Enzyme Defects in Hereditary Porphyria

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ABSTRACT

Heme is an important prosthetic group for proteins concerned with energy metabolism. All cells in the body probably make heme, but nucleated erythroid and hepatic cells have been studied the most. Feedback control of heme formation differs in the red cells and in the liver. About eight enzymes have a place in the formation of heme. Defects in the enzyme pathways may be the result of genetic abnormalities and phenotypically occur as hereditary porphyrias. If the major defect occurs in the red cell line, erythropoietic porphyrias occur; if the liver has the major defect, then hepatic porphyrias are present. There are probably three erythropoietic porphyrias and four hepatic porphyrias which are genetically determined. However, some are not clearly classified,—with erythropoietic protoporphyria involving hepatic and erythroid cells and porphyria cutanea tarda not being a clear cut genetic abnormality, at least some of the time. Elucidation of the genetic enzymatic defects introduces new diagnostic tools and also has led to at least one revolutionary new treatment for some hepatic porphyrias.

Introduction

In recent years, great strides have been made in elucidating the enzymes and substrates involved in the synthesis of heme, the prosthetic group of hemoproteins such as hemoglobin, myoglobin, cytochrome oxidases, catalases, peroxidases, etc. Hemoproteins are involved in basic energy reactions, such as transport of molecular oxygen (hemoglobin), activation of oxygen (cytochrome oxidases, tryptophan oxygenase, and mixed function oxygenases such as microsomal cytochromes), activation of peroxide (peroxidases), decomposition of hydrogen peroxide (catalase), and transport of electrons (mitochondrial cytochromes).15,16,31

Heme formation has been detected in a number of tissues, but it has especially been studied in hepatic and nucleated erythroid cells (figure 1). Although the pathway seems the same in both of these tissues, its regulation differs in each. Delta aminolevulinic acid synthetase (ALA synthetase) represents the rate limiting enzyme normally in both erythroid and
hepatic pathways. Feedback inhibition of this by heme as a corepressor is the major controlling factor in the hepatic path. Additionally in the liver, aminolevulinic acid (ALA) synthetase is induced by lipophilic drugs and chemicals,9,14,21,22,32 and fasting. On the other hand, glucose inhibits induction.33 Drugs inducing ALA synthetase activity have two properties in common. The first is they decrease intracellular heme and hemoproteins (e.g., cytochrome p450, catalase), either by inhibition of synthesis or by increasing breakdown. Second, they are lipid soluble and as such as potential inducers of hepatic microsomal enzymes.

Erythroid heme formation is controlled differently. The feedback inhibition of ALA synthetase by heme is not of primary importance. Rather, heme produces feedback inhibition by preventing dissociation of iron from transferrin.27 This results in prevention of iron uptake by the reticulocyte. Erythroid ALA synthetase is induced by hypoxia and erythropoietin which are not factors in induction in the liver.6

Only heme and possibly 5β-H steroids influence synthetase in both erythroid and hepatic tissues. This explains why erythropoietic and hepatic genetic porphyrias differ in their clinical presentations.16,18,34

**Enzymes in the Formation of Heme**

ALA synthetase is the first enzyme in the system. It brings about a condensation of glycine with succinyl CoA formed particularly in the tricarboxylic acid cycle. This is the only reaction in the formation of heme requiring both a vitamin cofactor and the introduction of energy. The former is pyridoxal phosphate and the reaction is oxygen dependent. Delta aminolevulinic acid (ALA) is formed.13,16,24,30,33 The formation involves a reaction between a sulfhydryl group in the active site of the enzyme and the vitamin cofactor. The resulting compound forms a Schiff base with glycine and this condenses with succinyl CoA. There follows

![Figure 1. Heme formation in hepatic and nucleated erythroid cells.](image-url)
a decarboxylation of the glycylcarbonyl group. The entire process takes place in the mitochondrion.

The ALA formed moves into the cytosol, and two molecules condense under the influence of delta aminolevulinic acid dehydratase [(dehydrase) (ALA dehydratase)] to form porphobilinogen (PBG). First, an intermediate, a Schiff base, is formed by condensation of one molecule of ALA and one subunit of ALA dehydratase. Next, in the cytoplasm, uroporphyrinogen is formed by the action of uroporphyrinogen I synthetase (URO I synthetase), acting on four molecules of PBG. This leads to the formation of one molecule of uroporphyrinogen I and four molecules of ammonia. The action of URO (I) synthetase and uroporphyrinogen (III) cosynthetase (URO III cosynthetase) forms uroporphyrinogen III from PBG. By itself, the cosynthetase cannot utilize either PBG or uroporphyrinogen I as substrate. There is a disagreement as to whether URO I synthetase produces an intermediate on which URO III cosynthetase acts or whether uroporphyrinogen I and III are formed with different dipyrrylmethanes (conjugates of two molecules of porphobilinogen differently linked) and then the cosynthetase alters the pattern of PBG condensation. If the latter putative mechanism is correct, as favored by the extensive work of Frydman, the synthetase will produce different isomers and the cosynthetase is a modifier protein and not an enzyme.

Uroporphyrinogens I and III are oxidized to uroporphyrin I and III, respectively. The reduced substances are colorless and the oxidized products are colored but are found in minor amounts in the body normally. Next, uroporphyrinogen I and III are decarboxylated stepwise enzymatically by uroporphyrinogen decarboxylase (URO decarboxylase) until four acetic acid side chains are removed and coproporphyrinogens I and III, respectively, are formed. These steps produce porphyrinogens with seven, six, and five carboxyl groups (hepta, hexa, and pentaporphyrinogens). The more carboxyl groups a porphyrin has, the more water soluble it is, accounting for the predominantly urinary or fecal distribution of various porphyrins. At any one time, the concentration of these intermediates is small, since the $K_m$ of the decarboxylating enzyme is low and a high turnover of products with more than four carboxyl groups occurs. Coproporphyrinogen I and uroporphyrinogen I and their intermediates are not able to be utilized in the heme metabolic pathways and, with their intermediates, are excreted as I isomers.

Coproporphyrinogen III moves back into the mitochondrion from the cytosol, and it is converted into protoporphyrinogen IX by the action of the mitochondrial enzyme coproporphyrinogen oxidase (COPRO oxidase). This enzyme requires oxygen to decarboxylate oxidatively two propionyl groups of coproporphyrinogen.

Protoporphyrinogen IX is converted to protoporphyrin IX by protoporphyrinogen oxidase (PROTO oxidase) in the mitochondrion. The rapid introduction of iron into protoporphyrin IX is catalyzed by ferrochelatase (heme synthetase) which is associated with the inner mitochondrial membrane. Pyridoxal phosphate and copper are needed for full activity. Once iron is introduced, heme is formed.

In the past, genetic defects of porphyrin metabolism have been classified on the basis of symptoms and family studies. A further delineation has been accomplished by the almost complete elucidation of the enzymatic pathway for heme formation. The pathogenesis of the symptomatology and the clinical

$K_m = $ Michaelis constant.
course has become much clearer.\textsuperscript{8,26,31} It is now easier to understand the different excretory patterns of the hereditary porphyrias. Enzymatic defects lead to accumulation of substances in the heme synthetic pathway upstream from the deficiency. Also, new knowledge has given more specific direction to treatment.\textsuperscript{36}

Tests have been developed for measurement of most of the enzymes in the heme cycle.\textsuperscript{7,8} As yet no procedures for measuring PROTO oxidase is available. The test for URO III cosynthetase is cumbersome and not generally performed even in research laboratories.\textsuperscript{23}

Those enzymes located in the mitochondrion can be measured in leukocytes.\textsuperscript{8} These are ALA synthetase (EC 2.3.1.37), COPRO oxidase (EC 1.3.3.3), and ferrochelatase (EC 4.99.1.1). The cytosolic enzymes are measured in the mature red cell and comprise ALA dehydratase (EC 4.2.1.24), URO I synthetase (EC 4.3.1.8), and URO decarboxylase (EC 4.1.1.37).

### Classification

One of the common classifications of the hereditary porphyrias is shown in Table I.\textsuperscript{7,26,31}

The classification is by no means universally accepted. Meyer and Schmid consider E.C.P. as a variant of C.E.P. and P.C.T. as a third subgroup along with the erythropoietic and hepatic sub-groups.\textsuperscript{26} Eales considers C.E.P. and P.C.T. as erythrohepatic porphyrias.\textsuperscript{10}

Before the enzymatic pathways became clearer, the erythropoietic porphyrias were recognized by their characteristics of cutaneous photosensitivity and intense fluorescence evoked most effectively by long wave ultraviolet radiation (around 400 nm) acting on uro, copro, or protoporphyrin which accumulate in excess upstream from the specific block in each defect. In C.E.P., an autosomal recessively inherited condition, the abnormality is an imbalance between ALA synthetase and/or URO I synthetase and URO III cosynthetase with an increase in one or both of the first two or/and a decrease of the third.\textsuperscript{4,10,26,31,35} Watson favors the increase theory while Levin claims an etiologic role for cosynthetase decrease.\textsuperscript{23} The imbalance has been demonstrated in cultured fibroblasts as well as in mature red cells.\textsuperscript{7} There is an accumulation of uroporphyrinogens. Since normally there is great excess of cosynthetase, heme for hemoglobin formation is still synthesized in adequate amounts despite some quantitative decrease. Thus, anemia from lack of hemoglobin formation does not occur.

In E.P.P., an autosomal dominant condition, there is a partial deficiency of ferrochelatase.\textsuperscript{7,10,26} Since this is a

### Table I

<table>
<thead>
<tr>
<th>Classification of Hereditary Porphyrias</th>
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<tr>
<td>Type</td>
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<tr>
<td><strong>ERYTHROPOIETIC PORPHYRIAS</strong></td>
</tr>
<tr>
<td>C.E.P. Cutaneous</td>
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<tr>
<td>E.P.P. Mild cutaneous</td>
</tr>
<tr>
<td>E.C.P. Mild cutaneous</td>
</tr>
<tr>
<td><strong>HEPATIC PORPHYRIAS</strong></td>
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<tr>
<td>A.I.P. GI &amp; neurologic symptoms</td>
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<tr>
<td>V.P. GI &amp; neurologic symptoms</td>
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<tr>
<td>H.C.P. GI &amp; neurologic symptoms</td>
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<tr>
<td>P.C.T. Cutaneous photosensitivity &amp; hepatic abnormalities</td>
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AR = Autosomal recessive
AD = Autosomal dominant
C.E.P. = Congenital erythropoietic porphyria
E.P.P. = Erythropoietic protoporphyria
A.I.P. = Acute intermittent porphyria
V.P. = Variegate porphyria
H.C.P. = Hereditary coproporphyria
P.C.T. = Porphyria cutanea tarda
Refer to figure 1 for additional legends.
mitochondrial enzyme, the defect is found in immature red cells and has also been demonstrated in leukocytes, in cultured fibroblasts, and in hepatocytes.\(^7\) This latter finding is the reason Eales considers the condition an erythrohepatic porphyria. The lack of anemia in this condition is said to be due to secondary regulating mechanisms leading to adequate heme formation.

Little is known of E.C.P., and no extensive enzyme studies have been done on this.\(^26,31\) It is said to be inherited as an autosomal dominant, but only a few cases have been reported.

The hepatic porphyrias are characterized by bouts of abdominal pain and neurologic symptoms of acute onset.\(^26,31,36\) Increased ALA, PBG, and other monopyrroles formed upstream from the enzyme defect accumulate in the central and peripheral nervous systems owing to ALA-synthetase increase. They are concentrated in the hypothalamus and inhibit brain and blood Na/L ATPase affecting transport across cellular membranes and altering neuromuscular functions. Monopyrroles can cause spasm of intestinal tract through their effect on the nervous system.\(^7\) The syndrome of inappropriate ADH secretions (S.I.A.D.H.) is also found often.\(^7\) Characteristically, the hepatic porphyrias are inducible, the term applied by Watson to those cases where ALA synthetase is increased because the heme formed is inadequate to shunt it off (feedback control), and acute neurological symptoms are produced.\(^7,36\) Since heme probably does not exert important feedback control over the red cell line, the erythropoietic porphyrias are not inducible in this sense. Symptoms in hepatic porphyrias are often set off by substances inducing ALA synthetase in the face of deficient heme formation.

A.I.P. is inherited as an autosomal dominant disease and is a manifestation of 50 percent deficiency of URO I synthetase. The defect has been demonstrated in red cells, cultured skin fibroblasts, and amniotic cells as well as in hepatocytes.\(^7,36\)

V.P. is also inherited as an autosomal dominant and is probably due to ferrochelatase deficiency.\(^7,11,36\) The defect is said to occur in erythroid precursors and in leukocytes, but Goldberg et al have been unable to verify this and they feel that the defect may be one of PROTO oxidase.

H.C.P. is inherited as an autosomal dominant also.\(^36\) The deficiency is in COPRO oxidase and can be demonstrated in cultured fibroblasts and leukocytes.\(^7,8\) No significant studies of the hepatocyte content of the enzyme have been conducted.

P.C.T. is not an inducible condition by the Watson definition and the status of ALA synthetase here is unclear.\(^1,2\) The defect is one of uroporphyrinogen decarboxylase. Excess excretion of uroporphyrinogens especially is noted along with large amounts of heptacarboxylic porphyrins. The enzymatic defect has been found in the hepatocytes and in red cells. For this reason and because of cutaneous symptoms, the exact place of this in the overall classification is in dispute. The red cell defect has not been confirmed in all cases and at present there is debate as to the exact status of P.C.T. Some feel the disease is inherited\(^1\) and some say it is acquired.\(^2,14\) Eales and coworkers feel that some cases are inherited as autosomal dominant and others, the vast majority, are acquired.\(^2\) For the defect to be manifested clinically, there must be an added abnormality in the liver such as alcohol associated hepatic disease or iron overload.

Studies of the enzyme pathway of heme formation have given more insight into the genesis of hereditary porphyrias, have pointed ways to greater assurance in diagnosis, and have lead to the development of a better form of treatment. For the inducible porphyrias, namely acute intermittent porphyria, variegate porphyria, and hereditary coproporphyria, acute attacks
of neurological symptoms and abdominal pain which at times may be fatal have been aborted or rapidly terminated by administration of hematin.36 This is based on the premise that heme (and its derivative hematin) acts as a feedback mechanism to shut off δALA synthetase; in practice this has proved to be so. In time, perhaps prevention by enzyme replacement may be developed. Certainly, studies in the last ten years have resulted in better understanding of this complex group of hereditary metabolic diseases.

References


