The Porphyrias: Recent Advances

J. Thomas Hindmarsh

Recent research has elucidated several of the hitherto poorly understood steps in heme synthesis. This review describes this metabolic pathway and pinpoints the enzymatic blockages in the various porphyrias. Recent advances in the understanding of the etiology of porphyria cutanea tarda are discussed, as are the abnormalities of porphyrin metabolism seen in chronic renal failure and in lead poisoning. An outline is given of the clinical and biochemical abnormalities seen in the porphyrias. Included is an algorithm to aid in the differential diagnosis of these diseases, and a brief review of the new analytical techniques available for the identification and quantification of porphyrins and their precursors in body fluids.

Additional Keyphrases: porphyria cutanea tarda · erythropoietic porphyria · lead poisoning · chronic renal failure · metabolic pathways · heme synthesis · neurotoxicity · hepatic iron accumulation · alcoholism · estrogen-induced effects · heritable disorders · harderpomphrya

Heme Synthesis

The principal sites for heme synthesis in the human are the hemapoietic tissues and the liver. The synthetic pathway is a series of irreversible reactions, some of which occur in the cell mitochondria and some in the cytoplasm (Figure 1). Intramitochondrially, the reactions are mainly oxidation–reduction, whereas the extramitochondrial steps are condensation and decarboxylation. The enzyme 5-aminolevulinate (ALA) synthase (EC 2.3.1.37) exhibits much less activity than the other synthetic enzymes and controls flow through the pathway; its activity is inhibited by heme or hemin. After release from the enzyme surface, δ-aminolevulinic acid diffuses into the cytoplasm and, catalyzed by ALA dehydratase (EC 4.2.1.24), two molecules condense to form the mono-pyrrole porphobilinogen. ALA dehydratase is a zinc-dependent metalloenzyme, and zinc partly protects this enzyme against the adverse effects of lead in vitro (1) and possibly also in vivo (2).

The enzymes porphobilinogen (PBG) deaminase (EC 4.3.1.8) and uroporphyrinogen-III synthase (EC 4.2.1.75) act together to polymerize four molecules of porphobilinogen to form the cyclic tetrapyrrole, uroporphyrinogen III. PBG deaminase appears to catalyze the condensation of four PBG molecules in a symmetrical head-to-tail arrangement to form the tetrapyrrole, hydroxymethylbilane (Figure 2).

The enzyme uroporphyrinogen-III synthase then in some way "flips" the D pyrrole to an asymmetrical arrangement and closes the porphyrin ring (3, 4). In the absence of uroporphyrinogen-III synthase, hydroxymethylbilane spontaneously cyclizes to form the I isomer of uroporphyrinogen. Support for this synthetic pattern comes from the observations that uroporphyrinogen-III synthase cannot consume PBG as substrate nor can it convert the I isomer of uroporphyrinogen to the III isomer. Ordinarily, excess uroporphyrinogen-III synthase is present, which greatly favors the formation of the III isomer. Its activity in human and

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**Fig. 1.** The pathway for heme synthesis, including sites of enzyme insufficiency in the porphyrias

1. ALA dehydratase deficiency, tyrosinemia, lead poisoning; 2. acute intermittent porphyria; 3. congenital erythropoietic porphyria; 4. porphyria cutanea tarda, hemolysin and porphyric porphoria, toxic porphyria; 5. hereditary coproporphyria, lead poisoning; 6. hereditary porphoria, lead poisoning; 7. porphyria variegata; 8. protoporphyria, lead poisoning. *= probable step
pig livers reportedly is inhibited in vitro by ferrous compounds (5).

The decarboxylation of the acetate side-chains of uroporphyrinogen III appears to be a sequential process proceeding through 7-, 6-, and 5-carboxyl intermediates; it is catalyzed by the enzyme uroporphyrinogen decarboxylase (EC 4.1.1.37). The acetate side-chains of uroporphyrinogen I are also decarboxylated, ultimately to form coproporphyrinogen I, after which the metabolic pathway of this isomer series proceeds no further. Uroporphyrinogen decarboxylase can react only with uroporphyrinogen as substrate and not the corresponding porphyrin. Ferrous compounds are reported to inhibit the enzyme in vitro (6, 7), although one study has reported the opposite (8).

Coproporphyrinogen oxidase (EC 1.3.3.3) converts coproporphyrinogen III to protoporphyrinogen, in which the propionate side-chains in the A and B pyrrole rings are oxidized and decarboxylated to vinyl groups. This reaction probably proceeds with the preliminary formation of harderporphyrinogen, an intermediate compound with only one vinyl group (on the A pyrrole). Protoporphyrinogen is oxidized to protoporphyrin by protoporphyrinogen oxidase (EC 1.3.3.4). The final step is chelation of protoporphyrin with ferrous iron to form heme, catalyzed by the enzyme ferrochelatase (EC 4.99.1.1). Other divalent metals (cobalt, zinc, copper) also may be chelated with protoporphyrin.

Control of the rate of heme synthesis is achieved largely by a negative-feedback effect of heme on the enzyme ALA synthase. PBG deaminase also has a lower activity than the other enzymes in the pathway and probably also exerts some control. ALA synthase activity appears to be increased as a compensatory effect in most, possibly all, of the porphyrias, and this action is responsible for the excess production of intermediary metabolites proximal to the enzymatic blocks in these diseases. Brodie et al. (9) and Moore (10) have postulated that variability in the activity of PBG deaminase explains the different clinical features of the acute and nonacute porphyrias. Their thesis is that in the acute porphyrias, ALA and PBG accumulate in the tissues, whereas this is not a feature of the nonacute porphyrias; moreover, this accumulation in nervous tissue is probably responsible for the neurotoxic features of the acute disease. Because heme synthesis is potentially suboptimal in all porphyrias, these diseases carry the potential for an accumulation of excess intermediary metabolites proximal to the enzymatic block. However, in the nonacute porphyrias, PBG deaminase activity appears to be increased (at least in erythrocytes), thereby preventing the accumulation of porphyrin precursors (ALA and PBG), whereas in the acute porphyrias the activity of this enzyme is normal (hereditary coproporphyria and porphyria variegata) or subnormal (acute intermittent porphyria), and therefore excess ALA and PBG accumulate. Although this is an attractive hypothesis, it is not certain that heptatic PBG deaminase is similarly increased in the nonacute porphyrias, and further work is needed before the theory can be fully accepted.

The mechanisms whereby the porphyrin precursors (ALA and PBG) produce neurotoxicity have not been fully elucidated, but clinical neuropathy is related to their accumulation. Neuropathy occurs in diseases in which ALA alone accumulates (ALA dehydratase deficiency); ALA or a metabolite may be the responsible agent. There have been problems in producing comparable neurotoxic effects in vivo in experimental animal models (11), but toxicity may be related to the ability of ALA to inhibit competitively the binding of the central nervous system neurotransmitter, γ-aminobutyric acid, to synaptic membranes in brain tissue (12, 13).

Factors known to precipitate acute attacks in porphyric subjects include alcohol, stress, infection, starvation, hormonal changes, and the administration of certain drugs. It is not completely understood how drugs precipitate acute attacks. The mechanisms may be related to the depletion of the mitochondrial free-heme pool by decreased heme synthesis or increased utilization of hemoproteins such as cytochrome P450, resulting in activation of cellular ALA synthase (11).

The Porphyrias

The porphyrias traditionally are classified according to whether the liver or the erythropoietic tissue is the main source of excess porphyrin production (14), and this approach has some merit: it is simple. The classification shown in Table 1 is based on whether patients with the diseases suffer acute attacks, dermatological manifestations, or both. Any classification should include an account of the related enzyme deficiencies.

Congenital Erythropoietic Porphyria

Patients with this rare disease suffer from mutilating photo-induced skin lesions, are excessively hirsute, and often have hemolytic anemia. Erythrocytes—erythrocytes exhibiting fluorescence when activated with the appropriate light energy—are present in the blood, and the teeth and bone marrow may also exhibit fluorescence. Studies of erythrocytes and skin fibroblasts have shown the activity of uroporphyrinogen-III synthase to be markedly decreased in
this condition, resulting in overproduction of the I isomers of uroporphyrinogen and coproporphyrinogen. Excessive uroporphyrinogen and coproporphyrinogen are found in the urine.

Pimstone and Kushner (15–17) described an unusual case of congenital erythropoietic porphyria in which erythrocyte uroporphyrinogen-III synthase activity was within normal limits but uroporphyrinogen decarboxylase activity was subnormal by approximately 50%. Urinary porphyrins were mainly the III isomers, and fecal isocoproporphyrin was greatly increased. Seventy percent of bone marrow normoblasts showed nuclear porphyrin fluorescence; liver porphyrins were not increased. These authors believe that two inherited abnormalities coexist in this patient: erythropoietic uroporphyrinogen decarboxylase deficiency and a dys-erythropoietic anemia.

Porphyria Cutanea Tarda (PCT)

This disease is associated with reduced liver uroporphyrinogen decarboxylase activity. There appear to be two types: a sporadic, and a rare inherited form (familial PCT). In the latter, erythrocyte uroporphyrinogen decarboxylase activity is also reduced (18, 19). The enzymatic defect in familial PCT is inherited as an autosomal dominant trait (20). One study of familial PCT has reported that the amount of immunologically reactive enzyme in erythrocytes is decreased along with the enzymatic activity (21).

Sporadic PCT is the commonest porphyria in North America. Its onset is often precipitated by ethanol overindulgence or, more rarely, by therapy with estrogen or use of the contraceptive pill. However, only a small proportion of persons exposed to these substances is affected, indicating that other etiologic factors are also operating.

The role of iron in the causation of PCT is confusing and controversial. Most patients with active PCT have increased hepatic iron stores; hepatic siderosis was present in 80% of the patients in the study of Grossman et al. (22). The increase in hepatic iron varies from a modest twofold increase to concentrations similar to those seen in idiopathic hemochromatosis and appears in many cases to be the consequence of increased intestinal absorption (23). The iron is especially prominent in the hepatic parenchymal cells and the portal tract macrophages, less so in the Kupffer's cells (23). Decreasing hepatic iron stores by repeated phlebotomy produces clinical and biochemical remission of the disease—amelioration of the symptoms and signs and a decrease in urinary uroporphyrin excretion (24). Also, Sweeney et al. (25) have shown that mice rendered iron-deficient by repeated bleedings are protected against the porphyrinogenic effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin. However, iron plays a "permissive" rather than simply a causal role in PCT, because even though alcoholics commonly have increased hepatic iron stores (26), the etiology of which is unclear, this finding is more common than PCT is in this group. PCT is also uncommon in patients with idiopathic hemochromatosis. Another finding irreconcilable with a simple causal role for iron is that, although phlebotomy produces clinical improvement in patients with PCT, it does not increase the low activity of hepatic uroporphyrinogen decarboxylase in these patients (27). Hepatic siderosis does not appear to be limited to the ethanol-induced variety of PCT, because it was reported in at least one patient with estrogen-associated PCT (28). Thus iron overload cannot be attributed solely to alcoholism. How often hepatic iron stores are completely normal in active PCT is not clear, but it probably is uncommon. Hepatic iron accumulation was not seen in the study of Elder et al. (29) of rats rendered porphyric by hexachlorobenzene, so increased iron loads are not essential for porphyria development, at least in their model. The protective effect of phlebotomy in a similar mouse model has already been mentioned (25).

The etiology of PCT has recently been reviewed by Pimstone (30) and Sweeney (31); clearly, it is multifactorial. The key problem is failure to decarboxylate uroporphyrinogen adequately. Uroporphyrinogen decarboxylase activities are decreased by about 50% in livers of patients with sporadic PCT and in the livers and erythrocytes in familial PCT.

In familial PCT there is a clear pattern of autosomal dominant inheritance of the enzymatic defect, and Sassa et al. (32) have shown that the immunoreactive enzyme content of erythrocytes, at least, is similarly decreased. This
disease, then, is associated with an inherited deficiency of uroporphyrinogen decarboxylase activity and synthesis. However, the disease is not clinically expressed in all family members who inherit the enzyme deficiency, so other factors such as increased hepatic iron stores and alcoholism must contribute to the impairment of uroporphyrinogen decarboxylation.

The etiology of sporadic PCT is more complex. Diminished hepatic uroporphyrinogen decarboxylase activity does not recover, even though the clinical state improves when iron stores are reduced by phlebotomy, so these patients also seem to have an irremedial enzyme defect. This may also be inherited, but the pattern of inheritance is not clear. As was the case in familial PCT, the enzyme deficiency in sporadic PCT does not produce clinical disease unless the patients also have hepatic siderosis and (or) have some other disease-precipitating factor such as alcoholism or take estrogens.

An interesting hypothesis, supported by HLA marker studies, has been advanced by Kushner et al. (33). They propose that, in sporadic PCT, a defect in uroporphyrinogen decarboxylase is inherited as a recessive trait. To develop the disease, the patient must be homozygous for the enzyme defect and also inherit one allele, perhaps on a different chromosome—i.e., be heterozygous—for the autosomal recessive disease, idiopathic hemochromatosis. Hepatic siderosis, perhaps the consequence of the recessive state for hemochromatosis, has a further inhibitory effect upon the already compromising enzyme (33, 34). It is not clear whether alcohol and estrogens aggravate the enzyme insufficiency by further inhibiting the enzyme directly or by promoting further hepatic iron deposition. All these factors combine to produce clinical disease from a relatively innocuous enzyme deficiency. The complex inheritance pattern explains why sporadic PCT does not appear in several members of a family; the autosomal dominant inheritance of the enzyme deficiency in familial PCT explains why it does.

The complex interactions of the etiological factors in PCT have also recently been reviewed by Mukerji et al. (34). They suggest that iron may further compromise uroporphyrinogen decarboxylation, in two ways. Firstly, ferrous iron directly inhibits uroporphyrinogen decarboxylase by combining with sulfhydryl groups on the enzyme. Secondly, in the presence of oxygen, binding of ferrous iron to cellular sulfhydryl groups promotes the generation of superoxide radical anions, which might further inhibit enzyme activity and (or) oxidize porphyrinogens to porphyrins that cannot be acted upon by the enzyme.

The symptomatology of PCT is very variable but the patient usually shows some cutaneous blistering and fragility in the light-exposed areas, along with some hirsutism. Diagnosis is usually made from a combination of a classical clinical picture and increased urinary porphyrins, with uroporphyrin exceeding coproporphyrin. It is sometimes difficult to distinguish patients with PCT from those with porphyria variegata, as there may be no clear history of inheritance in the latter disease. In porphyria variegata the urinary excretion of coproporphyrin usually exceeds that of uroporphyrin, but in the phase of recovery from an acute attack, urine uroporphyrin may exceed coproporphyrin, perhaps because of nonenzymatic conversion of porphyrin precursors to porphyrin. If there is no clear history of ethanol abuse, I usually use thin-layer chromatography to look for the presence of grossly increased amounts of isocopro-
in erythrocytes. Interpretation of the biochemical indicators of porphyrin metabolism is difficult in renal failure, when urinary excretion of porphyrins may be impaired and porphyrin concentrations in plasma correspondingly increased. Day et al. (39, 40) have recently reviewed porphyrin metabolism in chronic renal disease. Their studies show that uroporphyrin concentrations in plasma and urine are commonly supranormal in chronic renal failure, whereas values for coproporphyrin in urine and plasma are often subnormal. Plasma uroporphyrin concentrations in two of their three patients with renal failure and bullous skin lesions were similar to those reported for patients with PCT from other causes. However, the plasma uroporphyrin concentration in the third patient with skin lesions, although increased, was similar to those seen in some dialysis subjects without skin lesions. Poh-Fitzpatrick et al. (41) have also reported overlap of plasma uroporphyrin concentrations in patients with chronic renal failure, with or without skin lesions.

Day and Eales (39) reported that the increased uroporphyrin in chronic renal disease was the III isomer, in contrast to sporadic and familial PCT, in which the I isomer is usually found in excess. Uroporphyrinogen did not pass through the dialysis membrane, probably because of its binding to a plasma protein. Another surprising finding in their study (39) was the markedly decreased coproporphyrin concentrations in urine and plasma, which, associated with decreased fecal porphyrins, led them to conclude that the kidney might be a major source of coproporphyrin. In support of this thesis, they reported a patient with porphyria variegate who underwent renal transplantation for renal failure (40). The patient showed no accumulation of plasma coproporphyrin before transplantation, despite cessation of urinary coproporphyrin excretion; marked coproporphyrinuria occurred after transplantation. Why a kidney from a presumably nonporphyrin donor would produce excess coproporphyrin when transplanted into a porphyrin patient remains to be explained, as do many of the other features of this interesting hypothesis. Etiological factors contributing to the increased prevalence of PCT in chronic renal disease might include the iron overload sometimes seen in these patients as a consequence of therapy with iron and repeated blood transfusions (42). Aluminum is known to inhibit some enzymes in the heme synthetic pathway and, because it appears to be retained in some patients with chronic renal failure receiving aluminum hydroxide therapy, it may play some part as an etiological factor (43).

Toxic Porphyrinopathy

This disease resembles PCT. Hepatic uroporphyrinogen decarboxylase concentrations decrease in response to exposure to a toxin such as hexachlorobenzene or 2,3,7,8-tetrachlorodibenzo-p-dioxin. However, the prevalence of porphyrin in persons exposed to these toxins is much higher than in ethanol- and estrogen-associated PCT, and the mechanism presumably is a more direct effect of the toxin on the enzyme. Elder and Sheppard (44) have shown, in a rat-liver model, that the immunoactive uroporphyrinogen decarboxylase content remained unchanged, even though catalytic activity was inhibited by prior administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin and hexachlorobenzene.

Hepatoerythropoietic Porphyrinopathy

Hepatoerythropoietic porphyria clinically resembles congenital erythropoietic porphyria, presenting in childhood with severe photo-induced skin damage with inflammation and scarring associated with increased zinc protoporphyrin in erythrocytes and acetate-substituted porphyrins in the plasma, urine, and feces. The principal porphyrin in feces is isocoproporphyrin (45–47). Erythrocyte uroporphyrinogen decarboxylase concentrations are less than 10% of normal, and it is thought that this disease is a homozgous form of familial PCT. Some authors (21) have reported that immunological measurements of erythrocyte uroporphyrinogen decarboxylase are correspondingly decreased, whereas others (32) have reported them to be within normal limits. This could be due to genetic heterogeneity. The mechanism of how zinc protoporphyrin accumulates in erythrocytes is complex and has been discussed by Elder et al. (45) and Lim and Poh-Fitzpatrick (47).

Harderoporphyria

Few cases of this interesting disease have been reported. It presents with jaundice and hemolytic anemia at birth (48, 49), followed in one case by the development of mild photosensitivity at age 11 years. Large amounts of coproporphyrin are excreted in the urine and feces, but the pattern of fecal porphyrin is atypical, the major fraction being harderoporphyrin (>60%; normally <20%). Coproporphyrinogen oxidase activity in the lymphocytes of the patients was 10% of control values, suggesting a homozygous state. The enzyme exhibited about 50% of normal activity in both parents. In harderoporphyria a vinyl group replaces the propionate group in the A pyrrole ring only. In the normal subject, the propionate groups in the A and B pyroles probably are converted to vinyl groups in a stepwise manner; oxidation of the group on the A ring preceding that on the B ring; apparently this process is incomplete in harderoporphyria.

Protoporphyria

This disease usually presents in childhood with light-induced erythema or urticaria, which is often mild. These patients have an increased prevalence of gallstones. Recently it has been shown that, in long-standing cases, there are abundant porphyrin deposits in the liver, and liver damage and failure may occur (50). The excess erythrocyte protoporphyrin found in this disease is a free unchelated form rather than zinc protoporphyrin. The enzymatic defect appears to be a deficiency of ferrochelatase (51). Diagnosis rests on the combination of a typical clinical picture and the demonstration of increased erythrocyte protoporphyrin concentrations. Urinary porphyrin excretion is within normal limits unless hepatic cirrhosis impedes biliary porphyrin excretion sufficiently to increase urinary porphyrin. Two cases of a variant of protoporphyria described by Heilmeyer (52) were clinically similar to protoporphyria except that the erythrocyte porphyrin was mostly coproporphyrin. No further cases have been reported.

Erythrocyte zinc protoporphyrin is increased in lead poisoning and some anemias, including those due to iron deficiency, excessive blood loss, and chronic disease. When insufficient iron is available, or it cannot be readily chelated to protoporphyrin, small amounts of zinc protoporphyrin are formed and circulate as nonfunctioning hemoglobin (53).

Acute Intermittent Porphyria

Acute attacks of abdominal pain and neuropsychiatric manifestations are the main clinical features of acute intermittent porphyria; dermatological lesions do not occur.
Common features of the acute attacks are: acute abdominal pain (in 95%); vomiting (60%); constipation (60%); neuropathy (60%); tachycardia (60%); hypertension (40%); and mental changes (50%) (54). About 5% of attacks that are severe enough to require hospitalization end fatally, and repeated attacks in the same patient are common, so the mortality rate is around 15 to 20% (14). Residual paralysis, hypertension, and renal failure are long-term sequela. Acute attacks are more common in females, more frequently during the second and third decades and are rare before puberty. Attacks are commonly precipitated by drugs, particularly barbiturates and sulfonamides, but many other drugs are hazardous (11, 55). Because of the wide variety of drugs that are potentially hazardous to these patients, it is more advisable to provide the patient with a list of drugs known to be safe rather than a list of the hazardous compounds. Endocrine factors influence the course of the disease, and premenstrual attacks are common. Alcohol, infection, and fasting are other important precipitants. In an acute attack the diagnosis is made by the association of a typical clinical picture together with increased PBG and ALA in the urine. Diagnosis between attacks is more difficult because occasionally the amount of PBG in the urine is normal (56). If this is so and the clinical story is convincing, I usually proceed to measure erythrocyte PBG deaminase.

The disease is associated with a partial deficiency of the enzyme PBG deaminase in several tissues. The enzyme deficiency is inherited as an autosomal dominant trait, but only about 10% of the patients who inherit the enzyme deficiency suffer attacks of the disease (54), so other etiological factors are involved. It is important to identify potential or latent cases in relatives of patients with the disease, by measuring PBG deaminase in erythrocytes. The amount of enzyme present in circulating erythrocytes is probably related to the age and differentiation of the cells, and therefore measurements are unreliable in patients with a high reticulocyte count for any reason and in newborns (57). There is overlap between the normal and abnormal reference intervals, which limits the usefulness of the test (58).

Anderson et al. (59) have proposed that clinical expression of the disease is related to impairment of 5a-reduction of steroid hormones by the liver. Glucose (60) and hematin (61) are partially effective in treating the acute disease, probably by repression of ALA synthetase.

**ALA Dehydratase Deficiency**

This can occur as a primary inherited disorder in which the enzyme concentration in erythrocytes is less than 3% of normal; it presents as an acute porphyria (62). Bird et al. (63) have described a family with 22% to 41% of the normal concentrations of this enzyme in their erythrocytes; they were asymptomatic. Inheritance of this latter defect was autosomal dominant and may represent the heterozygous form of the disease described by Does et al. (62). ALA dehydratase (PBG synthase) deficiency may also be a consequence of lead poisoning. This deficiency may also be seen in tyrosinosis, where the abnormal metabolite succinyl acetone, present in excess, competes with ALA (a structural analog) for attachment to the active site of the enzyme, and patients suffer from acute porphyric symptoms (64).

**Porphyria Variegata**

The clinical features of porphyria variegata are variable and include acute attacks and light-induced cutaneous lesions. The disease does not usually present before puberty. The site of the enzyme defect has been argued, but Brenner and Bloomer (65) have demonstrated that protoporphyrinogen oxidase activity in fibroblasts is 50% of normal in patients with the disease. Inheritance of the enzyme defect does not necessarily mean that the patient will develop the disease; steroid 5a-reduction is commonly impaired in those in whom the disease has appeared (54). Urinary excretion of porphyrin precursors and porphyrins commonly increases during an acute attack but is usually normal between attacks. Fecal protoporphyrin and, to a lesser extent, coproporphyrin are invariably increased in patients who have developed the disease, even asymptomatic ones. A homozygous variety of protoporphyrin oxidase deficiency may exist (66).

McColl et al. (67) have described a family of acute porphyrins in which some family members have porphyrin excretion patterns typical of acute intermittent porphyria, some had patterns typical of porphyria variegata, and still others had an intermediate pattern. Only abdominal and neurological features were present in affected patients; none experienced photo-induced skin lesions. All had subnormal activities of PBG deaminase in erythrocytes and of protoporphyrinogen oxidase in leukocytes.

**Hereditary Coproporphoria**

Clinical features of this disease include acute neurovisceral attacks and photo-induced cutaneous lesions. Symptomatic disease does not usually appear before puberty and is commonly the consequence of drug therapy. Porphyrin precursors are commonly increased in the urine during an acute neurovisceral attack, as is fecal coproporphyrin. Concentrations of all can be normal between attacks (68). The biochemical defect appears to be a partial deficiency of coproporphyrinogen oxidase activity (50% of normal) in the tissues (69). Homozygous coproporphoria has been reported (70).

**The Effect of Lead on Heme Synthesis**

Although there has been much argument about the effects of low concentrations of lead on mental development, overt symptoms of lead poisoning do not usually appear until the concentration of lead in the blood reaches 600–800 μg/L (2.90–3.85 μmol/L). Lead appears to inhibit several steps in the heme synthetic pathway and ALA, coproporphyrin, and zinc protoporphyrin are produced in excess in the frankly lead-poisoned patient. Inhibition of ALA dehydratase activity can be demonstrated at blood lead concentrations as low as 100–150 μg/L (0.50–0.70 μmol/L) (71). However, urinary ALA and coproporphyrin excretion do not increase until the lead concentration in blood reaches 400 μg/L (1.90 μmol/L). Piomelli et al. (72) have recently reported that erythrocyte zinc protoporphyrin increases when the blood lead concentration reaches 150–180 μg/L (0.70–0.85 μmol/L). The laboratory evaluation of lead toxicity has recently been reviewed by Fell (73).

**Procedure in Investigation of a Suspected Case of Porphyria**

This has recently been reviewed by With (74), Moore (75), and Hindmarsh (76). My approach is outlined in Table 2. The investigator should determine if the patient has had any symptoms of acute porphyria, a history of skin lesions, and if there is a family history of the disease. If the patient is
suspected of currently having an acute attack of porphyria (severe acute abdominal pain, or neuropsychiatric manifestations, or both), the investigation is easy, because results of the Watson–Schwartz urine test will be positive for PBG. Many drugs, including chlorpromazine, produce false-positive reactions to this test, however, and I usually proceed directly to quantification of PBG and ALA in urine by column chromatography. If values for these are normal, the patient cannot be having an acute attack of porphyria.

If the patient is between acute attacks, however, it may not be possible to establish a diagnosis of “acute porphyria between attacks.” Values for PBG and ALA in urine are commonly normal between attacks in porphyria variegata and hereditary coproporphyria and occasionally are normal in acute intermittent porphyria. In porphyria variegata, fecal protoporphyrin excretion will be above normal, even between attacks. Hereditary coproporphyria is very rare; values for fecal coproporphyrin are usually increased between attacks, but may be normal. In acute intermittent porphyria, values for erythrocyte PBG deaminase will be decreased.

If the patient has a history of skin lesions, the investigator should determine whether these were the urticarial and (or) erythematous lesions of protoporphyria or the bulae and scars of congenital erythropoietic porphyria, porphyria cutanea tarda, porphyria variegata, or hereditary coproporphyria. A diagnosis of protoporphyria is made from the association of a typical clinical picture with increased protoporphyrin concentrations in the erythrocytes. PCT can usually be diagnosed on the basis of a clinical history of alcohol overindulgence, use of the contraceptive pill, and increased urinary porphyrins, with uroporphyrin exceeding coproporphyrin. If there is no history of precipitating factors, it may be necessary to identify excessive isocoproporphyrin excretion in feces by thin-layer chromatography. Porphyrin variegata and hereditary coproporphyria are differentiated by demonstrating increased concentrations of protoporphyrin or coproporphyrin (respectively) in feces. Congenital erythropoietic porphyria is very rare and is diagnosed by its typical clinical presentation and biochemical features.

### Table 2. Investigation of Suspected Porphyrin

<table>
<thead>
<tr>
<th>Presentation</th>
<th>Test</th>
<th>Result</th>
<th>Further action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain (with or without skin lesions)</td>
<td>Screen for increased urinary PBG</td>
<td>Negative</td>
<td>Excludes acute porphyria as cause of abdominal pain. However, if you suspect patient is between attacks, quantify urinary PBG and ALA. If increased, proceed as for positive screen; if normal, measure fecal porphyrins to exclude VP and HC. If you still suspect AIP, measure erythrocyte PBG deaminase.</td>
</tr>
<tr>
<td>Sun-induced urticaria or erythema</td>
<td>Measure erythrocyte protoporphyrin concn</td>
<td>Normal</td>
<td>Positive</td>
</tr>
<tr>
<td>Skin lesions: erosions ± bullae, hirsutism, pigmentation</td>
<td>Measure urinary porphyrins</td>
<td>Increased</td>
<td>Excludes protoporphyria: if strong clinical suspicion, exclude other cutaneous porphyrias by testing urine and feces.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Protoporphyria confirmed by excluding zinc protoporphyrin.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Determine porphyrin profile of urine and feces to differentiate PCT, VP, and HC. Measure erythrocyte porphyrin if CEP suspected.</td>
</tr>
</tbody>
</table>

AIP, acute intermittent porphyria; VP, porphyria variegata; HC, hereditary coroporphyria; CEP, congenital erythropoietic porphyria; PCT, porphyria cutanea tarda.

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### Analytical Methods for Porphyrins and Porphyrin Precursors

Methods of analysis for porphyrins and porphyrin precursors have recently been reviewed by Moore (75) and Bissel (77). Normal ranges are method-dependent. Although solvent-extraction methods can be used for the investigation of most cases of porphyria, they lack specificity, and “high-pressure” liquid-partition chromatography of porphyrins is preferable for those laboratories with this facility; ion-exchange chromatography remains the method of choice for PBG and ALA.

"High-pressure" liquid-chromatographic methods for porphyrin analysis have evolved in several steps. Early workers used ultraviolet–visible or fluorometric detectors with isocratic or gradient elution of methyl esters on a normal-phase silica column (78). This gives acceptable results, but esterification may be incomplete and possibly may alter the porphyrin pattern (79). The use of a reversed-phase column obviates the need to form esters, and gradient elution gives good and fast separations (80). Riboflavin interference is a problem if an ultraviolet–visible detector is used; however, a fluorescence detector gives more accurate separations, with good sensitivity.

Porphyrin metabolism has fascinated chemists since the turn of the century and new porphyrias continue to be described. An interesting new development is the use of porphyrins for the localization and photodestruction of tumors (81).

I am grateful to Dr. S. L. Perkins, Dr. A. Soriskey, Dr. T. G. Jones, Professor S. French, and Mrs. N. Meagher for advice and help during the preparation of this review, and to Miss M. Linton for typing the manuscript.

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