

THE PORPHYRIAS

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The porphyrias are a group of metabolic disorders that result from defects of specific enzymes of the heme synthetic pathway (1) (see website chapter [W-17](#)). Clinically they may be characterized by a propensity to acute neurovisceral crises, photosensitive skin disease, or both. As the pathophysiology, diagnosis, and management of the porphyrias are critically dependent on an understanding of the biochemistry and enzymology of the heme biosynthetic pathway it is necessary to consider this aspect first. Thereafter clinical disorders caused by aberrations in this pathway can be examined in detail.

PORPHYRINS AND HEME

There are a number of excellent reviews on the detailed chemistry and biochemistry of porphyrins (2–6). What fol-

lows is sufficient information to provide a relevant background to this chapter. Porphyrins are tetrapyrrole macrocycles consisting of four weakly aromatic pyrrole rings linked by methene bridges and characterized by their ability to fluoresce a bright red when exposed to ultraviolet light. The porphyrin macrocycle is a rigid planar structure with eight positions where side chains can be attached. The side chain substituents attached to the rings are important in determining the physical characteristics of the porphyrins. Consideration of various arrangements of side chain substituents around the porphyrin ring implies that there are a number of possible different isomeric porphyrin forms. Thus, uroporphyrinogen and coproporphyrinogen can occur in four isomeric forms, but only the I and III forms occur naturally in mammals. All biologically functional tetrapyrroles are derived from uroporphyrinogen-III and the type I isomers of uroporphyrinogen and coproporphyrinogen appear to be biologically useless. As biologic intermediates, the porphyrin tetrapyrroles exist as the partially conjugated, less stable hexahydro-reduced, colorless forms, the porphyrinogens.

An important property of the porphyrin macrocycle is the availability of ligand binding sites within. This attribute gives these compounds the ability to bind metals, particu-

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larly iron to form heme, magnesium to form chlorophylls, and cobalt to form vitamin B₁₂. Heme is an iron-containing complex of the protoporphyrin-IX molecule that associates with several proteins and is central to virtually all biologic oxidations. Heme, by virtue of its redox-active iron coordinately bound within the tetrapyrrole ring, plays specific roles in oxygen binding, electron transport, reduction of oxygen, and transfer of oxygen for hydroxylation reactions. These roles are in turn determined by the structure of the protein moiety of each specific hemoprotein, their substrates, and the intracellular milieu within which it functions (7).

HEME BIOSYNTHESIS

Heme biosynthesis continues to intrigue medical scientists around the world. The following sections highlight new findings and aspects of heme biosynthesis. Heme is produced by a well-defined pathway in mammals, initiated in the mitochondrial matrix, continuing in the cytosol and ultimately returning to the highly reduced environment of the mitochondrion. All cells are able to produce heme. However, in humans most heme is synthesized in the liver and bone marrow. Estimates of the rate at which hepatic heme is produced in normal humans suggests that in the steady-state situation in which the synthesis of heme is equal to its rate of degradation, heme is produced between 0.7 and 1.6 $\mu\text{mol/kg}$ body weight/day (6).

The key to understanding the conditions referred to as the porphyrias is the fact that each step of heme biosynthesis is catalyzed by an enzyme, or enzymes; deficiency of a particular enzyme (in most instances inherited) may thus lead to a specific pattern of porphyrin accumulation and a characteristic clinical (porphyric) syndrome.

It is thus pertinent to consider the enzymology and molecular biology of each individual enzymatically catalyzed step of heme biosynthesis in detail.

THE ENZYMOLOGY AND MOLECULAR BIOLOGY OF HEME BIOSYNTHESIS

Formation of the Pyrrole

Biosynthesis of 5-Aminolevulinic Acid

The condensation of succinyl-coenzyme A (CoA) and glycine to form 5-aminolevulinic acid (ALA) is catalyzed by the enzyme ALA synthase (EC 2.3.1.37) (ALAS). Glycine is bound through an essential pyridoxal 5'-phosphate cofactor as a stable Schiff-base carbanion on the enzyme surface, which can react with the electrophilic carbonyl group of succinyl-CoA to produce an α -amino- β -ketoacid with the release of CoA. The carboxyl carbon of glycine is then decarboxylated enzymatically to yield ALA.

Cloning, expression, and detailed characterization of the mouse enzyme have demonstrated a Schiff base linkage between the pyridoxal 5'-phosphate cofactor and a conserved lysine residue [murine ALA synthase (ALAS) Lys-313] in the protein and it is proposed that this lysine residue acts as a general base catalyst (electron "sink") to effect transient quinonoid intermediate formation during catalysis (8). While Lys-313 is thus essential for catalysis, it is not essential for binding the cofactor per se (9) and a conserved tyrosine residue (murine Tyr-121) is now thought to play this role (10). The electron sink function of the pyridoxal 5'-phosphate cofactor may be enhanced by the presence of an aspartate residue (murine ALAS Asp-279) and Arg 439 in the mouse is suggested as playing an essential role in substrate binding (10,11).

Alignment of the predicted amino acid sequences of the mammalian ALA synthases confirms that one should consider two domains. First, the C-terminal two-thirds of the mammalian proteins represents a conserved, ancestral core of the protein, as there is a high degree of homology in this region among ALA synthases. Second, in eukaryotes the N-terminal domain of the newly synthesized protein is not necessary for enzymatic activity and serves as the "presequence," facilitating translocation of the enzyme to the mitochondria. Thus, the human ALA synthase gene encodes a 640 amino acid protein (approximate molecular weight 70,000) in which the N-terminal 56 amino acids constitute the "presequence," which is cleaved on translocation of the protein to the mitochondria.

It is now recognized that there are two separate genes encoding ALA synthase. There is a ubiquitously expressed "housekeeping" (nonerythroid) isoenzyme (ALA-S1; molecular weight 64,600 d) and an erythroid-specific isoenzyme (ALA-S2; molecular weight 59,500 d) (12,13). Although the ALA-S1 and ALA-S2 isoforms share over 50% amino acid identity, they are localized to chromosomes 3p21 and Xp11.21, respectively (14,15). ALA-S1 is the only ALA synthase gene expressed in liver and other nonerythroid tissue and its activity is decreased during differentiation of erythroid cells. During erythropoiesis the gene for ALA-S2 is induced and its gene product is then the dominant form of ALA synthase (16,17).

It is widely accepted that the primary mechanism of regulation of heme synthesis is modulated by ALA synthase. This is therefore considered in some detail in a later section.

Biosynthesis of Porphobilinogen

The condensation of two molecules of ALA to form the monopyrrole PBG is catalyzed by the multisubunit, cytosolic enzyme ALA dehydratase (EC 4.2.1.24) [also referred to as porphobilinogen (PBG) synthase]. The combination of two molecules of ALA to form PBG occurs through a series of stages involving an aldol condensation and formation of a Schiff base.

ALA dehydratase has been purified from a variety of sources and been shown to exist as octamers. The human enzyme is an homo-octamer with subunits of approximately 36,000 d. The holoenzyme contains four catalytic sites and can be viewed as a tetramer of dimers with one active site per dimer. Each active site binds two molecules of ALA at two distinct positions, the A-site and P-site. The ALA molecule contributing the acetate group and the amino-methyl group of PBG binds at the A-site. The ALA contributing the propionate side chain and the pyrrolic nitrogen binds at the P-site. There is an ordered binding in which the keto group of the ALA contributing the propionate side chain forms a transient covalent bond with a conserved lysine (human Lys-252) in the P-site first. Once there is bound substrate at the P-site with an available 5-amino group, binding of the second ALA molecule onto the enzyme at the A-site may occur. The amino-nitrogen then gets incorporated into the pyrrole ring of PBG (18).

Binding of the second substrate at the A-site is dependent on the presence of a divalent metal ion, and removal of these divalent ions prevent binding at the A-site, resulting in loss of activity, but has no effect on ALA binding at the P-site (19). Typically the required metals are Zn^{2+} or Mg^{2+} . Thus in mammalian systems up to a maximum of eight Zn^{2+} ions can bind onto an ALA dehydratase octamer, four being required for catalysis (at the A-site) and four not. Although the binding of the latter four metal ions at the P-site may appear nonessential, they probably play a role in conformational stabilization of the enzyme (20). The two metal binding sites have been specifically identified on the *Escherichia coli* ALA dehydratase and are termed the α and β sites (21).

Cloning technology has allowed several of the genes encoding ALA dehydratases to be sequenced and their cDNAs and protein products characterized. The human gene has been mapped to chromosome 9q34 (22). Furthermore, in humans two tissue-specific forms of ALA dehydratase exist that are encoded by a single gene that contains separate erythroid and housekeeping promoters and can undergo alternative splicing (23,24). It has been proposed that this novel expression of erythroid-specific and housekeeping transcripts evolved to ensure that there is enough supply of heme for high-level tissue-specific hemoglobin production (24).

Cloning has also allowed the development of expression systems for various forms of ALA dehydratase, and ultrapure preparations of the recombinant enzyme have yielded the protein as a crystal, sometimes suitable for x-ray diffraction (25,26). The crystallization and initial x-ray characterization of the ALA dehydratases from *E. coli* and *Saccharomyces cerevisiae* of around 2 Å have been reported (27,28) as well as a more detailed structure down to 1.67 Å for *Pseudomonas aeruginosa* ALA dehydratase (29). In all cases the best crystals were obtained when these proteins were covalently bound to levulinic acid. The x-ray struc-

tures have confirmed that ALA dehydratase is a homo-octamer with each of its subunits adopting a "TIM" (triosephosphate isomerase) barrel fold with an N-terminal arm of 30 amino acid residues (28). The monomers formed asymmetric dimers with their "arms" wrapped around each other, and four of these dimers interact to form octamers with their active sites located on the surface.

In the *E. coli* enzyme, Lys-247 (equivalent of the essential Lys-252 at the P-site in human ALA dehydratase) formed a Schiff-base link with the bound levulinic acid at the active site. This is also the case in the yeast ALA dehydratase where x-ray analysis shows the formation of a Schiff base with Lys-263 (also equivalent to human Lys-252) (28).

P. aeruginosa ALA dehydratase structural analysis reveals that in each dimer the monomers differed from one another by having a "closed" and an "open" active site pocket. Whereas no metal ions were found in the active site of both monomers, a single well-defined and highly hydrated Mg^{2+} was identified only in the closed form about 14 Å away from the Schiff base forming a nitrogen atom of the active site lysine. Based on this information a structure-based mechanism of action involving Mg^{2+} allosteric binding at the active site and rate enhancement has been proposed (29).

Several ALA dehydratase inhibitors have clinical significance. The enzyme is highly sensitive to lead, which is believed to displace the Zn^{2+} ions. The clinical symptoms of lead poisoning are similar to those described in hereditary ALA dehydratase deficiency (see below), suggesting that ALA dehydratase inhibition is responsible for these effects. Patients with hereditary tyrosinemia also display similar symptoms. In these cases a deficiency in the enzyme 4-fumarylacetoacetate hydrolase causes accumulation of succinylacetone, which is a potent inhibitor of ALA dehydratase.

Assembly of the Tetrapyrrolic (Porphyrinogen) Macrocycle

Biosynthesis of Hydroxymethylbilane

Formation of the basic porphyrinogen tetrapyrrole is initiated in the cytosol by the assembly of four PBG molecules via a stepwise deamination and head-to-tail polymerization into a chemically reactive linear tetrapyrrole, hydroxymethylbilane by the enzyme PBG deaminase (EC 4.3.1.8) (also referred to as uroporphyrinogen-I synthase or hydroxymethylbilane synthase).

PBG deaminase has been purified from many sources, often as a complex together with the next enzyme in the pathway, uroporphyrinogen-III synthase (see next section). PBG deaminase is unique in that it contains a covalently attached dipyrromethane cofactor at the active site that binds substrate molecules during the sequential assembly of the linear tetrapyrrole molecule (30). The structure of the dipyrromethane cofactor and its sites of attachment to the enzyme have been characterized. The PBG deaminase

apoenzyme catalyzes the deamination and polymerization of two molecules of PBG at its active site (31). The resultant dipyrrole is covalently linked via a thioether linkage to the enzyme through a conserved cysteine (*E. coli* Cys-242) (32,33). This dipyrrolic cofactor then acts as a primer that gets elongated in a stepwise mechanism, one PBG unit at a time, through enzyme-substrate (ES) intermediate complexes, ES (with one PBG attached); ES₂ (two PBGs attached); ES₃ (three PBGs attached), and finally ES₄ (four PBGs attached) from which the tetrapyrrole product, hydroxymethylbilane, is released by hydrolytic cleavage, regenerating the enzyme-dipyrromethane intact (34). Thus, the two proximal PBGs (i.e., the dipyrromethane cofactor) remain covalently linked to the enzyme and are not turned over. The precise mechanisms by which the enzyme carries out this sequential manipulation of the four substrates and how the tetrapyrrole product is specifically cleaved leaving the intact dipyrromethane cofactor covalently attached to the enzyme have not been fully elucidated.

PBG deaminase was the first of the heme biosynthetic enzymes to benefit from the application of modern recombinant DNA technologic investigation (35). This was primarily driven by investigators attempting to derive diagnostic benefit in the realm of the clinically important diagnosis of acute intermittent porphyria (see below). Consequently, the gene for PBG deaminase has been sequenced and characterized from both prokaryotic and eukaryotic sources.

In mammals there is a single PBG deaminase gene consisting of 15 exons extending over 10 kilobases (kb) of DNA (36). In humans the gene has been mapped to chromosome 11q23, and two different transcripts, differing at their 5' ends, are produced from the single gene. The first is a ubiquitous, housekeeping messenger RNA (mRNA) transcript produced in all cells in which exon 1 is spliced to exon 3. The second form is specific to erythroid cells and initiates by alternate splicing at exon 2 (37). Activation of transcription of these two forms of PBG deaminase is controlled by two separate, independently regulated promoters.

In addition to the cloning and large-scale expression of recombinant *E. coli* PBG deaminase (38), allowing characterization of the dipyrromethane cofactor, crystallization of the protein was facilitated and the x-ray crystal structure of the *E. coli* PBG deaminase was determined to 1.76 Å resolution (32,39). The high-resolution structure revealed a protein folded into three α/β domains of approximately 100 amino acids each, linked to one another by flexible strands (18,32). Domains 1 and 2, which have similar overall topology, form a cleft at their interface. The dipyrromethane cofactor is bound by extensive contacts, including salt bridges and hydrogen bonds between these two domains in this cleft. Site-directed mutagenesis experiments have demonstrated that several of the salt bridges between arginine and the pyrrole acetates and propionates are important for enzymatic activity (40,41). Domain 3, which is an open-

faced antiparallel sheet of three strands containing the cysteine to which the cofactor is covalently bound, is situated deep within the cleft between domains 1 and 2. Deamination of PBG and formation of the methene bridge occurs here. Thus domain 3 can be considered as containing the single catalytic site. Importantly the crystal structure shows flexible boundaries between the 3 domains, which would allow conformational changes that accommodate each added PBG pyrrole until the tetrapyrrole is synthesized.

Closure of the Tetrapyrrole Ring

The regularly substituted linear tetramer, hydroxymethylbilane, is converted to an asymmetrically substituted cyclic tetramer, uroporphyrinogen-III, by uroporphyrinogen-III synthase (EC 4.2.1.75) (also referred to as uroporphyrinogen cosynthase or hydroxymethylbilane hydrolase), involving intramolecular inversion of the terminal D ring of the substrate. This inversion probably occurs via a chiral spiro intermediate (42).

Uroporphyrinogen-III synthase has been isolated and purified to homogeneity from many sources including human erythrocytes (43). All appear to exist as monomeric subunits with molecular weights around 30,000 d and are extremely thermolabile. Because of this instability, the protein has not been well characterized. Suffice it to say that there is no evidence for a cofactor of any sort. The human enzyme has an isoelectric point of 5.5 and a pH optimum of 7.4, and activity measurements show it to be present in excess over PBG deaminase, favoring the synthesis of the uroporphyrinogen-III over the series I isomeric form (44).

The full-length cDNA for uroporphyrinogen-III synthase has been cloned from a number of sources. The human gene has been isolated, sequenced, and the cDNA expressed in *E. coli* (45). The human uroporphyrinogen-III synthase gene has been mapped to chromosome 10q25.3 (46). In both the human and mouse there are 5' and 3' untranslated regions and an open reading frame spanning 10 exons and encoding a polypeptide of 265 amino acids. The mouse gene shares an 80% nucleotide and 78% amino acid identity with that of the human gene (47).

Modification of the Peripheral Side Chains of the Tetrapyrrole

Biosynthesis of Coproporphyrinogen-III

The stepwise decarboxylation of four acetate side chains in the 8-carboxylic (-COOH) uroporphyrinogen-III molecule through formation of 7-, 6-, and 5-COOH intermediates results in the formation of the 4-COOH coproporphyrinogen-III. This reaction takes place in the cytosol and is catalyzed by a single enzyme uroporphyrinogen decarboxylase. At physiologic substrate concentrations this reaction occurs

in an orderly manner with the carboxyl groups removed in a clockwise direction starting at ring D and proceeding through A, B, and C before the final formation of coproporphyrinogen-III. The precise mechanism of achieving this remains elusive especially as it doesn't have a cofactor requirement. The partially decarboxylated intermediates formed in this reaction are relatively stable porphyrinogen species that are detectable *in vivo*. Each intermediate acts as the substrate for further decarboxylation until the requisite coproporphyrinogen is formed. Both the series I and III isomers formed are suitable substrates for this enzyme, but the series III isomer is more rapidly decarboxylated (48). It should be emphasized, however, that only the coproporphyrinogen III isomer can continue in the pathway. The substrate specificity of the enzyme suggests that the active site is flexible, enabling the enzyme "to combine specificity with promiscuity" (49).

This enzyme has been purified from a variety of sources, including human erythrocytes (50). Most forms of the protein have been reported to be monomeric with molecular weights reported from 40,000 to 46,000 d. As with the other heme synthetic enzymes, cloning has followed this earlier protein characterization, and the uroporphyrinogen decarboxylase gene has been studied from a number of sources including human, mouse, and rat (51,52). The interspecies amino acid sequence homology is strong, especially near the N-terminus. The human gene encodes a 367 amino acid residue polypeptide (predicted molecular weight approximately 41 kd), is present as a single copy containing 10 exons within about 3 kb of DNA, and has been mapped to chromosome 1p34. Although two transcriptional start sites separated by six nucleotides have been identified, there is no evidence from either analysis of the gene structure or expression studies in different tissues to suggest tissue-specific promoters or isoforms of uroporphyrinogen decarboxylase.

Various inhibitor studies suggest that cysteine and histidine residues are important for enzyme activity. However, site-directed mutagenesis experiments indicate that no single cysteine is absolutely critical for the integrity of the catalytic site but one histidine residue (human H339) has been identified as important in imparting isomer specificity (53).

A recombinant human uroporphyrinogen decarboxylase, expressed in *E. coli* and purified has been crystallized and the initial data collected at 3.0-Å resolution (54,55). Subsequently, the crystal structure has been determined at 1.60-Å resolution (56). The 40.8-kd protein is composed of a single domain containing a (β/α) 8-barrel with a deep active site cleft formed by loops at the C-terminal ends of the barrel strands. Many conserved residues cluster at this cleft, including the invariant side chains of human Arg37, Arg41, and His339, which probably function in substrate binding, and human Asp86, Tyr164, and Ser219, which may function in either binding or catalysis. The crystal was dimeric, and assembly of the dimer juxtaposes the active

site clefts of the monomers, suggesting a functionally important interaction between the catalytic centers.

Biosynthesis of Protoporphyrinogen-IX

At this point the synthesis of heme reenters the mitochondria, where the enzyme coproporphyrinogen-III oxidase (EC 1.3.3.3) catalyzes the oxidative decarboxylation of coproporphyrinogen-III to form protoporphyrinogen-IX.

The two propionate residues on rings A and B of the tetrapyrrole molecule are converted into vinyl groups in a clockwise fashion, proceeding via a tricarboxylic porphyrinogen, tripropionate monovinyl porphyrinogen (trivially known as harderoporphyrinogen). It is a stepwise decarboxylation with the decarboxylation of the position-2 propionate side chain proceeding first and at a faster rate than that of position 4 (57).

Coproporphyrinogen-III oxidase has been purified to homogeneity from a number of sources. The gene encoding coproporphyrinogen-III oxidase has also been cloned from a number of sources, and alignment of the predicted amino acid sequences of a number of oxygen-dependent coproporphyrinogen-III oxidases indicates a high degree of interspecies conservation (57).

Human coproporphyrinogen-III oxidase has been expressed in *E. coli*, the purified enzyme characterized further, and attempts have been made at crystallization (57-60). Purified human coproporphyrinogen-III oxidase is a nearly globular homodimer (60) composed of subunits of molecular weight approximately 40,000 d. It does not appear to contain any redox reactive metal centers and there was no *in vitro* stimulation by either Fe^{2+} or Cu^{2+} (59,60). This contrasts to other studies that indicate that the mouse enzyme was a metalloprotein associated with Cu^{2+} ions as essential cofactors (61).

The complementary DNA (cDNA) sequence of the human gene encoding coproporphyrinogen-III oxidase has been cloned, sequenced, and characterized (58,59,62,63). In humans there appears to be a single copy of the gene with multiple transcriptional initiation sites. The human gene spans approximately 14 kb, and consists of seven exons and six introns (63). This gene has been mapped to chromosome 3q12 of the human genome. Potential regulatory elements have been identified in the GC-rich promoter region (six Sp1, one CACCC, and four GATA sites) and it is suggested that a single promoter may be differentially regulated in erythroid and nonerythroid tissue (58,62).

It is apparent that newly synthesized coproporphyrinogen-III oxidase has an N-terminal mitochondrial-targeting peptide that is cleaved during transport into the mitochondria. Although the length of this leader sequence was initially proposed to be 31 amino acid residues in length (58), it has more recently been established that it is an unusually long leader sequence of 110 amino acids (62,63).

Oxidation of Protoporphyrinogen-IX and Insertion of Iron

Oxidation of Protoporphyrinogen-IX to Protoporphyrin-IX

While auto-oxidation of protoporphyrinogen-IX to the fully conjugated, planar protoporphyrin-IX can occur in the presence of oxygen, the reducing environment inside cells where this reaction takes place requires catalysis by protoporphyrinogen oxidase (EC 1.3.3.4) (64,65). During this six electron oxidation, the methylene bridges in protoporphyrinogen-IX are converted into methenyl bridges.

There is little direct evidence to suggest a catalytic mechanism for protoporphyrinogen oxidase, but it is possible that diverse mechanisms may exist, especially in prokaryotes that can survive under both aerobic and anaerobic conditions. Three molecules of molecular oxygen, whose ultimate fate is hydrogen peroxide rather than water (66), serves as the final electron acceptor in the aerobic reaction in both eukaryotic and prokaryotic protoporphyrinogen oxidases (66–69). The reaction proceeds via three two-electron oxidations rather than a single six-electron oxidation (H. Dailey, personal communication, 2000).

Partial and, on very rare occasions, homogeneous purification of protoporphyrinogen oxidase from various sources has allowed partial characterization of the enzyme. Most have reported molecular weights within the range of 51,000 to 57,000 d, and it appears that most of these protoporphyrinogen oxidases exist either as monomers or homodimers. Subfractionation studies of rat liver protoporphyrinogen oxidase show it as an intrinsic protein of the inner mitochondrial membrane (67).

Evidence from spectral analysis and gene/protein sequence information shows that protoporphyrinogen oxidases are flavoproteins. Such evidence has come in the form of flavins extracted from various purified protoporphyrinogen oxidases and the identification of the $\beta\alpha\beta$ dinucleotide binding motif (-Gly-x-Gly-x-x-Gly-) near the N-terminal sequences of cloned protoporphyrinogen oxidase genes (68,70,71). Generally the flavin cofactors are noncovalently bound to these proteins and take the form of flavin adenine dinucleotide (FAD) (66).

All protoporphyrinogen oxidases are relatively specific for their natural substrate protoporphyrinogen-IX, although most will also oxidize the nonphysiologic dicarboxylic mesoporphyrinogen-IX.

Of great interest is the inhibition of protoporphyrinogen oxidase by several herbicidal compounds such as the diphenyl ethers. An important feature of these inhibitors is their structural similarity to protoporphyrinogen-IX; while studies on the herbicidal protoporphyrinogen oxidase (PPO) inhibitors showed that a bicyclic structure was the minimum structural requirement for recognition of many molecules by these enzymes (72), it was subsequently shown that some other, but not all, compounds with a

tetrapyrrole structure were also effective inhibitors of the enzyme. Thus PPO is effectively inhibited by other compounds such as heme and its metabolic products biliverdin and bilirubin (73). The mode of inhibition is generally competitive.

In recent years several PPO genes have been cloned and sequenced. Initially Hansson and Hederstedt (74) reported an open reading frame in the aerobic bacterium, *Bacillus subtilis*, (*hemY*) which was suggested to be involved in the oxidation of protoporphyrinogen-IX. Following on this Dailey et al. (68) successfully cloned and expressed this *B. subtilis hemY* gene in *E. coli* and showed that it indeed encoded a 53,000 d protein that had oxygen-dependent PPO activity, providing conclusive evidence that this protein was PPO.

Sequence comparisons between various PPO species indicate that most sequence homology is found in the N-terminal sequences containing the dinucleotide binding motif (66,68,75,76).

The cloning, sequencing, and expression of the prokaryotic PPOs from *B. subtilis* (68,69,74) and *E. coli* (77) facilitated the discovery and identification of the mammalian genes encoding mouse (78,79) and human PPOs (80–85). Genomic DNA fragments containing the whole coding sequence for human PPO (1,431 base pairs) have been cloned (85). This gene encodes a 51,000-d (477 amino acid residues) protein that exists as a 100,000-d homodimer (82).

The human PPO gene was mapped by fluorescence *in situ* hybridization to chromosome 1q22-q23 of the human genome (80,85). The human PPO gene has 13 exons spanning approximately 5 kb (85), although some disagreement exists over the lengths of introns 4, 7, and 9 (80,86). Northern blot analysis from a variety of tissues suggests a single mRNA transcript for human PPO of approximately 1.8 kb in length (81,82). These transcripts contain an approximately 300 bp long 5' untranslated region (UTR) and a short 3' UTR. Researchers have identified the start and termination codons, as well as a consensus polyadenylation signal and polyadenylation site downstream from the termination site (80,82,86).

The sequence contains no obvious membrane spanning regions and shows no typical mitochondrial targeting signals at the amino terminal (82). Recent data, however, demonstrate that mitochondrial targeting is facilitated by the amino terminal dinucleotide binding motif sequence (H. Dailey, personal communication). Sequence analysis of the protein database suggested that PPOs are members of a protein superfamily that includes plant and animal phytoene desaturases, and animal monoamine oxidases (76). These proteins all share significant homology in a 60 amino acid residue stretch that contains the dinucleotide binding motif. The mouse PPO is similar to the human enzyme in terms of its size, 51,000 d (477 amino acid residues), and homology, as the two proteins share 89% amino acid

sequence identity and both lack typical membrane targeting signals despite their localization to the mitochondrial inner membrane (78,80).

Cloning of the various PPO genes and overexpression of this protein in *E. coli* cells have resulted in the establishment of simple rapid purification procedures (87) and enabled researchers to produce large amounts of the protein. Although this has facilitated confirmatory and further characterization of this enzyme from various sources, information on the protein structure, and in particular the understanding of the catalytic mechanism at the enzyme active site, is still lacking.

Insertion of Iron

The terminal step in heme biosynthesis involves the incorporation of Fe^{2+} into the protoporphyrin-IX macrocycle by ferrochelatase (EC 4.99.1.1, protoheme ferrolyase) to form heme or protoheme-IX. Ferrochelatase is located on the matrix side of the inner mitochondrial membrane.

The mechanism of catalysis appears relatively conserved among ferrochelatase species. They have similar substrate specificity with the natural substrates being Fe^{2+} and protoporphyrin-IX (88).

In addition to Fe^{2+} , most ferrochelatases will utilize Co^{2+} and Zn^{2+} as substrates *in vitro*. Several divalent cations including Mn^{2+} , Cd^{2+} , Hg^{2+} , and Pb^{2+} are competitive for all ferrochelatases examined to date (89). Ferrochelatase is thought to use an ordered "bi-bi" reaction mechanism where iron binds prior to porphyrin (90). Following the binding of the metal ion to the enzyme, distortion of the porphyrin to a nonplanar conformation facilitates porphyrin metallation (91,92). This distortion has been demonstrated for the yeast ferrochelatase by Raman resonance to be a simultaneous tilting, or doming, of all four pyrrole rings (92,93). Metallation then occurs with the concomitant removal of the two pyrrolic protons.

Ferrochelatases have been purified and characterized from various sources and exhibit similar properties. The protein is generally encoded as a preprotein of approximately 47,000 d. During importation into the mitochondria the protein is proteolytically processed in an energy-requiring step into a mature protein of approximately 42,000 d (94).

The human ferrochelatase gene has been mapped to chromosome 18q21.3 (95). The characterized gene cDNA sequence consists of 11 exons with an approximate size of 45 kb (96). Both human and mouse ferrochelatase cDNAs contain two polyadenylation signals in the 3' noncoding regions. A series of elegant experiments reveal that a single ferrochelatase gene is regulated so as to provide for both housekeeping functions and erythroid-specific functions (95,97). The promoter region contains a CpG-rich island, and a Sp1-driven promoter appears to be sufficient for ferrochelatase expression in nonerythroid cell lines. Erythroid

specificity is mediated via GATA-1 and nucleosome transcription factor-E2 (NF-E2) elements. Further studies also suggest a role for chromosomal/chromatin structure and *cis*-acting elements in the regulation of the ferrochelatase mRNA production in erythroid tissue (98,99).

Dailey et al. demonstrated the presence of a labile [2Fe-2S] cluster in purified recombinant human ferrochelatase (100,101), and this feature has now been found in a variety of animal ferrochelatases (102–104). This cluster is readily destroyed by nitric oxide (NO), and its destruction results in the loss of enzyme activity (105). The biophysical properties of this cluster have been described (106), and the four cysteine residues that serve as ligands to the cluster have been identified by site-directed mutagenesis (104,107) and in the crystal structure (H. Dailey, personal communication). The precise function of the [2Fe-2S] cluster, which does not serve to donate iron to the active site, remains to be elucidated, but it does appear to play a role in the stabilization of the ferrochelatase homodimer.

The crystal structure of recombinant *B. subtilis* ferrochelatase, which is a water-soluble monomeric protein that lacks any cofactor or [2Fe-2S] cluster, has been solved to 1.9-Å resolution by Al-Karadaghi et al. (108). More recently the x-ray crystal structure of recombinant human ferrochelatase has been solved at 2.0 Å (H. Dailey, personal communication). The structure reveals that the enzyme is an 86-kd homodimer that contains one [2Fe-2S] cluster per subunit. Each monomer contains 48% α -helix and 14% β -sheet structure and is folded into two similar domains in a fashion that typifies the periplasmic binding protein family. Two differences that exist between the two domains is an additional 50 residues at the amino-terminal end that constitutes a portion of the active site, and a 30-residue addition at the carboxyl-terminus that participates in ligation of the [2Fe-2S] cluster and dimer stabilization. Each monomer contains an active site pocket whose entrance is composed of two hydrophobic lips. In the homodimer, both active sites are present on the same molecular face, and this surface is the largest hydrophobic region of the protein. A proposed function of this region is to serve as the site of membrane attachment. The result of such an organization is that both active sites are within the membrane proper and in position to accept the hydrophobic substrate protoporphyrin from either the hydrophobic milieu of the phospholipid bilayer or directly from the preceding pathway enzyme, protoporphyrinogen oxidase.

The active site pocket contains the majority of highly conserved residues. Key among these may be His263, which has been proposed to be involved in substrate iron donation (109), but most likely participates along with a highly conserved group of carboxylates in proton abstraction and not in iron donation (H. Dailey, personal communication). Substrate iron is proposed to be inserted from the opposite side of the pocket from His263. Residues involved in porphyrin macrocycle distortion remain to be unequivocally identified.

Regulation of Heme Biosynthesis in Nonerythroid and Erythroid Tissue

Heme synthesis is normally an extremely efficient, tightly controlled process in which the amount of heme produced closely matches the needs of the body. This implies that enzymes involved in heme synthesis are normally able to use all of the substrate presented to them, that they can handle an increased flux through the pathway, and that the pathway may be subject to some form of “feedback” control. Indeed, there is much evidence to suggest that, at least in the liver and all nonerythroid tissue, heme itself modulates its own rate of production, principally at the level of ALA synthase, which is considered the rate-determining enzyme of the pathway. This tight regulation of liver cell heme occurs by several mechanisms.

First, heme regulates its own synthesis by controlling the amount of ALA-S1 mRNA. In mammalian systems this occurs primarily at the transcriptional level (110,111), but studies in avian systems suggest the effect may also be on mRNA stability (112,113). The half-life of mammalian ALA-S1 is less than an hour and the half-life of the protein in mitochondria is even shorter. This is an important mechanism as many, but not all, drugs that induce cytochrome P-450 activity also induce transcription of ALA-S1 in mammalian systems and it is suggested that this may be via heme depletion that would accompany the synthesis of P-450 hemoprotein.

Second, heme regulates the translocation of ALA synthase from cytosol to mitochondria (114). This is mediated by two cysteine-containing heme regulatory motifs in the leader sequence (115; H. Dailey, personal communication).

In contrast the mechanisms of controlling erythroid ALA synthase activity are different (116). Transcription of ALA-S2 is controlled primarily by erythroid-specific transcription factors interacting with noncoding regions of the gene (12,117). Interestingly, the same factors are responsible for induction of globin synthesis, but this only occurs following the induction of heme synthesis (118), indicating the importance of heme per se as a regulatory molecule.

Posttranscriptional regulation of ALA-S2 also occurs differently to that of ALA-S1. There is a *cis*-acting regulatory

iron element in the 5' untranslated region (12), similar to the stem-loop structure occurring in the 5' untranslated region of ferritin mRNAs (119). A protein that binds to the iron regulatory element inhibits translation of the mRNA, but in the presence of iron the protein dissociates and the mRNA binds to ribosomes and is translated (120). In such manner is the translation of ALA-S2 mRNA coupled to the availability of iron.

Although heme does not appear to play a major role in the transcription and translation of ALA-S2, there are identical cysteine-containing heme regulatory motifs to those found in the ALA-S1 gene, in the leader sequence of ALA-S2, and *in vitro* experiments suggest a heme-mediated inhibitory effect (115). Thus translocation of ALA synthase to the mitochondrion may also be a controlled event in erythroid tissue.

CLINICAL DISORDERS ASSOCIATED WITH DEFECTS IN HEME BIOSYNTHESIS

Spectrum of Disorders

Defects in each of the seven heme synthetic enzymes are associated with characteristic clinical disorders. The porphyrias, the associated enzyme defects, and the clinical effects are summarized in Table 21.1. There are two characteristic forms of clinical expression: an acute neurologic syndrome known as the acute attack or porphyric crisis, and skin disease associated with photosensitivity.

The characteristic clinical picture of the acute attack is that it is episodic, may or may not be associated with an obvious precipitating event (such as the administration of porphyrinogenic medication or menstruation), and is marked by a typical constellation of symptoms, notably severe abdominal pain accompanied by few clinical signs and by an absence of peritonism, and features of autonomic neuropathy (121) (particularly hypertension and tachycardia, vomiting, ileus, and constipation). This may proceed to a typical motor neuropathy resembling the Guillain-Barré syndrome; in severe cases, this leads to a severe flaccid quadriplegia and respiratory failure requiring ventilation.

TABLE 21.1. SUMMARY OF THE PORPHYRIAS

Enzyme	Disorder	Inheritance	Clinical Effects
ALA dehydratase	ALA dehydratase deficiency (Doss porphyria)	AR	Acute attacks
PBG deaminase	Acute intermittent porphyria (AIP)	AD	Acute attacks
Uroporphyrinogen cosynthase	Congenital erythropoietic porphyria (CEP)	AR	Photosensitivity
Uroporphyrinogen decarboxylase	Porphyria cutanea tarda (PCT)	Sporadic AD	Photosensitivity
Coproporphyrinogen oxidase	Hereditary coproporphyria (HCP)	AD	Acute attacks, photosensitivity
Protoporphyrinogen oxidase	Variegate porphyria (VP)	AD	Acute attacks, photosensitivity
Ferrochelatase	Erythropoietic protoporphyria (EPP)	AD (AR?)	Photosensitivity

AD, autosomal dominant; AR, autosomal recessive; ALA, 5-aminolevulinic acid; PBG, porphobilinogen.

Pathologically, the neuronal injury is characterized by severe axonal necrosis, though at times there may be a lesser element of demyelination as well (122–126). Once established, the neuropathy is slowly reversible, and typically months to years are required before full function is regained. The clinical features of the acute attack itself are reviewed in detail elsewhere (127–129).

Pathogenesis of the Acute Attack

The pathogenetic mechanisms whereby the acute attack is established are poorly understood. The most likely hypotheses include ALA neurotoxicity and heme deficiency, acting either directly within the neuron or via a deficiency of one or more essential hemoproteins. The subject has recently been concisely and elegantly reviewed by Meyer et al. (128).

5-Aminolevulinic Acid–Induced Neurotoxicity

The invariable observation that the acute attack is always accompanied by an elevation in ALA concentrations has led authorities to suggest that ALA is in itself neurotoxic, though a causal link has not been proven. Many asymptomatic patients, particularly with acute intermittent porphyria (AIP), excrete levels of ALA in excess of those seen in other patients, typically those with hereditary coproporphyrin (HCP) and variegate porphyria (VP), who are actively experiencing symptoms (128), and administration of ALA to healthy volunteers, to subjects with AIP (130), and to experimental animals (131,132) does not precipitate the syndrome. Additionally, there is experimental evidence in rodents to suggest that the blood–brain barrier is poorly permeable to ALA (128). In patients with the acute attack, cerebrospinal fluid (CSF) ALA concentrations have been reported to be much lower than those measured simultaneously in blood, representing only 2% to 3% of blood levels (133). However, though this may protect the central nervous system (CNS) against the toxic effects of ALA, the peripheral nervous system would remain exposed to the full blood ALA concentration, a level at which *in vitro* effects on nerve function have been demonstrated (134).

Yet there are lines of evidence to suggest that under certain circumstances ALA *in vivo* is detrimental. Administration into the cerebral ventricle has excitatory effects (135) and reduces seizure latency in rodents (136). There is also evidence that ALA at low concentrations can alter neurophysiologic function, depress spinal reflexes, and induce depolarization of muscle in animals (134), and is neurotoxic in chick embryos (137). Yet ALA at considerably higher concentrations has not proved toxic to human spinal cord neurons in culture (138). The similarity in chemical structure between ALA, the inhibitory neurotransmitter γ -aminobutyric acid (GABA), and the excitatory neurotransmitter L-glutamic acid has suggested a possible effect on

neuronal function, but such an association has not been proven (139,140).

Heme Deficiency

It has been postulated that the acute attack represents the clinical effects of intracellular (and more specifically intraneuronal) heme deficiency (128), since there is experimental evidence in patients with porphyria of a functional heme deficiency in some heme-containing enzymes. Thus, in patients with VP, impaired cytochrome P-450–mediated drug metabolism has been demonstrated (141–143); this function is restored to normal following the administration of exogenous heme. Evidence of direct intraneuronal heme deficiency is lacking (144), and it appears more likely that a deficiency in hemoprotein activity outside the CNS is important. A candidate enzyme is tryptophan dioxygenase, reduced activity of which results in the increased production of serotonin, a known modulator of neuronal function (143,145). It has also been postulated that heme deficiency might lead to deficiencies in cytochrome-dependent energy production in neuronal tissue, leading to axonal degeneration (146), although the evidence is weak.

Treatment of the Acute Attack

This chapter does not describe the clinical management of porphyria in detail. Useful reviews of the general management of the acute attack of porphyria may be found elsewhere (127,147,148). However, the specific therapy of the underlying metabolic defect will be reviewed to throw some light on the physiologic disorders accounting for the porphyria.

The Glucose Effect

The earliest specific therapy advocated for the acute porphyric attack was carbohydrate loading. In early clinical studies, it was suggested that approximately 75% of patients would respond favorably to intravenous or oral glucose administration (149). Additionally, there is experimental evidence that ALAS activity and porphyrin synthesis are repressed when liver cells grown in culture are exposed to porphyrinogenic medication in the presence of high carbohydrate concentrations. Carbohydrate intake also inhibits the induction of hepatic ALAS in experimental porphyria and in human AIP. But no dose-response curve has been shown for the effect of glucose on the induction of hepatic ALAS. Conversely, there is evidence that deficient carbohydrate intake and fasting may precipitate the acute attack of porphyria, and may even increase porphyrin excretion both in healthy subjects and in experimental rats. This, the so-called glucose effect, is demonstrable in AIP, VP, and even in nonacute porphyrias such as erythropoietic protoporphyria (EPP) and porphyria cutanea tarda (PCT). In addition to glucose, fructose and glycerol appear to share this ability to reduce porphyrin synthesis.

Heme Administration

Heme administration in patients with acute attacks of porphyria results in decreased porphyrin production via a repression of ALAS by a process of negative feedback (150), and is clinically highly efficacious. The effect, however, is short-lived (151,152). Since heme is known to induce the enzyme heme oxygenase and thus to mediate its own metabolism, tolerance may result from frequent administration. This has suggested the use of inhibitors of heme oxygenase in clinical practice. A number of substituted metalloporphyrins including tin protoporphyrin, tin mesoporphyrin, and zinc mesoporphyrin will inhibit heme oxygenase. In animal and human experiments, these inhibitors markedly inhibit the induction of hepatic ALAS, the production of ALA and PBG, and the appearance of bilirubin (153,154). There is as yet insufficient experience for their role in the treatment of human porphyria to be defined, but the preliminary evidence suggests that they may be beneficial in unusual patients with severe, recurrent acute attacks (148,155).

THE SPECIFIC SYNDROMES OF PORPHYRIA

5-Aminolevulinic Acid Dehydratase Deficiency

Also known as plumboporphyria or Doss porphyria, this is a rare autosomal-recessive disorder of heme synthesis. Five cases have now been reported since the first description in 1979. The molecular defects have been reported in three of these patients; all have been shown to be compound heterozygotes bearing single base substitutions, unique to each patient, on each allele (118). The patients exhibit a predominantly neurologic syndrome similar to the acute attack, with abdominal pain and vomiting progressing to a motor neuropathy and paralysis. One presented in infancy, two in adolescence, and two at an advanced age.

Porphobilinogen Deaminase Deficiency

The clinical syndrome associated with a deficiency of PBG deaminase is acute intermittent porphyria. This is the commonest of the acute porphyrias and is transmitted as an autosomal-dominant trait. Clinical symptoms are restricted to the acute attack, and skin disease is not encountered. This is ascribed to the observation that it is only the precursors ALA and PBG that are elevated in this disorder, rather than the porphyrins. An overall prevalence for Europe of 1 to 2 per 10,000 has been suggested (148), but in Finland, the prevalence is higher, at 2 per 1,000 (156).

Molecular Biology

The human *PBGD* gene has been assigned to chromosome 11q24 (157). The gene contains two distinct promoters;

alternative splicing gives rise to two primary transcripts and the production of distinct erythroid and nonerythroid mRNAs, which give rise to two proteins of slightly varying lengths. More than 100 mutations in the *PBGD* gene have been reported in patients with AIP. These have recently been summarized by Grandchamp (158). Most mutations are found in single families. However, the W198X mutation is present in about 50% of AIP families in Sweden (159), and is thought to result from a founder effect for this mutation, and the R116W mutation accounts for a third of AIP cases in the Netherlands (160).

Uroporphyrinogen III Cosynthetase Deficiency

Congenital erythropoietic porphyria (CEP) is the clinical syndrome associated with a deficiency of uroporphyrinogen III cosynthetase (UROS). This is an autosomal-recessive condition and is rare. It is an erythropoietic porphyria that primarily affects heme synthesis in the erythroid compartment, and presents with skin disease alone. Patients are not at risk of developing an acute attack. Severe UROS deficiency results in a failure of production of physiologically relevant series III porphyrin isomers, and series I isomers of uroporphyrin and its decarboxylated derivatives accumulate as a result of spontaneous cyclization and decarboxylation. Characteristic clinical features include hemolysis, mild splenomegaly, anemia, and photomutilation that is usually severe.

Molecular Biology

The molecular basis of CEP has recently been reviewed by Desnick et al. (44). Eighteen mutations have been identified in the UROS gene, comprising deletions, insertions, splice mutations, and single base substitutions. All but one of the known CEP missense mutations occurred in apparently highly conserved UROS peptide sequences. Some patients are homoallelic for a specific mutation, whereas other patients are heteroallelic. CEP is encountered in populations around the world, and several shared mutations have been identified in patients from apparently unrelated families. Particularly striking is the C73R mutation, found in 22 unrelated families and homoallelic in five of the subjects. There is evidence of some genotype–phenotype correlation in CEP. Thus the C73R mutation results in the detection of less than 1% of normal activity when expressed in *E. coli*; human homozygotes for this mutation appear to demonstrate an extremely severe phenotype that may include profound anemia, hydrops fetalis, and transfusion dependency at birth. Patients who carry both C73R and a second mutation that appears to express somewhat more residual activity may demonstrate a moderately severe phenotype, whereas patients who are allelic for mutations with more residual activity have milder forms of CEP (44).

There is considerable interest in gene therapy for CEP, since it is a severe disorder for which therapy is at best largely ineffective. Wild-type UROS cDNA has successfully been introduced into retroviral experiments *in vitro*, which have proved that it is possible to increase greatly UROS activity in hematopoietic progenitor cells and early erythroid cells, as well as in mononuclear cells and fibroblasts from patients with CEP following transfection vectors (161,162).

Uroporphyrinogen Decarboxylase Deficiency

Uroporphyrinogen decarboxylase (UROD) deficiency is associated with PCT, the most common form of porphyria encountered in most countries. The clinical features therefore include blistering, scarring, and pigmentary changes in sun-exposed areas of the skin, and typically a low-grade hepatitis (163). Two forms, clinically indistinguishable, are recognized: familial and sporadic. In both forms hepatic UROD activity is reduced. Additionally, in familial PCT, erythrocyte UROD activity is also reduced, in keeping with a genetic predisposition expressed in all somatic cells, and mutations in the UROD gene are recognizable. In sporadic PCT, by contrast, the UROD gene is normal (164), reduced UROD activity is restricted to the liver, and, following removal of such precipitating factors as iron overload, may return to normal, suggesting that UROD is subject to the effects of an inhibitor whose effects may be reversed (163). The mechanism for this is not understood, and it is discussed further below.

Molecular Biology

Some 34 UROD gene mutations have been identified in patients with familial PCT. Most are restricted to single families. Where patients are homoallelic for UROD mutation, or are heteroallelic for two such mutations, a severe phenotype referred to as hepatoerythropoietic porphyria may result. This is associated with a severe deficiency in UROD activity in all tissues and is expressed clinically as severe photosensitivity with photomutilation, with onset in childhood (49,51). Familial PCT accounts for approximately 20% of all patients with PCT (165–167), and is inherited as an autosomal-dominant trait but with low clinical penetrance: most clinically expressed cases of familial PCT will be found to have associated risk factors known to produce sporadic PCT, though the average age of onset may be earlier. This suggests that in many cases the presence of a mutant UROD is not of itself sufficient to result in clinical symptoms, but that when exposed to factors thought to inhibit UROD, the threshold for development of symptoms is lower.

Conditions Associated with Sporadic Porphyria Cutanea Tarda

Sporadic PCT is clearly associated with a number of associated, and presumably causally related, factors. These

include hepatic iron overload; alcohol consumption; estrogen therapy; viral infection, particularly hepatitis C virus (HCV) and human immunodeficiency virus (HIV); certain hydrocarbon-based toxins such as hexachlorobenzene; and, rarely, some systemic disorders including systemic lupus erythematosus and lymphoma (163,168). In nearly all cases of PCT, at least one of these disorders is present; frequently, several are present in combination.

Iron Loading

In most cases, mild to moderate iron loading of the liver is demonstrable. It has been shown that there is an approximately double allele frequency for the C282Y mutation in the *HFE* gene associated with genetic hemochromatosis in patients of northern European extraction (169–171). This association is not seen in other populations: the H63D mutation may be associated in patients of southern European origin, and suggests that a high transferrin saturation level in a patient with PCT might imply homozygosity for the hemochromatosis gene in European populations (170–172).

Viral Infection

A high prevalence of HCV antibodies in patients with PCT has been demonstrated in several studies from southern Europe with seroprevalences ranging from 62% to over 90% (173). However, studies from northern Europe and South Africa suggest a much weaker association. An association with HIV infection has also been suggested, though it appears that most such patients have other risk factors for the PCT as well (174).

Alcohol, Estrogen, and Other Hepatotoxins

There is a strong association between heavy alcohol ingestion and PCT (168), as well as with the use of natural and synthetic estrogens and toxins such as hexachlorobenzene. Less common factors associated with PCT include some systemic disorders, including systemic lupus erythematosus and lymphoma, and chronic renal failure on hemodialysis.

Pathogenesis

The pathogenesis of PCT has recently been summarized by Elder (163). There is evidence that during the recovery from clinically expressed PCT, in both sporadic and familial forms of the disease, the specific activity of hepatic UROD increases; in sporadic PCT, it may return to normal (175). This suggests that overt disease results from progressive inactivation of a structurally normal enzyme within the liver. The link between factors such as alcohol abuse, iron overload, and viral infection and UROD inhibition is not yet understood. It seems likely that iron serves largely as a powerful oxidant with resultant formation of reactive oxygen species that may bring about the oxidation of uroporphyrinogen to uroporphyrin and of other products that

may themselves inhibit UROD (176,177). It is likely that the cytochrome P-450 family is involved. CYP1A is induced by the cyclic hydrocarbons associated with both toxic PCT in humans and its experimental counterpart in animals; in rodents, an isoform of CYP1A catalyzes the microsomal oxidation of uroporphyrinogen to uroporphyrin (178) and reaction is promoted by iron *in vitro*. In a schema suggested by Elder (163), induction of CYP1A results in the oxidation of uroporphyrinogen III to uroporphyrin III (which can therefore participate no further in heme synthesis) and accelerates the production of nonporphyrin oxidation products; these reactions are promoted by the presence of iron-derived reactive oxygen species. These porphyrin products result in the irreversible inhibition of UROD with consequent further accumulation of uroporphyrinogen III, providing further substrate for the oxidation and thus establishing a self-sustaining cycle. Removal of iron may interrupt this cycle, allowing a restoration of normal UROD activity and a reduction in intracellular uroporphyrinogen III. In this schema, iron may be considered a switch controlling the formation of inactivators of UROD.

Treatment

Unlike other forms of porphyria, PCT is treatable. Precipitating factors such as alcohol and estrogen exposure should be removed. Iron removal by venesection is highly effective, and can be shown to result in a restoration of hepatic UROD activity to normal levels in sporadic PCT. Oral administration of chloroquine will also induce remission in most cases by releasing uroporphyrin stored within hepatocyte lysosomes (179), but is not recommended as sole therapy as it will not reduce the potentially deleterious elevation in iron stores.

Coproporphyrinogen Oxidase Deficiency

A deficiency in coproporphyrinogen oxidase is associated with the human disease hereditary coproporphyria and is inherited as an autosomal-dominant trait. Both the acute attack and photosensitive skin disease are encountered. It is a less common condition than other AIP or VP. Frequencies in three different populations have been estimated to range from approximately 1 in 70,000 to 1 in 130,000. It is estimated that only approximately a third of these will be clinically manifest.

Molecular Biology

The gene for coproporphyrinogen oxidase is localized to chromosome 3q12. It has a single promoter region that may be differentially regulated in erythroid and nonerythroid tissues (62,63). In addition to five polymorphisms, at least 19 mutations have been characterized and found to under-

lie hereditary coproporphyria (180). Homozygous HCP has been seen. A variant coproporphyrinogen oxidase deficiency syndrome has been described and labeled harderoporphyrin (181). In this condition, hematologic manifestations predominate and include jaundice, severe hemolytic anemia, hepatosplenomegaly, and skin photosensitivity. The oxidation of coproporphyrinogen is a two-step reaction, proceeding via an intermediate harderoporphyrinogen. In harderoporphyrin, it appears that the responsible mutations result in a selective failure of the second step, resulting in accumulation of harderoporphyrin and this unusual syndrome.

Protoporphyrinogen Oxidase Deficiency

Protoporphyrinogen oxidase (PPO) is the penultimate enzyme of the heme synthetic pathway and is responsible for the oxidation of protoporphyrinogen to protoporphyrin IX. Deficiency is clinically associated with variegate porphyria (VP). VP is transmitted as an autosomal-dominant trait and is associated with both acute attacks and photosensitivity. In Britain, VP has been estimated to occur with a prevalence roughly one-third that of AIP, or approximately 0.5 per 100,000 (148). The prevalence is very much higher in South Africa, where it has been estimated to approach 0.6% in the European immigrant population. This is ascribed to a founder effect and has been traced back to a Dutch settler who arrived in the Cape of Good Hope in 1688 (147).

Molecular Biology

The gene for human PPO has been assigned to chromosome 1q22-23 (85). In South Africa a single mutation (R59W) predominates, and has been estimated to account for 95% of the subjects with VP in that country (182); haplotype comparison with the Dutch families with the R59W mutation supports the belief that the mutation was imported from Holland. However, a further nine mutations have been identified in South African families, three of which have been shown heteroallelic to the R59W mutation in compound heterozygotes (183). This suggests a considerable degree of heterogeneity for VP. More than 80 PPO mutations associated with VP have now been reported from around the world. A large study of patients with VP drawn from both England and France has been instructive (184). Most are private mutations, though an R168H mutation appears to have arisen independently on several occasions, as has an L15F mutation.

Kinetic Basis of the Acute Attack

The association between VP and the acute attack is not intuitively obvious, since it is always associated with elevations in the proximal precursors ALA and PBG. In PCT,

despite a significant accumulation of intermediate porphyrins, an elevation of ALA and PBG is never encountered and acute attacks are not a feature. Meissner et al. (185,186) demonstrated that in lymphoblasts derived from VP subjects, PBG deaminase activity was decreased by approximately 25% in addition to the expected 50% reduction in PPO activity and was accompanied by an abnormal sigmoidal substrate velocity curve, suggesting allosteric behavior. Furthermore, reduced PBG deaminase activity could be induced *in vitro* in VP-derived lymphoblasts by the addition of protoporphyrinogen IX and coproporphyrinogen III, but not uroporphyrinogen III, and normal kinetic behavior restored by the removal of these porphyrinogens. These findings are in complete agreement with the clinical observation that VP and HCP, the disorders in which coproporphyrinogen and protoporphyrinogen in particular accumulate, may both be associated with acute attack, whereas PCT, associated largely with the accumulation of uroporphyrinogen, is not.

Clinical Expression

In the absence of the ability to make a definitive diagnosis of inheritance of the porphyria at the molecular level, the proportion of patients in whom VP was expressed either clinically or biochemically was unknown. Two previous studies in Cape Town have examined the clinical expression of South African VP. Eales et al. (187) suggested that 17% had experienced only acute attacks, 52% showed evidence of skin disease alone, and 21% had suffered both. A subsequent telephone survey suggested that there had been a change over the period between the two studies, with a decreasing proportion experiencing acute attacks, correspondingly more experiencing skin disease only, and a significant group being clinically silent and only detected as a result of biochemical screening of affected families (147). Recently, using DNA-based testing as the basis of a diagnosis of VP, we have shown in a single large kindred carrying the R59W mutation that only 52% of adult gene carriers demonstrated abnormal stool biochemistry, though abnormal fluorescence peaks were demonstrable in 90%. Only 39% had ever experienced skin symptoms, and 4% had an unequivocal acute attack in addition to skin symptoms; thus 61% were clinically silent. No subject younger than 16 had either biochemical or clinical evidence of VP.

Homozygous Variegate Porphyria

Fourteen subjects with homozygous VP have been reported. The clinical features have been summarized by Hift et al. (188) and include the onset of photosensitivity in infancy, photomutilation, brachydactyly, neurologic features such as nystagmus and seizures, behavioral disorders, a sensory neuropathy, and, in some cases, mental retardation. An unex-

plained observation is that these subjects do not appear to develop acute attacks despite their severely reduced PPO activity. However, we have now identified two sisters, compound heterozygotes, who presented in adolescence, one of whom has experienced acute attacks.

Most of these subjects have now been described genotypically. A minority are true homozygotes; most are compound heterozygotes. Studies of the residual expression of the mutant enzymes support the hypothesis that co-inheritance of two mutations associated with complete loss of PPO kinetic activity is not compatible with life; in every case, a severe mutation was shown to be heteroallelic expressing some residual activity (189). It has not been possible to show a correlation between the phenotypic severity and the activity expressed by the mutant proteins. In all four South African patients with compound heterozygous VP, the common R59W mutation, known to be associated with near-complete loss of kinetic activity, was present on one allele; none of the other mutations were associated with clinically or biochemically expressed VP in the heterozygous state (183), suggesting that they are functionally less damaging; preliminary kinetic data from our laboratory support this. None of the mutations encountered in homozygous and compound heterozygous VP in Europe were associated with clinically expressed VP in heterozygotes, in keeping with the proposition that, in general, these mutations code for mutant enzyme with a variable degree of residual activity sufficient to avoid symptoms except when paired with a second mutant allele (189). Such "mild" mutations are those that appear to preserve 10% to 25% of activity of the wild-type enzyme.

Ferrochelatase Deficiency

This enzyme is responsible for the last step in heme synthesis, that is, the incorporation of ferrous iron into the protoporphyrin line macrocycles. Ferrochelatase deficiency is associated with the human disease EPP. EPP is associated with a characteristic form of cutaneous photosensitivity. In contrast to the skin disease of CEP, PCT, HCP, and VP, which is marked by a vesiculo-erosive pattern of skin injury, skin disease in EPP takes the form of an immediate hypersensitivity. Characteristically, patients who exceed an individual threshold of sun exposure manifest problems of burning, stinging, erythema, and edema in exposed areas, and learn to associate these unpleasant symptoms with sun exposure; they will voluntarily seek to avoid exposure. These features usually begin in childhood rather than postpubertally as in the case with the vesiculo-erosive forms of porphyria. Approximately 10% of clinically expressed cases show evidence of severe protoporphyrin accumulation in the liver, leading to hepatic injury and ultimately to liver failure. Typically liver decompensation occurs late in the illness, but once initiated, progresses rapidly.

Molecular Biology

The gene encoding human ferrochelatase has been mapped to chromosome 18q22 (190). Many mutations have now been identified in the ferrochelatase gene. In most cases, mutations are private and are limited to single families (191). The inheritance of EPP is complex. Though typically described as an autosomal-dominant trait with incomplete penetrance, there is evidence to suggest that, in some families at least, particularly those in whom liver damage occurs, an autosomal-recessive mechanism appears to fit the observed pattern of inheritance more accurately. Most patients with manifest disease have severely reduced ferrochelatase activity, suggesting a disorder carried on both alleles. This has led to the belief that co-inheritance of two defects—one, presumably more severe, from the biochemically abnormal parent and the second, less severe, from an apparently normal parent—is necessary before clinical EPP arises. Since only one parent can be shown biochemically to carry EPP, the disease appears to be dominant, yet its actual inheritance will be recessive (192). In one family a clearly mutated allele was complemented by a poorly expressed ferrochelatase allele associated with markedly reduced mRNA expression (193). It is not known, however, whether a similar mechanism is common to all families with EPP.

Therapy

Recommended therapy includes avoidance of light exposure, the use of topical photoprotectants, and administration of oral β -carotene with or without canthaxanthine. Anecdotally, many patients will report an improvement in light tolerance on β -carotene; this has been described both to be formation of a photoprotectant layer of pigmentation in the skin and to its free radical quenching properties. Yet, in a controlled trial, β -carotene could not be shown to be unequivocally effective (194). A significant intrahepatic circulation of porphyrins has been shown (195), which may be interrupted by the administration of sequestrants such as oral charcoal, cholestyramine, or colestipol (196) with a reduction in protoporphyrin levels. Other theoretically effective measures include the suppression of erythropoiesis by erythrocyte transfusions or the administration of hematin, or by plasmapheresis to reduce free protoporphyrin in the plasma. Liver transplantation has been reported for end-stage protoporphyrin hepatopathy; photosensitivity persists, which proves that the defect in the erythropoietic tissue alone is sufficient to produce symptoms. Furthermore, protoporphyrin-induced liver damage has recurred in the transplanted liver despite the correction of endogenous protoporphyrin production by the transplant. Bone marrow transplantation has not yet been reported in EPP. Of greatest theoretical benefit would be combined liver and bone marrow transplantation (191).

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