entirely similar.

Acute attacks can last from several days to several months. Intermittent remissions occur with most symptoms either absent or slight in degree. During acute attacks, hyponatremia may become an alarming complication. This electrolyte abnormality has been attributed to an ISADH syndrome. Hypomagnesemia, sufficiently severe to cause tetany, has been reported. Many patients have hypertension and during attacks, sinus tachycardia is frequently observed. The pulse rate has been found to be a good indicator for judging activity of the disease. Death may occur from respiratory paralysis and uremia.

The neurological manifestations (27-30) in porphyria may resemble the Guillain-Barre syndrome, but there are several distinguishing features. Initially, there is no cranial nerve involvement in the porphyric attack. Motorneuropathy affects upper extremities and proximal musculature more severely than lower extremities and distal musculature. The porphyric neuropathy is predominantly a motor neuropathy; but sensory findings have been documented in some patients. Impairment of the autonomic nervous system results in paralytic ileus, abdominal pain, tachycardia and a wide swing of blood pressure. The most striking neurohistological findings include (1) chromatolysis with massive vacuolization of the anterior horn cells of the spinal cords; (2) chromatolysis in the spanchnic motor cells of the lateral horns, medullary nuclei and dorsal vagal nuclei; (3) reduction in neurosecretory cells with mild astrocytic gliosis and vacuolization in the supraoptic and paraventricular nuclei; (4) either primary demyelination or primary axonal degeneration.

As possible mechanisms of the neural impairment have been listed: (1) neurotoxicity of porphyrin precursors (PBG, ALA), (2) unidentified toxic metabolites of porphyrins, and (3) a metabolic defect of heme biosynthesis in brain. At the present time, the mechanism underlying the neuropsychiatric

disorder is unknown. Although blood levels of PBG and ALA seem to correlate to the degree of neurological impairment, both compounds can not sufficiently penetrate the blood-brain barrier. Brain levels of these compounds barely reach 4-8% of the maximum blood levels.

(c) Laboratory Findings

The most characteristic finding in IAP is the large excretion of PBG and ALA in urine. Two tests are available for the determination of PBG, namely the Watson-Schwartz test and the Hoesch test (see Appendix for details). The Watson-Schwartz test, using Ehrlich's aldehyde, gives an intense red color with the colorless PBG. This product, in contrast to complexes formed with urobilinogen, is not extractable with chloroform or butanol. During acute attacks, 20 to several hundred milligram PBG/liter urine per day are excreted. There is a rough correlation of the amount excreted as well as the plasma levels of PBG and ALA to the severity of the symptoms. In addition to large amounts of PBG and ALA, probably other pyrrolic substances are excreted. PBG, on standing of urine under light and air, forms spontaneously porphyrins as well as porphobilin, a brown amorphous oxidation product of PBG. Liver and kidney tissue contain large amounts of PBG. All findings indicate that in IAP the metabolic expression of the primary genetic defect (deficiency of uroporphyrinogen I synthetase) is the over-production of porphyrin precursors rather than porphyrins.

Enzymatic measurements have revealed two enzymatic abnormalities: deficiency of UROgen I synthetase and secondarily induced ALA-synthetase.

Conventional liver function tests are normal except for increased bromsulphalein (BSP) retention.

Other metabolic abnormalities in the acute attack are hypercholesterolemia with increased low-density lipoprotein levels, increased serum thyroxin (without hyperthyroidism), abnormal glucose tolerance and defective 5α reduction of testosterone in liver.

(d) Treatment

1. General Measures

Avoidance and withdrawal of provocative drugs which may support or precipitate porphyric attacks (Table VII).

Supportive treatment with careful monitoring of fluid and electrolytes is important because of commonly occurring hyponatremia, hypomagnesemia and azotemia.

2. Carbohydrate Infusion

The treatment of acute porphyrias with massive glucose infusions was utilized following the observations that fasting may precipitate acute porphyric attacks and that glucose can ameliorate the biochemical abnormalities (porphyrin precursor excretion and ALA-S induction) in experimental porphyrias (see above discussion of the "glucose effect"). However, the chemical and clinical effects of high carbohydrate intake on the acute por-

Table VII (Ref. 6)

Drug Usage in Persons with Haem-deficient Porphyria (Acute Intermittent Porphyria, Hereditary Coproporphyria, Variegate Porphyria).[a]

May precipitate acute attacks[b]	Believed to be safe
Barbiturates	Aspirin
Griseofulvin	Bromides
Sulphonamides	Chlorpromazine
Glutethimide	Chloral hydrate
Hydantoins	Corticosteroids
Meprobamate	Diazepam
Oestrogens	Dicoumarol
Ergot preparations	Digoxin
Methyldopa	Diphenhydramine
Chloroquine	Ether
Chlorpropamide	Guanethidine
Chlordiazepoxide	Meperidine
Tolbutamide	Morphine
Ethanol	Neostigmine
Halothane	Nitrous oxide
Chloramphenicol	Penicillins
Pyrazinamide	Propranolol
	Tetracyclines

[a] The data are taken, in part, from Wetterberg.[86]
[b] Listed in decreasing order of importance as precipitants of acute attacks.

phyric attack have varied from a dramatic improvement in urinary porphyrin precursor excretion and symptomatology to essentially no change in either aspect of the disease (31). Nevertheless, the current strategy (26) is to routinely infuse as soon as possible and as long as biochemical abnormalities exist, 10-20 g glucose/hour (300-400 g/day). Although it has been common practice (26) to wait for 48 hours while administering glucose before deciding on the application of hematin therapy, Watsons group (32) has argued that hematin should be the first drug of choice. In favor of the latter is that (1) hematin infusions have been shown to be clinically safe and (2) that if the neurological lesions are permitted to progress in absence of early hematin treatment they may become irreversible. In any case, at the present time glucose infusion is a part of the standard treatment for acute porphyria.

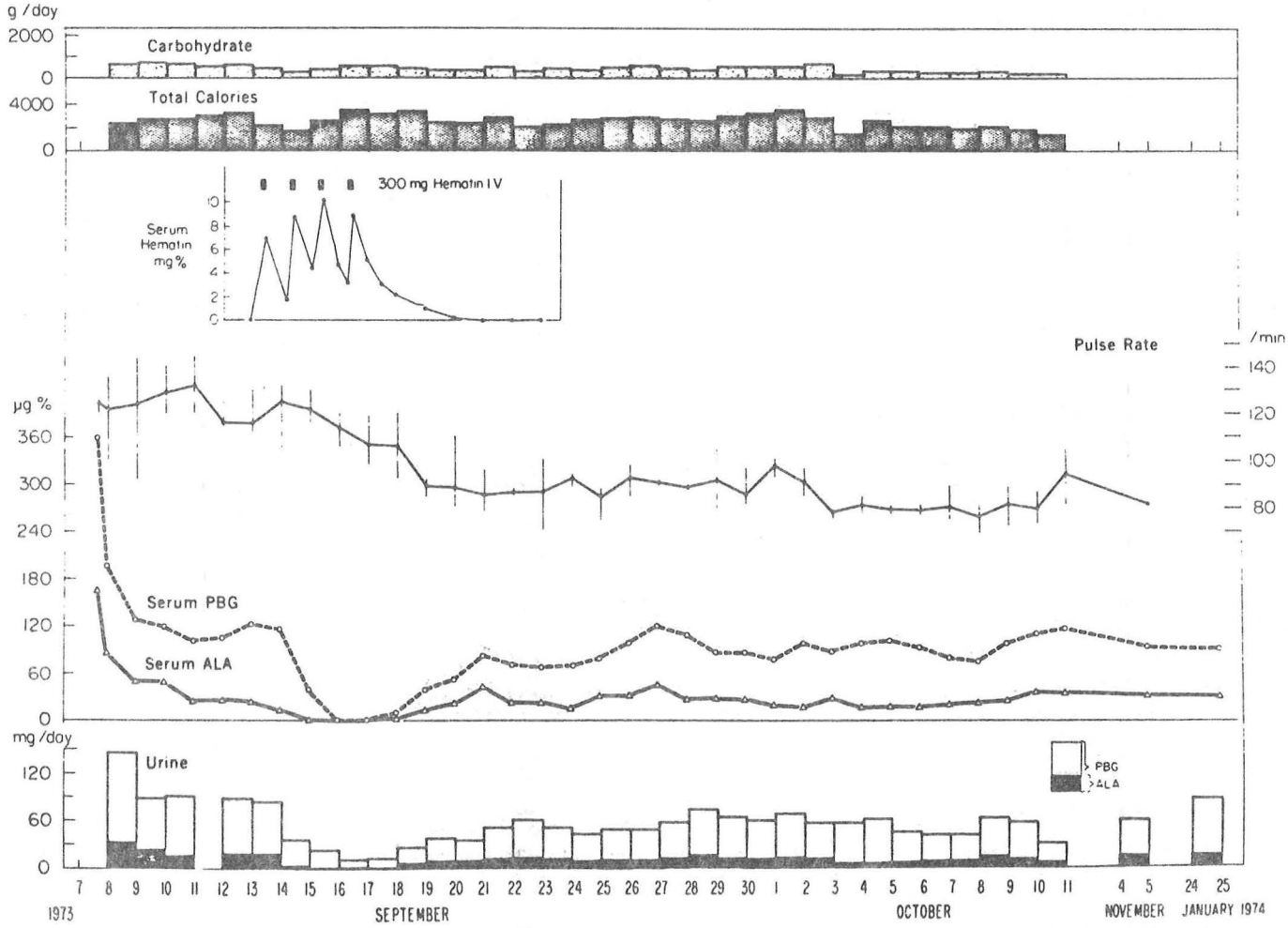
3. Propranolol Treatment

Striking and persistent hypertension and tachycardia most commonly accompany an acute porphyric attack. A strong correlation of pulse rate to clinical improvement has been observed. Treatment of these symptoms with beta-adrenergic blocking drugs (e.g. propranolol) is recommended as standard measure (26). In addition to this symptomatic treatment, several reports have shown (33,34) that propranolol treatment alone resulted in resolution of severe neurological manifestations (33) and prevention of attacks during drug maintenance (34). Acute porphyria patients have shown high resistence toward effects of propranolol. Up to 284 mg I.V. were administered over 18 hours until clinical remission was achieved (33), followed by 300-950mg/day p. o. for 5 days. In the twenty cases described by Menawat et al. (34), propranolol doses of 20-200 mg were required to achieve adequate control of pulse and blood pressure.

4. Hematin Treatment

To recapitulate, hematin has been demonstrated in experimental porphyria to effectively prevent induction of hepatic ALA synthetase and has been shown to reverse the biochemical abnormalities and cause clinical improvement within 48 hours in many patients (31, 32, 35-41) with acute porphyria. The effect of hematin could involve (1) direct inhibition of ALA-S, (2) inhibition of synthesis of ALA-S, (3) replenishment of the "regulatory heme" pool and (4) saturation of induced apoproteins of hemoproteins with heme which would relieve the relative heme deficiency state. Hematin infusions are clinically safe if administered at doses of up to 4 mg/kg Q12 hours. The only side effects observed were 4 instances of chemical phlebitis in 172 infusions (31) and one case of a transient, reversible renal failure (ATN) after administration of 1000 mg hematin. Watson's group has directly or indirectly participated in several hundred hematin infusions without morbidity or mortality. In many instances (31, 32, 36-41) rapid and often striking improvement of clinical symptoms was observed after hematin infusion, especially when in the presence of glucose treatment alone the neurological deterioration had progressed. In Figure 34, the typical course of the clinical and biochemical response of acute porphyria following hematin infusion is demonstrated (37).

Figure 34 (Ref. 37)



Effect of hematin in acute porphyric relapse (P91); mg% = mg/100 ml; µg% = µg/100 ml; ALA = δ-aminolevulinic acid; PBG = porphobilinogen.

4 mg/kg

The present treatment schedule recommended for hematin infusion calls for 4 mg/kg hematin I.V. over 15 min every 12 hours for 3 days (6 doses). If clinical and biochemical improvement is not satisfactory, four more doses are recommended. Monitoring of urinary and plasma porphyrin precursors is required. In addition, pulse rate is a good indicator of success. Additional details of the technical aspects and the clinical protocol are listed in the Appendix.

5. Management of Neurological Symptoms

Agitation and psychotic thought disorders occurring in acute porphyrias have been safely and successfully treated with Thorazine (chlorpromazine) (27). The abdominal pain has been successfully controlled by meperidine.HCl (Demerol). Seizures related to acute porphyrias have been safely treated with Valium, paraldehyde, chloralhydrate (27) and bromides (28). Phenytoin (Dilantin), Clonazepam and Valproic acid have been found to be porphyrinogenic (28).

6. Folic Acid Treatment

A recent report described treatment of intermittent acute porphyria with oral folic acid (30 mg/day) for 10 days (43). In all three patients treated, clinical and biochemical improvement immediately followed folic acid administration. The mechanism of action proposed is (1) stimulation of UROgen I synthetase and/or porphobilinogenase which diverts excess PBG.

7. Determination of Carrier State

If a patient with acute hepatic porphyria has been diagnosed, family studies (in cooperation with specialized centers) should be initiated to uncover additional carriers of the genetic enzyme dificiencies (44). The patient as well as carriers of the inherited disorders should be educated with regard to risks following the exposure to environmental chemicals, drugs or steroids.

B. Hereditary Coproporphyria (HCP)

(a) Definition

Hereditary coproporphyria (HCP) is a hepatic porphyria where the enzyme defect of the heme synthetic pathway is represented by a 50% deficiency of the mitochondrial enzyme coproporphyrinogen III oxidase (Figure 8). This enzyme catalyzes the decarboxylation of the two propionic acid side chains of the pyrrole rings A and B of coproporphyrinogen III, thereby converting them to vinyl groups (Protoporphyrinogen IX). The mode of inheritance is autosomal dominant. Many carriers of this genetic defect remain asymptomatic. The overt disease occurs in both sexes from age 7-75 years. In a study of 86 patients by Jaeger et al. (45) including 25 families, more than half were asymptomatic or presented with abdominal and neuropsychiatric manifestations indistinguishable from IAP. The fewer than 100 cases reported are probably a significant underestimation of the true incidence of this disease.

(b) Clinical Presentation

The acute clinical presentation is identical to the hepatic porphyrias IAP and VP. As in these syndromes, abdominal pain and neuropsychiatric attacks are predominant. But in contrast to IAP, skin photosensitivity occurs in approximately one-third of patients with overt HCP (26) which

results in erythema and blister formation. Precipitation of an HCP attack is similar as in IAP induced by ingestion of barbiturates, estrogens, sulfonamides or with onset of pregnancy. The distinguishing feature from IAP is the specific excretion pattern of porphyrins in urin (and photosensitivity, if it occurs).

(c) Laboratory Findings

Patients with HCP excrete large amounts of coproporphyrin III in the feces. The majority (95%) of the fecal coproporphyrin consists of the type III isomer. In addition to these diagnostic findings, feces also contain increased amounts of hepta-, hexa- and pentacarboxylic porphyrins. If the capability of specific enzyme measurements is available, one will find an increase (induction) of ALA synthetase as well as a 50% decrease of coproporphyrinogen III oxidase. Direct, diagnostic measurements of the latter enzyme have been performed in cultured skin fibroblasts (46) and in leukocytes of patients with HCP (47). The Watson-Schwartz and Hoesch tests for porphyrin precursors are positive during the acute attack.

(d) Treatment

The clinical management of patients with acute HCP attacks is identical to the treatment prescribed for AIP (see above). In addition, oral β-carotene appears to be beneficial to improve protection against skin photosensitivity.

C. Variegate Porphyria (VP)

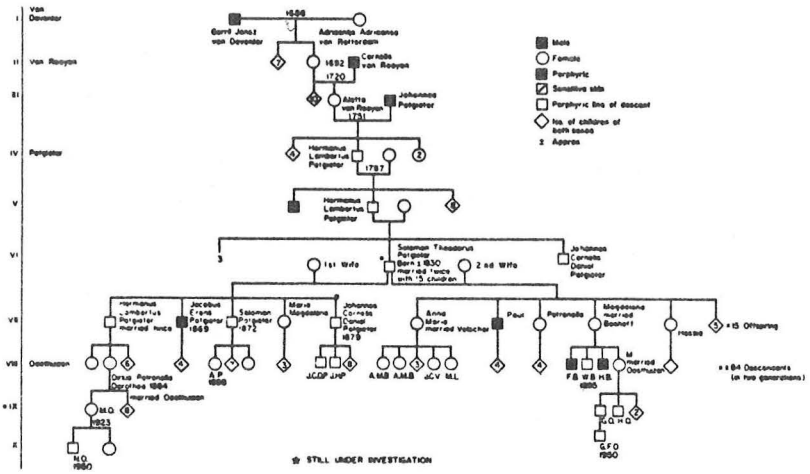
(a) Definition

Variegate porphyria is an autosomal dominant inherited disease which is classified as hepatic porphyria. According to the excretion pattern of porphyrins in urine and feces, the presumed (but not proven) enzyme defect(s) in the heme synthetic pathway is most likely a deficiency of either protoporphyrinogen IX oxidase or heme synthetase (ferrochelatase) or both (Figure 8). However, recent studies in human cultured skin fibroblasts of patients with VP revealed a normal heme synthetase activity but a suppression of porphyrinogen IX oxidase to 43% of normal (48). The disease has been very common in South Africa where the relative frequency of VP in the white population is approximately 3:1000. Many cases in South Africa have been identified as descendants of a woman who emigrated to Cape Town from the Netherlands in 1688 (Figure 35). However, VP has now also been identified in many other parts of the world.

(b) Clinical Presentation

The majority of porphyric attacks occur during the second and third decade of life. The clinical symptoms and signs of overt VP is almost identical to IAP and HCP (Table VI) with abdominal pain and neuropsychiatric symptoms. In contrast to IAP and HCP, one finds in VP a very high incidence of cutaneous lesions (80% of patients). In a recent study of 300 cases of VP in South Africa (49), the incidence of attack was slightly in favor of females (171:129) compared to males. One of the major clinical problems associated with the acute attack were electrolyte disorders including a very high incidence of severe hyponatremia, hypochloremia and azotemia. In addition, a substantial incidence of alkalosis and hypokalemia was observed.

Figure 35 (Ref. 5)



Family tree showing one of the four main lines of descent of South African VP (from 1688 to 1973). The inheritance pattern is that of an autosomal dominantly inherited disease with high penetrance and expressivity.

The skin lesions of VP consist of dermal abrasions, superficial erosions and blister formation after trivial mechanical trauma. The skin fragility appears to be limited to light exposed areas. Depigmentation, scarring and secondary infections may occur. Women often present with hypertrichosis, and hyperpigmentation of the face and hands are common. The skin lesions are identical to porphyria cutanea tarda (PCT; see below).

(c) Laboratory Findings

During the acute attack, the urinary excretion of ALA and PBG is increased. In addition, there is elevated urinary output of coproporphyrin and uroporphyrin. Urinary porphyrins include porphyrine-peptide conjugates.

VP patients also excrete in feces increased amounts of proto- and coproporphyrins as well as some ether-insoluble porphyrins ("X-porphyrin"), the precise structure of which is not known. Stool may also contain porphyrins with seven, six, five and three carboxyl groups (9). Erythrocyte porphyrins are normal, allowing distinction from protoporphyria (PP).

(d) Treatment

Clinical management of acute VP attacks is identical as described for IAP and HCP. Watson's group (35) has successfully treated VP patients with hematin infusion, thereby preventing further progression of the neuropsychiatric disorder. The treatment was followed by total remission.

D. Porphyria Cutanea Tarda (PCT)

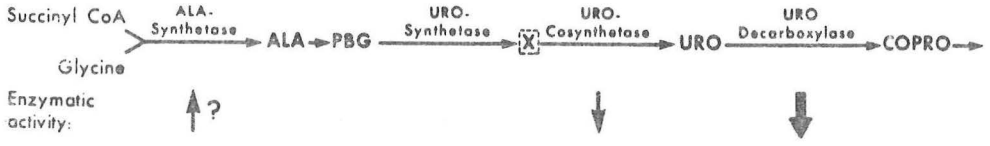
(a) Definition

Porphyria cutanea tarda (PCT) is the most common form of porphyria and has been described all over the world. The characteristic clinical presentation of PCT includes chronic skin lesions frequently associated with alcoholism

and hepatic disease (and hepatic siderosis). Neurological disorders are absent. The urinary excretion pattern of porphyrins is distinct. The disease is caused by a deficiency of uroporphyrinogen III decarboxylase (Figure 8).

The genetics and incidence of the disease have recently been more clearly defined. In the past, PCT has generally been considered as an acquired disorder of porphyrin metabolism because most patients have no familial history of the disease. In two recent family studies (50, 51) it was shown that PCT may exist in two forms: the sporadic and the familial type. The familial type reveals autosomal dominant inheritance whereas the sporadic type may be more dependent on exposure to chemical toxins (such as TCDD; 2,3,7,8-tetrachloro-dibenzo-p-dioxin). It is unclear whether a deficiency of UROgen III decarboxylase can also be acquired by exposure to chemical toxins. Nevertheless, enzyme deficiency (independent of etiology) of UROgen III decarboxylase, which catalyzes conversion of uroporphyrin III to coproporphyrinogen III by various decarboxylation steps (Figure 36) as described earlier, leads to an impairment of hepatic heme synthesis. This is followed by skin photosensitivity only after additional exposure to other factors such as iron overload, mostly in association with liver disease (26). The enzymatic defect of PCT (in contrast to IAP, HCP and VP) does not result in severe alteration of the heme synthetic pathway and does not lead to induction of ALA synthetase. Therefore, the usually normal urinary excretion of ALA and PBG as well as the lack of sensitivity to drugs such as barbiturates is not unexpected.

Figure 36 (Ref. 9)



Enzymatic abnormalities in porphyria cutanea tarda (PCT). Reduced uroporphyrinogen decarboxylase activity is suggested as the primary genetic defect.

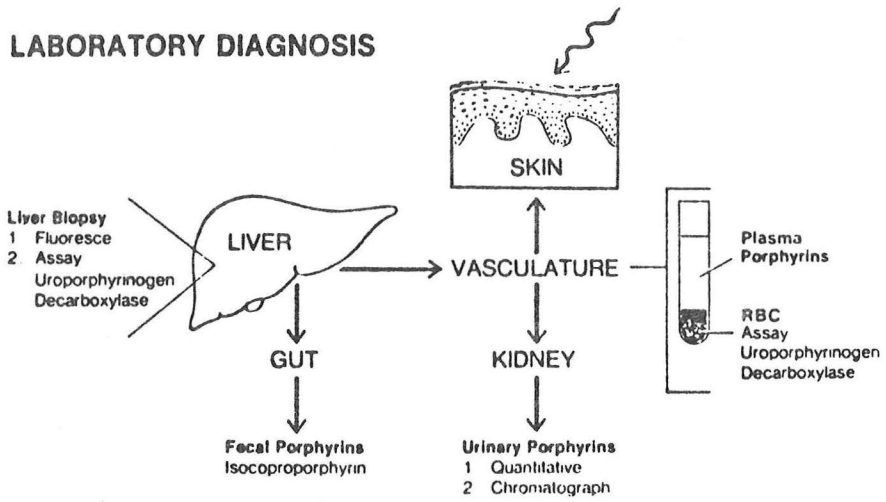
(b) Clinical Presentation

The major clinical manifestation of PCT is skin photosensitivity (52). Males are much more affected than females. Sunlight exposed areas such as face, neck and back of hands present with itching blisters after mechanical insult. These blisters soon become greatly enlarged. Ulcerations from scratching develop mostly in the face. Since the site of the blisters is subepidermal, healing results in scar formation. Hypertrichosis develops in the face around the eyes, ears and cheeks. Overall, the skin lesions are similar as described in variegate porphyria. A summary of a work-up to establish the diagnosis of PCT is shown in Figure 37 (53).

(c) Laboratory Findings

A liver biopsy of a PCT patient will reveal several distinct features. Using a Wood's light (ultraviolet lamp), the cylindrical tissue specimen reveals a glowing orange-red fluorescence. Histopathology of the PCT liver presents the following abnormalities (54, 55): In all untreated cases, bifringent acicular cytoplasmic inclusions (needle-like) were apparent

Figure 37 (Ref. 53)



Sources of specimens useful in the laboratory diagnosis of porphyria cutanea tarda.

which seem to be specific for PCT; other distinct features include constant, mild periportal siderosis, focal lipofuscin deposition, focal lobular necrosis associated with groups of pigment-laden macrophages (hemosiderin, ceroid), focal steatosis, marked hepatocyte hyperplasia and periductal lymphocyte aggregates (43% of all cases).

Abnormal laboratory function tests are listed in order of their frequency in Table VIII.

Table VIII (Ref. 53)

Laboratory Abnormalities in 40 Patients with Porphyria Cutanea Tarda

LABORATORY TEST	PER CENT ABNORMAL
Bromsulphalein	63
Serum iron/total iron-binding capacity	62
Serum SGPT	61
Serum SGOT	60
Abnormal glucose tolerance test	60
Antinuclear antibody	38
Serum gamma glutamyl transpeptidase	36
Serum alkaline phosphatase	35
Serum lactic dehydrogenase	33
Serum bilirubin	13

The urinary excretion pattern of porphyrin precursors reveals usually a negative Watson-Schwartz and Hoesch test (normal PBG, ALA). The excretion of uroporphyrin and less so, coproporphyrin, is increased in urine. Urine color may be pink or brown. Also intermediary porphyrins (such as hepta carboxylic porphyrins) are found. In feces, the increases of porphyrin excretion are mostly restricted to the coproporphyrin fraction.

The definitive diagnosis of familial PCT can be established by specific measurement of the red blood cell uroporphyrinogen III decarboxylase (56), which was shown to be present at subnormal level in PCT patients only (58% of normal) compared to controls.

The syndrome of "toxic acquired porphyria", which resembles PCT, has occurred in several thousand individuals accidentally exposed to hexachlorobenzene, polychlorinated biphenyls, tetrachlorodibenzo-p-dioxin (TCDD), and other polychlorinated hydrocarbons.

(d) Treatment of PCT

1. Abstinence from alcohol leads in many patients to clinical improvement.

2. Phlebotomy treatment: This mode of treatment has been successfully performed in several hundred patients (57, 58), followed by normalization of porphyrin excretion in urine and remission of the skin lesions. Although the pathogenesis of PCT has always been associated with impaired iron metabolism, it is interesting that in patients with hemochromatosis an impairment of porphyrin metabolism is usually absent. It has also been shown (58) that phlebotomy was useful in PCT patients where increased iron stores were not present. The working hypothesis by Ippen (57) assumes that removal of blood increases the rate of hemoglobin synthesis in the bone marrow which subsequently results in utilization of hepatic porphyrins and porphyrin precursors for protoheme production. Another possibility is that frequent phlebotomy removes toxic porphyrin metabolites which are responsible for the skin lesions.

The phlebotomy schedule calls for removal of 500 ml blood weekly until urinary uroporphyrins approach normal values or the hemoglobin concentration falls below 11 grams/dl. Clinical remission may be observed after removal of as little as 2-3 g iron (58). In some patients, treatment may have to be extended to one or two years. Usually, remission remains stable for several years afterwards.

3. Hydroxychloroquine or Chloroquine

Recently, low dose hydroxychloroquine treatment was successfully applied to achieve remission in PCT patients (59). Five to 13 months of treatment reversed the cutaneous lesions and urinary excretion pattern to normal. No adverse effect on routine laboratory tests were observed. The treatment schedule called for 100 mg QOD for one month; 200 mg QOD for the second month; 200 mg QD from second to fourth month; 300 mg QD after 4-7 months; 400 mg QD after another 4 months. The duration of treatment depended on the degree of remission. Discontinuation of the drug led frequently to relapse.

Low dose chloroquine treatment (125 mg twice weekly for 3-8 months) achieved reliable clinical and metabolic remission and did not involve any fundamental laboratory or histopathological changes in liver (60).

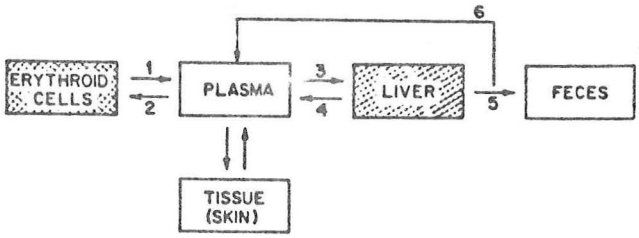
E. Protoporphyria (PP)

This disease will not be discussed at great length, since it represents a syndrome focusing mostly on the erythropoietic system.

(a) Definition

Protoporphyria, also called erythropoietic protoporphyria or erythrohepatic protoporphyria, has generally been conceived as a genetic disease with autosomal dominant inheritance which occurs in both sexes. Although the biochemical expression of the genetic enzyme defect in the heme synthetic pathway was previously almost exclusively assigned to the erythropoietic system, more recent observations have demonstrated that protoporphyrins may accumulate in some patients in liver. The enzyme defect responsible for the expression of the disease is a deficiency in heme synthetase (ferrochelatase). The major clinical findings are mild skin photosensitivity and increased protoporphyrin levels in erythrocytes. Over the last 15 years, over 300 cases have been described (9). Biochemically, the disease is characterized by elevated concentrations of protoporphyrin in erythrocytes, plasma and feces. It is still impossible to decide whether and to what extent the liver participates in the excess protoporphyrin production. The difficulty is apparent from the current working hypothesis (Figure 38) involving various pools for the distribution of protoporphyrin in human PP.

Figure 38 (Ref. 61)



Model for the distribution of protoporphyrin in human protoporphyria. Five distinct protoporphyrin pools are represented by the *boxes*. Transport of protoporphyrin between different pools is represented by *arrows*. Sites of production of protoporphyrin, erythroid, and liver, are distinguished by *shaded boxes*.

(b) Clinical Presentation

Acute attacks of neuropsychiatric dysfunction, characteristic of most forms of hepatic porphyria, do not occur in PP.

The skin photosensitivity, beginning in early childhood, is produced by a narrow band of near-ultraviolet light in the 400 nm region. As a result, stinging, burning and pruritus occur after sun light exposure. This photosensitivity is also called "solar urticaria" or "solar exzema". Chronic exzematous lesions may also occur. In some patients, the skin lesions show only reddened edema. The hyaline deposits (52), which suggest hyalinosis cutis et mucosae are conspicuous on the face and hands. An amorphous PAS-positive material of unknonw origin is deposited in and around capillary walls of sun-exposed skin.

Cholelithiasis is not an infrequent occurrence in PP-patients. The gallstones consist partially of precipitated protoporphyrin. Patients are sometimes symptomatic for cholelithiasis at an early age. Recently reviewed 34 PP-cases revealed four patients with gallstones (62).

Hepatic disease is fairly common in PP. In a recent report, chronic liver disease with progression to cirrhosis and even massive hepatic necrosis was described in patients with erythropoietic protoporphyria (63). Autopsy revealed a black, firm liver, finely nodular. Histology revealed massive pigment deposits in Kupffer cells, portal macrophages, bile canaliculi and occasionally in parenchymal cells. Various degrees of inflammatory changes were observed. Pigment deposits were most likely protoporphyrins.

(c) Laboratory Findings

The most striking finding is the increase in protoporphyrin concentration in erythrocytes, plasma, and feces. The level of free protoporphyrins (9) in circulating erythrocytes may be raised 100-fold. Red fluorescence is mostly present in reticulocytes and less so in mature cells. Porphyrin-containing erythrocytes are readily hemolyzed by exposure to light of 400 nm wavelength in vitro.

Large amounts of protoporphyrin are excreted in stool; occasionally, fecal coproporphyrin is also increased. Patients with overt liver disease have increased coproporphyrin in urine; therefore, coproporphyrinuria may indicate early hepatic involvement.

(d) Treatment

At the present time, only the skin lesions are subject to treatment. Oral β-carotene substantially improves sunlight tolerance (26).

ACKNOWLEDGMENTS

I thank Mrs. Renate Davis for her superb typing and editorial work, and I am grateful to Mrs. Harshika Shah-Bhatt for proof-reading. I also appreciate the help of Dr. P. Bergstresser, Dr. M. Bissell, Dr. G. Krejs and Dr. A. Ware for obtaining picture materials; and last but not least, I thank Ms. Irene Bossenmaier of the Watson Laboratory in Minnesota for supplying all the relevant information regarding hematin infusion.

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