

Environmental Chemical Exposures and Disturbances of Heme Synthesis

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Porphyrias are relatively uncommon inherited or acquired disorders in which clinical manifestations are attributable to a disturbance of heme synthesis (porphyrin metabolism), usually in association with endogenous or exogenous stressors. Porphyrias are characterized by elevations of heme precursors in blood, urine, and/or stool. A number of chemicals, particularly metals and halogenated hydrocarbons, induce disturbances of heme synthesis in experimental animals. Certain chemicals have also been linked to porphyria or porphyrinuria in humans, generally involving chronic industrial exposures or environmental exposures much higher than those usually encountered. A noteworthy example is the Turkish epidemic of porphyria cutanea tarda produced by accidental ingestion of wheat treated with the fungicide hexachlorobenzene. Measurements of excreted heme precursors have the potential to serve as biological markers for harmful but preclinical effects of certain chemical exposures; this potential warrants further research and applied field studies. It has been hypothesized that several otherwise unexplained chemical-associated illnesses, such as multiple chemical sensitivity syndrome, may represent mild chronic cases of porphyria or other acquired abnormalities in heme synthesis. This review concludes that, although it is reasonable to consider such hypotheses, there is currently no convincing evidence that these illnesses are mediated by a disturbance of heme synthesis; it is premature or unfounded to base clinical management on such explanations unless laboratory data are diagnostic for porphyria. This review discusses the limitations of laboratory measures of heme synthesis, and diagnostic guidelines are provided to assist in evaluating the symptomatic individual suspected of having a porphyria. — *Environ Health Perspect* 105(Suppl 1):37–53 (1997)

Key words: biological markers, diagnosis, environmental exposure, hexachlorobenzene, laboratory, lead, metals, multiple chemical sensitivity, occupational exposure, porphyria, porphyrins

Manuscript received 8 July 1996; manuscript accepted 29 August 1996.

The authors wish to extend their sincere gratitude to Ellen K. Silbergeld, University of Maryland, for her thoughtful, critical review of this article during its preparation. Statements made in this article do not necessarily represent Dr. Silbergeld's opinions. This article was adapted from the *Working Document: Evaluation of Individuals with Environmental Chemical Exposures and Suspected Abnormalities of Heme Synthesis* (11 February 1996), which was prepared through the Office of the Medical Director at the Washington State Department of Labor and Industries, Olympia, Washington. A statement of potential conflicts of interest was included with this article at the time it was submitted for consideration by this journal.

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Abbreviations used: ADP, ALA-D deficiency porphyria; AIP, acute intermittent porphyria; ALA, δ -aminolevulinic acid; ALA-D, ALA dehydratase; CEP, congenital erythropoietic porphyria; CHP, chronic hepatic porphyria; Copro-O, coproporphyrinogen oxidase; 2,4-D, 2,4-dichlorophenoxyacetic acid; EPP, erythropoietic protoporphyria; FEP, free erythrocyte protoporphyrin; HCB, hexachlorobenzene; HCP, hereditary coproporphyria; HEP, hepatoerythropoietic porphyria; MCS, multiple chemical sensitivity (syndrome); PBB(s), polybrominated biphenyl(s); PBG, porphobilinogen; PCB(s), polychlorinated biphenyl(s); PCT, porphyria cutanea tarda; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; TCDD(s), tetrachlorodibenzodioxin(s); Uro-D, uroporphyrinogen decarboxylase; VP, variegate porphyria; ZPP, zinc protoporphyrin.

Introduction

Porphyrias are inherited or acquired metabolic disorders in which the clinical manifestations are attributable to characteristic patterns of overproduction of specific heme precursors and their accumulation in certain tissues as a consequence of decreased activity of a specific enzyme(s) in the heme synthesis pathway, usually in association with stimulation of the initial stage of the heme-forming system by endogenous or exogenous stressors. When clinically active, and in some cases even when latent or in clinical remission, porphyrias induce high levels of heme precursors in blood, urine, and/or stool.

Porphyrias are relatively uncommon conditions but are probably underrecognized. The prevalence of genetic predisposition to porphyria probably has been underestimated in the general population because of the limited availability of clinical tests for specific heme-synthesis enzymes and because prevalence studies generally have focused more on family members of affected individuals and less on the general population. The symptomatic manifestations of porphyria, particularly the noncutaneous manifestations, are often nonspecific and may not be accompanied by supporting physical signs. Most clinicians encounter and recognize few if any cases of porphyria in the course of their careers, and in general their levels of suspicion for these conditions are low; yet diagnosis of porphyria is critically dependent on the clinician first suspecting it as a possible cause of a patient's symptoms and then ordering the specific essential diagnostic tests.

Some of the inherited porphyrias occur commonly as toxicogenetic conditions, where the genetically acquired trait is clinically latent until clinical manifestations of porphyria are triggered idiosyncratically by exposure to certain therapeutic drugs, chemicals, or alcohol. Porphyria also can occur as an acquired toxin-induced condition, where biochemical and clinical manifestations of the porphyria are actually caused by exposure to certain chemicals in individuals with no evident genetic predisposition. In addition, certain chemicals can produce disturbances of heme synthesis that are associated with alterations of subcellular structure and functions and characteristic changes in patterns of heme-precursor excretion. These measurable changes offer potential biological markers for detecting harmful effects of specific

chemical exposures while their biological effects are still preclinical and potentially reversible. Most clinicians, however, including specialists in occupational and environmental medicine, are relatively uninformed about these phenomena.

It has recently been proposed that a variety of chemical-associated illnesses for which there are no widely accepted specific diagnostic tests or etiologic explanations—such as multiple chemical sensitivity (MCS) syndrome, Persian Gulf War illnesses, conditions associated with silicone breast implants, and various fatigue syndromes—may represent either mild chronic cases of porphyria, or, at least in part, manifestations of acquired abnormalities in heme synthesis. Although diagnoses of porphyria and porphyrialike conditions in such cases are commonly dismissed by other clinicians and researchers, these diagnoses are increasingly put forth as objective justification for recommending major lifestyle modifications and therapeutic interventions in individual cases.

This article reviews pertinent medical and scientific literature in order to provide a foundation to address current issues related to environmental chemical exposures and possible disturbances of heme synthesis. The first three sections of this review provide necessary background information about heme synthesis and the classification and biochemistry of porphyria; more detailed information is available in the cited general references (1–10).

Heme Synthesis

Heme is a biological compound that, when combined with certain proteins, plays a central role in a variety of vital physiologic functions, including oxygen binding and transport (hemoglobin and myoglobin), respiratory electron transport (cytochromes a, a₃, b₁, c, and c₁), activation and decomposition of hydrogen peroxide (catalase and peroxidase), and other oxidation-reduction functions (cytochromes P450 and b₅). Heme synthesis, which is often also referred to as porphyrin metabolism, occurs in all human cells. It is especially productive in the erythropoietic cells in bone marrow, where nearly all of the heme is used for hemoglobin, and in the liver, where the heme is primarily needed in cytochrome P450.

Heme, or iron-protoporphyrin IX, is produced by a metabolic pathway that involves eight enzyme-controlled steps (see below and Figure 1).

- Succinyl CoA and glycine are combined to form δ-aminolevulinic acid

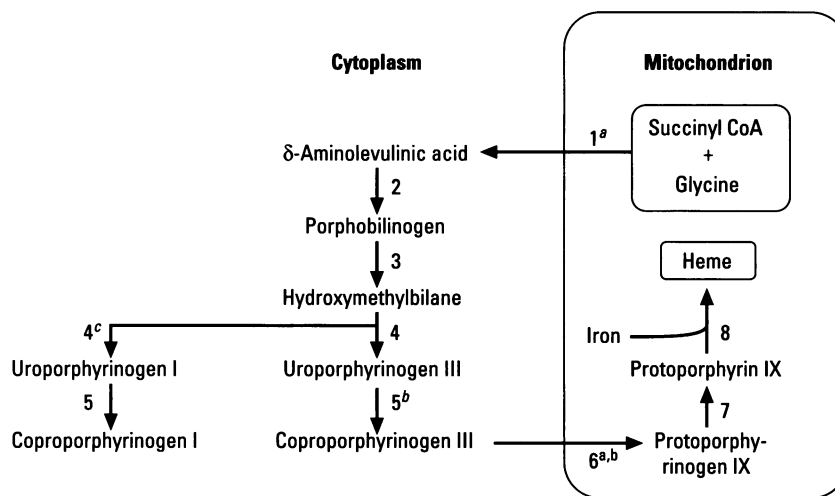


Figure 1. Heme Biosynthesis Pathway. Numbers designate individual enzyme-catalyzed steps of heme biosynthesis. Enzyme name, step 1, ALA synthase; step 2, ALA dehydratase; step 3, PBG deaminase (uroporphyrinogen synthase); step 4, uroporphyrinogen-III cosynthase; step 5, uroporphyrinogen decarboxylase; step 6, coproporphyrinogen oxidase; step 7, protoporphyrinogen oxidase; step 8, ferrochelatase. ^aALA is synthesized within mitochondria in step 1 and then traverses to cytoplasm, where step 2 occurs. Coproporphyrinogen III is synthesized in cytoplasm in step 5 and then traverses into mitochondria, where step 6 occurs. ^aIntermediate precursors not shown for step 5 (7-, 6-, and 5-carboxyl porphyrinogens) or step 6 (3-carboxyl porphyrinogen). ^bAlternative pathway at step 4 (hydroxymethylbilane to uroporphyrinogen I) is not enzymatically catalyzed.

(ALA), an amino acid committed exclusively to heme synthesis.

- Two ALA molecules are condensed to form porphobilinogen (PBG), a monopyrrole.
- Four PBG molecules are polymerized to form the linear tetrapyrrole hydroxymethylbilane.
- Hydroxymethylbilane is converted enzymatically to the cyclic tetrapyrrole uroporphyrinogen III (or nonenzymatically to an isomer, uroporphyrinogen I, which does not act as an intermediate in heme synthesis).
- Sequential decarboxylations produce a series of 7-, 6-, and 5-carboxyl porphyrinogens and then coproporphyrinogen III.
- Further sequential decarboxylations produce 3-carboxyl porphyrinogen and then protoporphyrinogen IX.
- Protoporphyrinogen IX undergoes oxidation to protoporphyrin IX.
- Protoporphyrin IX is chelated with ferrous iron to produce heme.

Heme and protoporphyrin IX are the only directly formed intermediates in the heme synthesis pathway that are actual porphyrins rather than porphyrinogens. However, porphyrinogens are readily oxidized to the respective porphyrin forms, particularly when removed from the body. Most laboratory assays measure and report

porphyrinogens in porphyrin form. For the convenience of a summary term, we use the term heme precursors loosely to include measured porphyrin forms of porphyrinogens as well as the true intermediates of heme synthesis.

Classification of Porphyrias

Most types of porphyria are known to occur only as inherited conditions; however, one type of porphyria [porphyria cutanea tarda (PCT)] is known to occur in either an acquired or an inherited manner. The inherited porphyrias are attributable to an autosomal dominant or autosomal recessive genetic defect affecting a single enzyme in the heme synthesis pathway but rarely are attributable to coexistent defects separately affecting two different heme-synthesis enzymes.

Porphyrias historically have been classified in various ways (see Table 1), including: specific enzyme(s) principally involved; nature of underlying inherited defect, if any (autosomal dominant, autosomal recessive, acquired); principal origin(s) of the excess heme precursors (hepatic, erythropoietic, mixed); usual temporal pattern of symptomatic manifestations (acute or chronic); and principal nature of symptomatic manifestations (neurologic, neurocutaneous, cutaneous). These classifications overlap and are not mutually exclusive.

Table 1. Classification of porphyrias and lead intoxication.

	Incidence pattern	Enzyme deficiency step no. ^a	Principal origin of excess heme precursors		Symptom pattern		Symptoms	
			Erythropoietic	Hepatic	Acute	Chronic	Neurologic	Cutaneous
Porphyrias								
ADP	AR	2	Unknown	Unknown	+		+	
AIP	AD	3		+	+		+	
CEP	AR	4	+			+		+
PCT	AD or Acquired	5		+		+		+
HEP	AR	5	+	±		+		+
HCP	AD	6		+	+		+	±
VP	AD	7		+	±	±	±	±
EPP	AD	8	+	±	±	+		+
Lead intoxication	Acquired	2,6,8 ^b	+	+	+	+	+	

Abbreviations: AD, autosomal dominant inheritance; AR, autosomal recessive inheritance; +, usually present; ±, variably present. ^aHeme synthesis enzyme designated by step number (see Figure 1). ^bThere is apparent interference by lead with these steps. In cases of lead intoxication, there may be mechanisms other than lead inhibition of the enzymes at steps 6 and 8 [Rossi (17)].

Porphyrias with Neurologic Manifestations

Symptoms in the neurologic porphyrias [ALA dehydratase (ALA-D) deficiency porphyria (ADP) and acute intermittent porphyria (AIP)] and noncutaneous symptoms in the neurocutaneous porphyrias [hereditary coproporphyrinuria (HCP) and variegate porphyria (VP)] tend to occur in intermittent acute attacks, with variable degrees of symptom presence between attacks. Clinical onset of the neurologic and neurocutaneous porphyrias usually occurs in postpubertal adolescents or adults; attacks are more common in women than men. Only six cases of ADP have been reported (7). AIP is one of the more prevalent types of clinically manifested porphyria, at least in the United States.

All the neurologic and neurocutaneous porphyrias (except ADP) are classified as hepatic porphyrias because the liver is a principal site in which the enzyme deficiency manifests itself biochemically. Liver function abnormalities are common but are generally mild or moderate (12). Several studies have indicated that individuals with AIP are at risk for developing hepatocellular carcinoma (13–15). The principal site of enzyme expression has not been identified clearly for the small number of patients reported to have ADP.

The neurologic manifestations of acute attacks are indistinguishable for the various neurologic and neurocutaneous porphyrias, and these conditions are differentiated by biochemical or genetic testing. The principal manifestations reflect broad dysfunction of the nervous system. Frequent symptoms include: abdominal pain, constipation, nausea, vomiting, tachycardia, hypertension, fever (i.e., autonomic nervous system);

weakness, back and extremity pains, localized or extensive pareses or paralysis, paresthesias, hypoesthesias (i.e., peripheral nervous system); and psychiatric or behavioral symptoms (i.e., central nervous system). Hyponatremia may reflect inappropriate secretion of antidiuretic hormone (i.e., hypothalamus). The intensity of symptoms, particularly pain, can be severe; associated physical signs, however, are commonly either absent or disproportionately less intense. Symptoms are generally less severe in HCP (7).

Most individuals who possess the genetic defect associated with one of the neurologic or neurocutaneous porphyrias show no recognizable clinical manifestations of porphyria throughout their lives. Only about 10% of individuals genetically predisposed to AIP or VP, and probably no more than one-third of those predisposed to HCP, ever develop the characteristic attacks of acute symptoms (4,7,16–18). If and when symptoms develop in a genetically predisposed individual, the initial onset and any subsequent recurrences often are linked to precipitating factors such as use of a therapeutic drug, exposure to a provocative chemical, alcohol consumption, tobacco smoking, an infection, the menstrual cycle, or fasting (1).

The frequency and severity of symptom attacks vary widely both among and within individuals. In most cases, symptom attacks have discernible onsets, are characterized by moderate or severe pain, last several days or longer (up to weeks or months), and are followed or separated by periods of clinical remission. Attacks are usually recurrent but not necessarily (18). Some manifestations of acute attacks can persist after the other clinical and biochemical

manifestations subside or resolve; this is particularly true for peripheral motor neuropathy, which can take up to 1 year to resolve or can become permanent (19,20). Permanent neurological deficits are not necessarily accompanied by heme precursor abnormalities when a patient's porphyria is otherwise in remission.

In contrast to the typical acute presentation, symptoms in some cases can occur nondistinctly in time and/or be relatively mild and some symptoms, particularly pain or psychiatric manifestations, can become chronic, often with variable severity over time (7,9,21). Given such complex presentations as well as the frequent paucity of physical signs, the diagnosis of porphyria can be delayed or missed entirely. Accordingly, screening surveys of psychiatric inpatients have reported a relatively high prevalence of individuals with previously unrecognized porphyria (22–24). One recent study, however, found no significant increase in major psychiatric illness among 344 consecutive patients with AIP (25).

Porphyrias with Neurologic and/or Cutaneous Manifestations

The neurocutaneous porphyrias, VP and HCP, can manifest cutaneous lesions as well as acute noncutaneous symptom attacks. One review of 110 cases of HCP reported that 30% experienced acute photosensitive responses, usually in association with acute symptom attacks (16). In contrast, acute photosensitivity is not common in VP; the cutaneous lesions tend to be chronic and they occur more often in individuals who do not have acute symptom attacks than in those who do (26,27). The cutaneous lesions of VP are particularly common in hot climates—about 75% in a

series of South African patients with clinically active VP (27). The cutaneous lesions of VP and chronic cutaneous lesions of HCP cannot be distinguished clinically or histologically from PCT (see below).

Porphyrias with Cutaneous Manifestations

The cutaneous porphyrias include all the porphyrias classified as erythropoietic [congenital erythropoietic porphyria (CEP), hepatoerythropoietic porphyria (HEP), and erythropoietic protoporphyria (EPP)] plus the nonerythropoietic porphyria PCT. The hereditary and acquired forms of PCT usually manifest first in adulthood, whereas clinical onset of the erythropoietic porphyrias usually occurs in infancy or childhood. There is no gender predilection in PCT, although in the past it has tended to occur more often in men. The erythropoietic porphyrias are relatively uncommon: fewer than 200 cases of CEP and fewer than 20 cases of HEP have been reported (7,28,29).

The cutaneous manifestations of CEP, PCT, and HEP (and VP and HCP) can be difficult to distinguish on the basis of clinical or histologic appearance, although the lesions of the autosomal recessive conditions, CEP and HEP, are usually more severe. Cutaneous manifestations include: skin fragility, vesiculobullous skin eruptions evolving into crusted ulcers, and residual hyperpigmentation or scarring, primarily occurring in sun-exposed areas of the body (7,30). Facial hypertrichosis is also common. The pattern of EPP differs substantially and is generally less severe than in the other cutaneous porphyrias; it is characterized by acute photosensitivity manifesting as pain, pruritus, and erythema with exposure as short as minutes, and generally without vesiculobullous lesions or scarring unless sun exposure is prolonged (30,31). Liver injury is usually not found with the cutaneous porphyrias other than in some patients with EPP, in which case it can be severe and potentially fatal.

Porphyria Cutanea Tarda

Porphyria cutanea tarda is one of the most prevalent types of porphyria; it occurs more often as a sporadic condition than as a familial inherited condition. The genetic defect associated with the familial form is inherited in autosomal-dominant manner. Hepatic uroporphyrinogen decarboxylase (Uro-D) activity is decreased in all forms of PCT; erythrocyte Uro-D activity, however, is normal in the sporadic form but is decreased in all but a small subgroup of

familial cases (32). The sporadic and familial forms of PCT usually occur in association with exogenous factors such as alcohol, oral estrogens, iron, and certain chemicals, or with certain medical conditions, particularly liver diseases; both are successfully treated in the same manner (2,7). Familial PCT, therefore, can be regarded at least partially as an acquired condition in which the inherited enzyme deficiency may only increase an individual's susceptibility to disease (33).

PCT is usually associated with some degree of liver abnormality (2,3,8). Liver function tests are nearly always abnormal to some degree. Moderate siderosis and various degrees of fibrosis or necrosis are often found upon biopsy, and cirrhosis develops in a small proportion of patients. It is common for PCT to develop in individuals with preexisting liver disease of a variety of types, particularly alcoholic liver disease or chronic hepatitis C (34–38). Patients with chronic liver disease also can develop mild or moderate degrees of porphyrinuria (increased urine porphyrins) in biochemical patterns that can be consistent with PCT but without associated cutaneous lesions (see "Chronic Hepatic Porphyria") (2,39). Patients with PCT are reported to be at risk for developing hepatocellular carcinoma (13,40–42); conversely, some benign or malignant liver tumors can overproduce uroporphyrin and induce PCT (43–45).

Biochemistry of Porphyrias

Deficient activity of a heme-synthesis enzyme results in accumulation of the heme precursors proximal to the deficiency, although overall production of heme is generally adequate (1–10,46). The water solubility of the heme precursors decreases progressively with successive steps of heme synthesis. Excess protoporphyrin is excreted exclusively in stool; coproporphyrinogen, uroporphyrinogen, and the 7-, 6-, and 5-carboxyl porphyrinogens are excreted in both urine and stool, and ALA and PBG are excreted predominantly in urine. Porphyrins in stool undergo varying degrees of transformation by normal enteric bacteria (47,48). When a heme-synthesis enzyme deficiency is expressed more in one tissue than another, heme precursors that accumulate in one tissue can be transported in blood to other tissues, with subsequent conversion to later intermediates in the heme synthesis pathway; experimental ALA loading, for example, leads to prompt coproporphyrinuria in humans (49–51).

In general, the neurologic (and neurocutaneous) porphyrias are characterized by excessive excretion of the porphyrinogen precursors, ALA and PBG, in urine; the cutaneous (and neurocutaneous) porphyrias are characterized by the accumulation of porphyrins in blood and excessive excretion of porphyrins in urine and/or stool (46,52–54). Individual types of porphyria can be differentiated by the pattern of heme precursors (i.e., the absolute values and the ratios to each other) in a patient's urine, blood, and stool, if collected either during or soon after attacks of neurologic symptoms or while porphyric skin lesions are actively manifested. Excretion of ALA and PBG in urine usually normalizes within several weeks following an acute attack in VP or HCP (16,26,27); in AIP, urine excretion commonly subsides but might not completely normalize during remissions (19,20). Compared to an acute attack, urine porphyrinogen precursors (i.e., ALA and PBG) may be lower in VP when solely cutaneous lesions are present, but urine porphyrins are still increased to diagnostic levels (27). Porphyrins are always increased in the plasma of patients with active cutaneous lesions (10).

When an individual has symptomatic manifestations of a porphyria (i.e., when clinically active and not latent or in remission), the level of the most excessively excreted heme precursor is typically at least several-fold greater than the values reported for the upper limit of normal. In reports describing the neurologic porphyrias, for example, urine ALA and PBG in AIP increased acutely to 8 to 150 and 30 to 200 mg/day, respectively (normal upper limits, 3 to 7.5 mg/day, depending on the laboratory) (7,20,26,55–57); and in ADP, urine ALA was markedly elevated and urine and erythrocyte porphyrins were elevated up to 100 times (7,10). In reports of the neurocutaneous porphyrias, urine coproporphyrin in HCP increased acutely to 4 to 190 times normal (16,58). In VP, one small case series reported the lowest values of urine ALA, PBG, uroporphyrin, and coproporphyrin in acute attacks to be 9, 12, 190, and 27 times the mean control values, respectively (26), which was consistent with the higher average values reported in a larger series (27). In reports of the cutaneous porphyrias, urine uroporphyrin in PCT increased to about 10 to 375 times normal in over 300 reported cases (except in one case (59), where a single value was within normal range) (60–70), and in EPP, reported erythrocyte protoporphyrin values ranged from 2.4 to

90 times normal (31,65,71), with the exception of a single patient in whom it was elevated by only a factor of 1.4 (31). Finally, in reports of the less common (autosomal-recessive) cutaneous porphyrias, urine porphyrins in CEP were 20 to 60 times normal (7) and in HEP, urine uroporphyrin increased by at least 20-fold and erythrocyte porphyrins increased by 5- to 10-fold (28,29,72). Plasma porphyrin concentrations are markedly increased when there are active skin lesions due to any cutaneous porphyria (10).

Secondary Porphyrinuria

Some of the tests used to diagnose the porphyrias are nonspecific and are abnormal in a variety of circumstances other than the porphyrias. Porphyrinuria can be caused by porphyrias, by a number of other medical conditions, especially those affecting the liver or bone marrow, and by a variety of exogenous factors such as alcohol and certain drugs and chemicals that disturb heme synthesis or stress heme-dependent metabolism (1,73-76). The term secondary porphyrinuria is commonly applied to the porphyrinuria occurring with conditions and factors lacking a primary enzyme defect in heme synthesis. It usually involves mild or moderate coproporphyrinuria, with no or little excess uroporphyrin in urine, and is also often called coproporphyrinuria or secondary coproporphyrinuria.

The detection of porphyrinuria has potential utility as a biological indicator of exposure to chemicals with porphyrinogenic properties and as a staging measure for sub-clinical development and progression of chronic hepatic porphyria (see "Chronic Hepatic Porphyria and Environmental Chemicals and Effects on Measures of Heme Synthesis"). However, with the noteworthy exception of lead poisoning (see "Environmental Chemicals and Effects on Measures of Heme Synthesis"), the porphyrin excess in secondary porphyrinuria has no recognized clinically detectable consequences of its own. Most reviewers attribute symptoms associated with secondary porphyrinuria (other than lead poisoning) to the condition or agent causing the porphyrinuria or to an unrelated cause, not to a disturbance in heme synthesis (2,3,10,75,77-81). Still, although the porphyrinuria itself may be benign, an associated medical condition may be far from benign.

Accumulation or excessive excretion of heme precursors does not necessarily mean there is a deficiency of a heme pathway

enzyme(s); other possible mechanisms exist. Increased erythropoiesis can produce increases in urine coproporphyrin, as demonstrated by induced anemia in experimental animals (82). Coproporphyrin normally is excreted in bile and in urine, and impaired biliary excretion in hepatobiliary disease leads to increased coproporphyrin excretion in urine (83). Stimulation of hepatic heme synthesis (e.g., by certain drugs) in the absence of deficiency of any heme-synthesis enzymes can lead to increased coproporphyrin excretion. The kidney (primarily the epithelium of the proximal tubules) has been identified as a major source of porphyrins (primarily coproporphyrin) in the urine of normal and porphyric individuals as well as lead- and mercury-poisoned individuals (84-87). It is conceivable that toxic effects on renal tubular function could lead to increased urinary loss of porphyrins along with other substances handled by renal tubules without inhibition of heme synthesis.

Chronic Hepatic Porphyria

Based on clinical experience (77,88), and with supporting evidence from animal experiments (89), Doss (39,75,90) has described the potential for chronic nonspecific disturbances in hepatic heme synthesis to make a transition from secondary coproporphyrinuria and progress through several clinically latent stages of chronic hepatic porphyria [(CHP) Types A, B, and C] to PCT (CHP Type D). Each stage in the Doss model (75,88) is differentiable by urine porphyrin quantities and patterns (i.e., initial accumulation of uroporphyrin and later heptacarboxyl porphyrin in the liver), progressively greater degrees of sub-clinical liver injury, and ultimately the occurrence of cutaneous lesions. The transition from secondary coproporphyrinuria to CHP requires either a genetic defect or toxic inhibition of Uro-D activity in the liver, generally in combination with liver disease and precipitating factors such as alcohol or estrogens.

The Doss model, however, is not uniformly accepted as valid. The evidence in humans for the Doss model comes from cross-sectional studies of patient populations, not longitudinal studies (77,88). There are no well-documented cases of individual patients actually progressing through CHP Types A, B, and C to PCT, and it has not been demonstrated that mild degrees of porphyrinuria, particularly coproporphyrinuria, predict the potential for an individual to develop PCT. The early stages in

the CHP model are characterized primarily by coproporphyrinuria, which can occur nonspecifically with liver disease and may reflect a separate phenomenon. There is no evidence or reason to predict that hepatic Uro-D deficiency is manifested initially by coproporphyrinuria before uroporphyrinuria. In addition, there is evidence that the kidney may be the primary source of coproporphyrin and other porphyrins excreted in normal urine and may also be a major source in certain porphyrias and toxin-induced porphyrinurias (84,91,92).

Environmental Chemicals and Effects on Measures of Heme Synthesis

In individuals who are genetically predisposed to developing an acute or cutaneous porphyria (e.g., inheriting one allele), the biochemical and clinical manifestations of porphyria can be triggered by a variety of exogenous factors including certain chemicals and therapeutic drugs, alcohol consumption, tobacco smoking, infections, and dietary factors, as well as by certain medical conditions and endogenous factors such as the menstrual cycle (1). Exposure to the sun can trigger cutaneous manifestations of porphyria if an excess of porphyrins already exists. Therapeutic drugs are particularly well recognized as possible precipitants of acute porphyria and current lists of drugs classified as unsafe and drugs thought to be safe for use in acute porphyrias are maintained (93-95). Exogenous factors can also cause changes in the heme synthesis pathway, even in the absence of genetic predisposition; in some cases, these acquired changes have been reported to cause PCT.

A number of chemicals, including halogenated hydrocarbons and metals, are known to be porphyrinogenic (i.e., capable of inducing changes in heme synthesis, with subsequent overproduction and excessive excretion of heme precursors) in experimental animals, generally with exposure by ingestion and with doses much greater than the range of human experience (96-98). In addition to possible interspecies differences, the differences in dose and pattern of exposure limit the ability to extrapolate from experimental animal studies in order to assess the potential risks of specific chemicals for humans. Unfortunately, there has been only limited systematic study of the subject in humans. In humans, with the noteworthy exceptions of porphyria caused by hexachlorobenzene (HCB) ingestion and the porphyrinuria

caused by lead, reports of porphyria or porphyrinuria attributable to chemical exposures have been infrequent. The reported findings have generally been linked to chronic industrial exposures, industrial accidents, or environmental exposures that were much higher than usually encountered.

Hexachlorobenzene

The most noteworthy incident of chemical-induced porphyria in humans occurred in Turkey during the late 1950s, when approximately 3000 cases of PCT developed from the ingestion of seed wheat treated with fungicides containing about 10% HCB (99–106). The wheat was treated in anticipation of use for planting but was instead used for human consumption. The syndrome commonly consisted of weight loss, muscle wasting, weakness, hepatomegaly, thyromegaly, arthritic changes, gross porphyrinuria, hypertrichosis, and photosensitive dermatopathy (99,102). The dermatopathy was usually hyperpigmented and had vesiculobullous lesions that eventually ulcerated, were commonly complicated by infections, and left depigmented scars and often disfiguring contractures. Active skin lesions tended to abate within a month after HCB exposure ended but often recurred subsequently in summer months, even in the absence of any new exposure to HCB. Children were affected disproportionately more than adults. Some reports noted the absence of abdominal crises, mental disturbances, and other neurologic complications (99,100); others noted the presence of abdominal colic and muscle weakness during the acute phase (102). Follow-up studies up to 30 years later reported that a majority of the patients studied had cutaneous residua, arthritic deformities, thyromegaly (particularly in women), and neurologic manifestations including weakness, paresthesias, and sensory shading; smaller proportions of patients had myotonia or cogwheel rigidity without other extrapyramidal signs (103–106).

Administration of HCB to experimental animals causes a deficiency of hepatic Uro-D and a pattern of porphyrin accumulation closely resembling that in human PCT (89,107–111).

Dioxin

Laboratory experiments have demonstrated that tetrachlorodibenzodioxins (dioxin(s) or TCDDs) in high ingested doses are porphyrinogenic in rodents (112,113), but the evidence in humans is mixed. The strongest

human evidence is from two workplace studies. In 1964, a study at a New Jersey chemical plant that produced the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) described two workers with PCT and another with possible PCT, and also reported that urine uroporphyrins were positive in 8 of 26 other workers (114). Most workers studied had chloracne, prototypically caused by dioxin, which was then a frequent contaminant of 2,4,5-T production. Another study of 55 workers arriving at a hospital from a Czechoslovakian herbicide manufacturing plant found a high prevalence of chloracne, 11 workers with PCT, and slightly increased urine uroporphyrin ($\leq 100 \mu\text{g}/24 \text{ hr}$; reference range not stated) in 21% of those tested (115). One worker in a TCDD-contaminated workplace developed PCT, sarcoma, and probable chloracne; the combined occurrence of these rare conditions suggested an etiological relation to the TCDD exposure (116,117). Other workplace studies, however, have been less demonstrative. A later study at the same New Jersey plant found no active cases of PCT and no workers with abnormal urine excretion of heme precursors except one worker who formerly had severe PCT (118). Maintenance workers, who had the highest chemical exposures, were found to have higher average urine coproporphyrin levels than other workers, but their individual levels were all within normal limits. Two separate later studies of herbicide production plants (one of which included the New Jersey plant mentioned above) (119) were both negative (120).

Limited evidence is also available from community studies. An explosion at a chemical plant in Seveso, Italy, in 1976 contaminated the surrounding environment with TCDDs. Two related individuals who were found to have a preexisting familial defect in Uro-D were reported to manifest PCT after the explosion without another evident precipitating factor (121). Among 60 Seveso residents from other families, 8 were reported to have secondary coproporphyrinuria and 5 had a transition constellation to CHP Type A (121) (see "Chronic Hepatic Porphyria"). Another study, of 115 Seveso residents, found that 84% had urine porphyrin patterns consistent with coproporphyrinuria or CHP Type A, with the greatest prevalence and degree of abnormality in residents from areas of highest contamination (122). A later study, however, reported chloracne

but no PCT attributable to TCDDs among Seveso children and adolescents (123,124). In two studies of Missouri communities where TCDD-contaminated waste oil had been sprayed for dust control over several years, there were no cases of PCT, but one study found significantly higher mean uroporphyrin levels in urine and higher prevalence of elevated urine uroporphyrin values ($> 13 \mu\text{g}/\text{g}$ creatinine (cre) in 16%, vs 7% controls) among 154 residents of a contaminated mobile home park compared to matched control subjects (125,126).

Other Halogenated Hydrocarbons

A number of halogenated hydrocarbons have been demonstrated experimentally to be porphyrinogenic in exposed animals or in *in vitro* tests; these are well summarized in Strik et al. (96) and Marks (97) and will not be described in this review. The reported human experience is less extensive. Haberman et al. (127) mentioned that a case of PCT manifested itself soon after agricultural application of DDT. Lynch et al. (128) reported a case of PCT in association with hepatitis, which was attributed to chronic toxic exposure and presumed to be a polychlorinated phenol unintentionally synthesized on a regular basis by mixing cleaning agents containing benzylchlorophenol and sodium hypochlorite. A worker with severe acute methyl chloride intoxication had marked increase in urine and fecal excretion of coproporphyrin but not uroporphyrin or protoporphyrin; the coproporphyrinuria decreased in association with clinical improvement (129). Evaluation of 46 workers exposed to vinyl chloride in a polyvinyl chloride production process found that 36 had coproporphyrinuria ($\leq 820 \text{ nmol}/24 \text{ hr}$; normal (nl) ≤ 130), including 4 who also had mildly increased excretion of uroporphyrin and heptacarboxyl porphyrin in urine (130). A study of Michigan farm families exposed to polybrominated biphenyls (PBBs) by ingestion of contaminated meat and dairy products found that 11 of 126 persons had coproporphyrinuria (93–218 $\mu\text{g}/\text{liter}$; $\text{nl} \leq 78$), including 4 with mild uroporphyrinuria (29–59 $\mu\text{g}/\text{liter}$; $\text{nl} \leq 24$) (131). Of 20 individuals in Taiwan, 2 years after poisoning with polychlorinated biphenyls (PCBs) in food oil, 3 had elevated uroporphyrin in urine (50–139 $\mu\text{g}/\text{liter}$; controls, 7–22), 1 of whom, plus 5 others, had elevated coproporphyrin (61–255 $\mu\text{g}/\text{liter}$; controls, 2–48) (132). Two separate studies of 87 similarly poisoned individuals

in Yusho, Japan, about 10 years after the event, found no differences in urine porphyrins compared to those of controls and identified PCT in 1 person whose history was complicated by alcohol use (133,134). Total urine porphyrins were not significantly different from those of controls in 168 chemical plant workers exposed to either allylchloride, hexachlorocyclopentadiene, epichlorohydrin, or endrin (135).

Lead

Lead absorption, both acute and chronic, is well documented to affect heme synthesis (11). Lead causes accumulation of zinc protoporphyrin (ZPP) in erythrocytes and large increases of ALA and coproporphyrin in urine. Lead reversibly inhibits ALA-D and also appears to interfere with the function of coproporphyrinogen oxidase and ferrochelatase, possibly by mechanisms other than direct enzyme inhibition (11,98,136,137). It is conceivable that the coproporphyrinuria occurs by the same mechanism as in ALA loading of normal subjects (Biochemistry of Porphyrins).

There is evidence that ALA-D polymorphism may influence lead toxicokinetics. The ALA-D enzyme is comprised of eight identical subunits coded by an autosomal gene, at which there are two common alleles (138). The ALA-D1 allele has about 80 to 90% prevalence in studied European and American population samples; the ALA-D2 allele, about 10 to 20% (138-142). The three associated phenotypes (1-1, 1-2, 2-2) have similar enzyme activities in erythrocytes. A number of studies have reported that individuals with the ALA-D2 allele, particularly the homozygous ALA-D 2-2 phenotype, have higher blood lead levels on average than individuals with the ALA-D 1-1 phenotype and comparable lead exposures (142-145). The possible mechanism of this reported phenomenon is not known, although it has been speculated that ALA-D subunits coded by the ALA-D2 allele might bind lead more efficiently (146). The possible clinical relevance also is unclear. Some investigators have suggested that ALA-D2 might render individuals more susceptible to lead poisoning (143,145); others have suggested that ALA-D2 might protect against lead effects (147).

Lead intoxication is generally classified as a secondary porphyria rather than an acquired porphyria, although some investigators have classified it as a porphyria, noting clinical and biochemical similarities with the acute porphyrias (see Table 1),

particularly ADP (39,148-150). It is not known to what degree the manifestations of lead poisoning might be attributable, if at all, to the associated disturbance in heme synthesis apart from the directly neurotoxic properties of lead, but there is evidence the disturbed heme-forming system may have some contributing role (151,152).

The reported interaction between lead exposure and genetic deficiency of ALA-D is noteworthy. ADP is attributable to homozygous autosomal-recessive defects affecting ALA-D, with near total loss of enzyme activity; heterozygous carriers of the defect have only partial loss of ALA-D activity and usually have no associated symptoms (153). However, the heterozygous defect appears to increase the vulnerability of the individual to the effects of exogenous agents such as lead, which reduce ALA-D activity. In case reports of individuals who had a heterozygous defect affecting ALA-D and were exposed to lead, the biochemical abnormalities and clinical features of illness were consistent with lead intoxication but were much more severe than expected for their mildly elevated blood lead levels (22-43 µg/dl) (149,154,155). The prevalence of the heterozygous defect in the general population has been estimated variably to be below 1% (149) or as high as 2% (156).

Other Metals

Mercury, arsenic, and other metal exposures are also reported to affect heme synthesis in humans, although the associated levels of porphyria are lower than those seen with lead. In a survey of dentists attending an annual professional meeting, those who were found to have >20 µg/liter mercury in spot urine samples also had 2- to 3-fold higher levels of coproporphyrin in urine (42 ± 18 µg/g cre or 74 ± 8 µg/liter; mean ± SD) than those with no urinary mercury (coproporphyrin 28 ± 12 µg/g cre and 23 ± 6 µg/liter) (87). Urine uroporphyrin excretions did not differ significantly; however, pentacarboxyl porphyrin and an atypical porphyrin tentatively identified as keto-isocoporphyrin were significantly elevated in urine. A study of random urine samples from 52 workers exposed to inorganic and organic mercury (median urine mercury about 500 µg/liter) found that 23% had elevated coproporphyrin levels (>70 µg/liter and up to 159 µg/liter) compared to only 3% of control subjects (157). Similarly, a study of arsenic-exposed smelter workers (urine arsenic 129 ± 109 µg/g cre in the high-exposure

group) found more than twice as much coproporphyrin (63 ± 30, range 18-171, µg/g cre) but comparable amounts of uroporphyrin in morning urine specimens, relative to those of nonexposed control subjects (coproporphyrin 27 ± 14, range 2-57, µg/g cre) (158). In another study, individuals exposed to arsenic in drinking water (urine arsenic mean 65, range 1-130 µg/liter) were reported to have no significant increase in urine porphyrin levels relative to those of a control group, although most exposed individuals did have inversion of the urine coproporphyrin/uroporphyrin ratio (76%, vs 32% of controls) (159). Downey (160) reported a case of acute intermittent porphyria (laboratory results not presented), with symptoms including transient dermatitis, severe abdominal pain, diarrhea and vomiting, dyscoordination, memory loss, and visual hallucinations, all of which developed soon after placement of dental prostheses (containing 76% palladium and 10% copper) and resolved "almost immediately" after removal of the prostheses.

Experimental animal and *in vitro* studies have demonstrated a number of other metals to be porphyrinogenic, including aluminum, cadmium (with arsenic or lead), cobalt, gallium arsenide and others; again, these data are summarized in detail in other reviews [Marks (97), Woods (98), Fowler et al. (161)].

Other Chemicals

Bleakley et al. (162) noted, in describing elevated fecal porphyrins in rats with subchronic cutaneous exposure to diazinon, "in a number of cases seen [of PCT in humans] ... a history of contact with pesticides, in particular with diazinon." A study of 17 pulp production workers exposed to hydrogen sulfide and methylmercaptan reported reduced activities of ALA synthase, ALA-D, and ferrochelatase (relative to reference ranges) in the reticulocytes of 8, 1, and 5 workers, respectively, as well as low erythrocyte protoporphyrin levels in 7 workers compared to control values (163). In two reported case series of acute porphyria, paints and solvents were described as triggers of acute symptoms in a number of genetically predisposed individuals (26,164).

Measures of Heme Synthesis as Biological Markers of Chemical Exposure

Measurements of the status of heme synthesis, particularly measurements of excreted heme precursors, have potential

field and clinical utility as biological indicators of chemical exposure and chemical effect on body functions (98,165). This is well illustrated by the extensive experience with lead, where measurements of protoporphyrin in blood [e.g., free erythrocyte protoporphyrin (FEP) and ZPP] and ALA and coproporphyrin in urine have been used, and in the case of ZPP are still used, as sensitive markers of lead intoxication. Animal experiments have demonstrated that a number of metals produce characteristic patterns of change in the urinary excretion of heme precursors, that the induced disturbances in heme synthesis are typically associated with ultrastructural changes and alterations of other subcellular processes, and that these measurable changes occur before the effects of metal exposure would be clinically evident (98,161). Therefore, measurements of heme precursors may offer a means of detecting harmful effects of specific chemical exposures in humans at stages of biological response to exposure that are still preclinical and are presumably most reversible. As the preceding sections indicate, however, only a small number of studies in humans have attempted to assess the utility and validity of heme precursor measurements or other measures of the status of heme synthesis as markers of exposure or preclinical disease in chemically exposed humans.

Unexplained Chemical-associated Illnesses and Measures of Heme Synthesis

It has recently been proposed that a variety of chemical-associated illnesses for which there are no widely accepted specific diagnostic tests or etiologic explanations—such as MCS syndrome, Persian Gulf War illnesses, conditions associated with silicone breast implants, and various fatigue syndromes—may represent either mild chronic cases of porphyria, or at least in part, manifestations of acquired abnormalities in heme synthesis (166–177).

The MCS syndrome (178–180) has been defined by Cullen (181) as “an acquired disorder characterized by recurrent symptoms, referable to multiple organ systems, occurring in response to demonstrable exposure to many chemically unrelated compounds at doses far below those established in the general population to cause harmful effects.” No physiologic test has been widely accepted as correlating with symptoms in MCS syndrome,

although a variety of immunologic and neurologic tests have been described as abnormal or diagnostic based on clinical series data. Of note, however, one blinded prospective controlled study evaluated a panel of immunologic tests offered by a laboratory with recognized interest in the evaluation of individuals with chemical sensitivities, and found that the tests did not distinguish between MCS patients and control patients (182). Still, in spite of continued debates about the origins of MCS syndrome, there is little doubt that it can be associated with substantial symptomatic distress, major lifestyle disruption, and severe degrees of inability to perform usual activities of work and daily life.

To date, the hypotheses of porphyric mechanisms in these otherwise unexplained syndromes are based only on individual cases or case series characterized by relatively mild increases in porphyrin excretion and/or decreases in activity of various heme-synthesis enzymes. The data reported to date have not identified any characteristic unifying pattern(s) among the laboratory test results. Donnay and Ziem (171) reported in 1995 that two medical practices in Washington State had found excess porphyrins and/or enzyme abnormalities in patterns that did not match those of any inherited porphyrias in over 70% of more than 150 MCS patients with symptoms of porphyriopathy. Morton (172) described laboratory findings for 38 individuals with chemical sensitivities and deficient activity of at least 1 of 5 tested heme-synthesis enzymes. Although he did not distinguish between the marginal and low laboratory reference ranges in defining deficiency (see “Limitations of Laboratory Measures of Heme Synthesis”), 22 (58%) had deficiency of coproporphyrinogen oxidase (Copro-O), 13 (34%) had deficiency of protoporphyrinogen oxidase, and 27 (71%) had at least 1 excess porphyrin in feces or urine (values reported only as yes or no). Of note, 7 of the 22 individuals with Copro-O deficiency and 4 of the 16 individuals with deficiency of enzymes other than Copro-O had no detected excess of porphyrins in feces or urine. Ziem and McTamney presented supporting clinical data at a recent scientific meeting; those data are not yet available for circulation (177).

Downey (168) described hereditary coproporphyrin in 13 patients (plus 7 family members) who arrived at an oral stomatology clinic with unexplained oral conditions and multiple systemic complaints: 16 of 19

tested had abnormal Copro-O activity, 3 of those 16 had one other abnormal heme-synthesis enzyme, and 14 of 14 tested had elevated heme precursors in urine or stool. Of the 16 abnormal Copro-O activity values, 14 were in the marginal reference range (0.06–0.09 relative units) and the other 3 values were just below the marginal range (0.05 relative units) (182). Of the 21 elevated heme precursor values (most often coproporphyrin), 17 (81%) were 1.1 to 1.8 times higher than the respective value reported for the upper limit of normal, and 4 (2 in stool and 2 in urine) were 2.0 to 2.3 times higher (182). Downey (167) mentioned that about 90% of 62 patients (including the above-described 19) were found to have one or more heme-synthesis enzyme abnormalities.

Two types of hypotheses have been put forth in explanation of such laboratory findings occurring with otherwise unexplained chemical-associated illnesses. The first hypothesis is that these illnesses are actually porphyrias, in which illness is precipitated by environmental chemical exposures in genetically predisposed individuals (160,167,172). Proponents suggest that the latent genetic traits for various porphyrias are much more common in the general population but were not recognized until the recent increased availability of laboratory assays for the activity of heme-synthesis enzymes. It is hypothesized that such genetically predisposed individuals can manifest porphyria symptoms in low-grade smoldering or chronic and slowly progressive patterns (without classically recognizable acute attacks of severe symptoms) in response to exposures to a variety of substances such as “formaldehyde, other aldehydes, heavy vehicle exhaust fumes, perfumes, other fragrances, chlorine, chlorinated cleaning agents, possibly any chlorinated hydrocarbon, substances in poorly ventilated buildings, and anything that triggers porphyria symptoms” (172). It is contended that these chronic symptom patterns are not necessarily accompanied by diagnostic abnormalities of heme precursors, possibly because a “limited attack especially on a small number of cells or on cells where the heme pathway is not a major functioning system or where the affected cells do not have ready access to external excretory processes may not raise porphyrin precursors above normal levels” (167); measurement of heme-synthesis enzyme activities, therefore, is regarded as critical to diagnosis.

The second hypothesis is that genetic predisposition may not be an essential

component of a porphyric mechanism in MCS syndrome, and that reported reductions in activity of heme-synthesis enzymes might represent an acquired deficiency attributable to exogenous toxic agents or stressors, manifesting as either an intoxication porphyria or some other as-yet undefined disorder of porphyrin metabolism (169,171). It is hypothesized that "the heme pathway may be just one of many sites in the body adversely affected by exposure to toxic chemicals, and that this interaction could account for many of the cutaneous, neurological and psychological symptoms reported by MCS patients, including the most controversial symptom of chemical sensitivity itself" [A Donnay, personal communication; (166)]. Some cases of MCS syndrome are predicted to be porphyric and the others nonporphyric (171).

Limitations of Laboratory Measures of Heme Synthesis

In evaluating the symptomatic patient with a suspected disturbance of the heme synthesis pathway, the interpretation of laboratory tests must consider the conditions of sampling and the limitations of those tests.

Methodologic Limitations

Laboratory test results, in general, can be compromised by a variety of factors, including specimen integrity (reflecting conditions of specimen collection, processing, transport, and storage), analytical quality, limitations of analytical methods, and the applicability and specificity of reference ranges, including the range of normal intraindividual variability. Issues of specimen integrity may be particularly relevant when specimens are collected and processed at one site and then transported to a geographically distant reference laboratory, as in nearly all the cases described in "Unexplained Chemical-associated Illnesses and Measures of Heme Synthesis." Specimen integrity is a particular concern in enzyme activity assays. There is the risk of obtaining falsely low measurements of enzyme activity because of the potential lability of enzymes after removal from the body. Because of these risks, an abnormal test result generally should be confirmed by analysis of a second specimen. The need to repeat a test, of course, must be tempered by the degree of support for a diagnosis from other clinical and laboratory data, and by the feasibility of repeating the test

(i.e., the appropriate clinical circumstances should still be present).

Reference Ranges

In addition to considering methodologic limitations, the interpretation of any laboratory test should consider potential limitations of the test reference range. First, reference ranges usually do not include all possible values for normal people (184). It is common laboratory practice to define a reference range as the mean \pm 2 SD, based on the distribution of test results in an ostensibly normal reference sample; therefore, it is expected that 5% of normal individuals, and possibly more if the distribution is skewed, will have low or high outlying values. Second, the range of possible test values may overlap for normal and affected individuals, and many tests might be more appropriately characterized as having an inconclusive range or a continuum between normal and diagnostic ranges, rather than simply being dichotomized (185–187). Third, the usual ranges of test values for normal individuals and for individuals with a given disease may differ so substantially that intermediately abnormal values might be regarded as nondiagnostic for that disease.

It is particularly important to recognize that there can be considerable normal intraindividual variation in physiologic tests (183); often for specific tests either this has not been characterized or the degree of expected variability has not been made known to the clinician. Any such variation, however, can limit the clinician's ability to interpret a test result confidently as abnormal when an individual's single result is outside the reference range by an amount that is small relative to expected intraindividual variability on that test. One study, for example, reported a 3-fold difference between the highest and lowest total porphyrin measurements in 24-hr urine specimens (normally comprised mostly of coproporphyrin) collected over 7 consecutive days from the same person; all values were normal except one that exceeded the value reported for the upper limit of normal by about 25% (131).

Finally, because a reference range may be unique to the assay method and the laboratory performing the test, test results should be interpreted relative to the laboratory-specific reference range and/or, if sufficient general clinical experience exists, against accepted absolute reference standards. However, a reference range may have limited representativeness for test subjects,

even when derived from a large sample of normal individuals, if test subjects differ from the reference sample in terms of the circumstances affecting specimen integrity or in terms of potentially confounding personal factors. For example, the study of PBB-exposed Michigan farm families (see "Environmental Chemicals and Effects on Measures") found total urine porphyrins were significantly higher on average than those in the Dutch reference sample, but they were no different than those in a contemporary control group of Wisconsin farm families (131).

Enzyme Activity Measurements

The diagnostic value of activity measurements for heme-synthesis enzymes depends on the presence or absence of symptoms and on other clinical and laboratory data, particularly the level and pattern of any associated overproduction of heme precursors. Individuals who are genetically predisposed to a porphyria usually will have deficient activity of the associated enzyme, yet a substantial proportion will never develop symptoms of a porphyria. Therefore, identification of the genetic trait alone in a symptomatic patient is not by itself sufficient evidence of symptom causality. In addition, reductions in enzyme activity do not necessarily reflect a genetic trait; certain exogenous agents such as alcohol or lead can inhibit or interfere with the activity or alter the synthesis of specific enzymes in the heme synthesis pathway (11,74). Conversely, normal enzyme activity measurements can reduce the likelihood, although they do not completely eliminate the possibility, that a person has porphyria. There is typically a substantial degree of overlap in enzyme activity values for normal individuals and for individuals with a porphyria characterized by deficiency in that enzyme (73,188). Also, some porphyrias do not necessarily manifest the associated enzyme deficiency in erythroid cells, even though it may be present in other tissues (e.g., variant AIP, when a mutation is in or near exon 1 of the PBG deaminase gene and affects only the nonerythroid enzyme) (10).

The measurement of specific heme-synthesis enzyme activities is commonly considered a second-line test in the evaluation of porphyrias (189). Enzyme activity measurements are most often used for identifying a genetic trait for porphyria in family members of a person with diagnosed or suspected porphyria to identify individuals who should be counseled on the need to

minimize or avoid exposure to factors known to precipitate porphyria, even though they may have been asymptomatic to date (7,188,190,191). Enzyme activity measurements also can be useful when a symptomatic person is diagnosed as having a porphyria of some type—based on symptom pattern and a substantial increase in excretion of heme precursor(s)—but the pattern of heme-precursor excretion does not allow differentiation of the specific type of porphyria; proof of deficient activity for a specific heme-synthesis enzyme can facilitate determination of the specific porphyria. Measurements of enzyme activity otherwise have limited utility in the evaluation of suspected porphyria in symptomatic individuals.

Coproporphyrinogen Oxidase Activity

Decreased Copro-O activity is the most frequently identified enzyme finding, and in many cases is the sole basis for diagnosing a disturbance of heme synthesis in the above-mentioned cases of unexplained chemical-associated illnesses with reportedly abnormal measures of heme synthesis (see “Unexplained Chemical-associated Illnesses and Measures of Heme Synthesis”).

The assay for Copro-O activity is the subject of some controversy. At present, only one laboratory offers a Copro-O activity assay on a commercial basis. That assay is performed by the incubation of ALA substrate with a lysed peripheral blood cell specimen, followed by analysis of the porphyrins formed; a low yield of protoporphyrin and a normal or increased yield of coproporphyrins indicate a deficiency of Copro-O (183). The commercial assay, therefore, uses coproporphyrinogen synthesized enzymatically *in situ* from ALA added to the incubate. In contrast, research laboratories that perform Copro-O activity assays in peripheral blood cells do so in isolated leukocyte or lymphocyte fractions, using externally synthesized coproporphyrinogen as specific substrate (16,192–194).

It is widely believed that heme synthesis in humans is confined to nucleated cells and that Copro-O is a mitochondrial enzyme. However, there is little published information that addresses the relative degrees of Copro-O activity in reticulocytes, mature erythrocytes, and leukocytes (16,195,196). Heme synthesis in peripheral blood cells is expected to occur only in leukocytes and perhaps in reticulocytes, and not in mature erythrocytes, which lack nuclei and mitochondria and which constitute about 99% of peripheral blood cells.

Mitochondria are present in nucleated blood cells but are not normally present in mature erythrocytes. Developers of the lysed-blood assay maintain, however, that circulating erythrocytes can transform 10 to 40% of available coproporphyrinogen III to protoporphyrinogen during 3-hr incubations, with reticulocytes having at least twice as much Copro-O activity as older erythrocytes, and with overall erythrocyte activity far exceeding that seen in lymphocytes (RD Ellefson, personal communication). The subcellular distribution of the reported Copro-O activity in erythrocytes is unclear; remnants of mitochondria are one speculated possibility.

It is generally agreed that because the percentage of reticulocytes varies among normal persons and can change dramatically in a number of disease states, interpretation of test values from the lysed-blood assay should at least be adjusted for the reticulocyte count (183). It is also agreed that regardless of the technique used to assay activity, Copro-O is a particularly labile enzyme after removal from the body. Vigorous specimen processing or failure to properly maintain the specimen during transport or storage can cause enzyme damage and produce falsely low (i.e., false positive) test results (183).

Evaluating the Symptomatic Patient in Whom Porphyria Is Suspected

The most important first step toward diagnosing or ruling out porphyria in a symptomatic patient is for the clinician to maintain a high index of suspicion for a possible diagnosis of porphyria, whether symptoms are classic for a porphyria or are vague or unexplained. The conclusive diagnosis of a porphyria should be based on a systematic approach incorporating medical history, physical examination, and biochemical data, and including genetic evaluation if necessary. Certain symptom patterns, physical findings, and elements of the exposure history may raise the degree of suspicion for porphyria; however, the lack of supporting information from these sources cannot exclude a diagnosis of porphyria. Therefore, the systematic approach to evaluating a symptomatic patient with suspected porphyria must include laboratory evaluation (see also “Biochemistry of Porphyrias”).

Laboratory Evaluation— Diagnosis of Porphyria

The nature and pattern of a patient's symptoms and physical signs may provide

some guidance in the selection of tests for evaluating the symptomatic patient with suspected porphyria. However, the neurologic and cutaneous manifestations of porphyrias can be nonspecific or atypical and caution is necessary to avoid being overly focused on the basis of clinical appearance in initial test selection. Reviewers make slightly different recommendations regarding the appropriate panel of first-line tests for the evaluation of suspected porphyria. The most common recommendation—when symptoms suggest possible neurologic manifestation(s) of an acute porphyria—is for the measurement of PBG with or without ALA in urine (46,52,54,189). Most reviewers also recommend quantification of total or individual porphyrins in urine and, routinely or supplementally, in stool—particularly when symptoms or signs suggest possible cutaneous manifestations of porphyria. Measurement of protoporphyrin in blood is often recommended, depending on the degree of suspicion for erythropoietic protoporphyria. Anderson (10) alternatively recommends measurement of total plasma porphyrins, plus urine PBG and ALA, to determine the presence or absence of porphyria. A blood lead level, with or without a ZPP level, should also be considered because of the similarity of symptoms in lead poisoning and porphyrias with neurologic manifestations.

It is generally less difficult to determine whether a patient has porphyria than it is to differentiate which specific type of porphyria is present. The presence or absence of increases in urinary ALA and PBG and the relative increases in the individual porphyrins are particularly helpful in diagnosis. The nature and pattern of reported symptoms can assist in differentiation. The cited general references and review articles (1–10,46,52,54,189) provide information regarding the patterns of laboratory abnormalities to consider in attempting to differentiate the specific type of porphyria in the patient who has laboratory and clinical evidence consistent with a porphyria. We will not discuss further this level of differential diagnosis; we will focus on preliminary screening steps in the diagnostic evaluation of a possible porphyria.

Laboratory Evaluation— Excluding Porphyria as the Cause of Symptoms

As discussed in “Biochemistry of Porphyrias,” when a porphyria (or another clinically important disturbance of heme synthesis, e.g., lead intoxication) of any recognized

type is symptomatic, it is almost always accompanied by substantial overproduction and increased excretion of heme precursors, typically at least several-fold greater than the value reported for the upper limit of normal. One possible but infrequent exception occurs after a patient with acute porphyria develops a neurologic deficit that becomes permanent while the porphyria is otherwise in remission; biochemical parameters may return to normal ranges.

Conversely, in a patient who is currently or recently symptomatic and who is suspected to have a porphyria, it is not probable that the patient's symptoms are attributable to a porphyria of any type unless a measurement on at least one of the following tests is greater than twice the value reported for the upper limit of normal: ALA, PBG, uroporphyrin, or coproporphyrin in urine; blood total porphyrins; or fecal coproporphyrin (see "Biochemistry of Porphyrias") (90,189). A blood lead level should be checked to determine the possibility of lead intoxication if lead exposure is possible, if excretion of coproporphyrin or ALA is increased, or if blood porphyrins (e.g., ZPP) are increased. If a person is currently or recently symptomatic and has reduced activity of a specific heme-synthesis enzyme, but laboratory testing does not reveal overproduction of heme precursors in a pattern and levels consistent with the porphyria associated with deficiency of that enzyme, then the reduction in measured enzyme activity has no probable causative relationship to the person's symptoms.

Satisfaction of these 2-fold-threshold screening criteria does not necessarily establish a diagnosis of porphyria. Depending on the degree and pattern of abnormalities on these tests, additional testing may be necessary to establish or exclude a diagnosis of porphyria. It is possible that an individual could have an abnormal heme-precursor measurement with this degree of abnormality as a consequence of something other than porphyria (or lead intoxication). Other medical conditions can cause secondary porphyrinuria of this magnitude. Blood porphyrins can also be increased by this magnitude in conditions other than porphyria; for example, iron deficiency commonly produces an increase in blood ZPP.

Conversely, failure to satisfy these 2-fold threshold-screening criteria does not necessarily exclude a diagnosis of porphyria. Heme-precursor measurements in the range of one to two times the value reported for the upper limit of normal

should not be interpreted as normal but rather as indeterminate or nondiagnostic. The timing of sample collection relative to the occurrence of symptoms is critical. When a patient with suspected porphyria is not currently or recently symptomatic, the levels of heme-precursor excretion are generally lower and can even normalize with time (i.e., within days to weeks). If a patient's last symptoms occurred remotely in the time relative to specimen collection, it may be necessary to repeat the tests during or as soon as possible after future symptoms. In view of expected interindividual variations and the potential processing and analytic limitations of laboratory tests, particularly at the low range of abnormality, mildly abnormal levels of heme-precursor excretion generally should be repeated before utilizing them as justification for further diagnostic assessment.

Secondary Porphyrinurias

Indeterminate or nondiagnostic levels of porphyrin excretion might represent a secondary porphyrinuria. With the noteworthy exception of lead poisoning, the porphyrin excess in secondary porphyrinuria has no recognized clinically detectable consequences of its own. Medical conditions that appear to have only secondary effects on the heme synthesis pathway are appropriately evaluated, with attention focused on the primary condition. Similarly, when chemical exposures are suspected as the cause of a patient's symptoms or medical condition, the exposure relationship can be characterized more specifically by exposure assessment or by quantification of the suspected chemical (or its metabolite) in blood or urine than by measurement of heme precursors. Lead poisoning, for example, is probably the most commonly recognized condition that can result from a chemical exposure and that is accompanied by abnormal measures of heme synthesis. However, even though these measures provide sensitive indicators of lead exposure and effect, the blood lead level is a more sensitive and more specific test for the diagnosis and management of lead poisoning.

Exposure Relationships

If it is ultimately determined that a symptomatic patient has a specific porphyria or another clinically important disturbance of heme synthesis (e.g., lead intoxication) and if the possibility of exposure relationship or work relationship is at issue, then the exposure history and any

independently available exposure data should be reviewed to assess the likelihood that an exogenous chemical(s) might have triggered or caused the diagnosed condition. The potential complexity of the exposure assessment process is beyond the scope of this review. Exogenous agents that are known or suspected to cause or to trigger porphyria are discussed above. Given that there has been only limited systematic study of potentially porphyrinogenic chemicals in humans, however, consideration of possible exposure relationships should not be confined to chemicals known or suspected to affect heme synthesis.

Conclusions

There is little question that individuals who are genetically predisposed to a porphyria can have clinical manifestations of porphyria triggered by exogenous chemicals. Most of the experience with such chemical triggering of porphyria has involved alcohol consumption and pharmacological doses of drugs. However, other chemical exposures have also been reported to trigger porphyrias with neurologic manifestations as well as those with only cutaneous manifestation—PCT in particular.

It is also clear that certain exogenous chemical exposures can actually cause porphyria in the absence of genetic predisposition. This is demonstrated most convincingly by the Turkish epidemic of PCT caused by HCB intoxication. Other chemicals have also been linked to cases of porphyria in humans; the reported cases, however, have been infrequent, have all involved PCT, and have generally been linked to chronic industrial exposures, industrial accidents, or environmental exposures that were much higher than normally encountered. Chemical exposures have not been established as a cause (i.e., not just a trigger in the presence of genetic predisposition) of porphyrias with primarily neurologic manifestations, although lead intoxication is noteworthy as a possible exception.

There is no doubt that lead absorption can cause substantial disturbance of heme synthesis. Lead intoxication has prominent manifestations as well as biochemical abnormalities that mimic the manifestations of the neurologic porphyrias. It is not known to what degree the neurologic manifestations of lead poisoning might be attributable (if at all) to the associated disturbance in heme synthesis, apart from the directly neurotoxic properties of lead, but there is evidence the disturbed heme-forming system may have some contributing role.

It must be acknowledged that there have been only a limited number of systematic attempts to identify porphyria or even subclinical porphyrinuria in chemically exposed humans, and that a large number of chemicals have been identified as porphyrinogenic in experimental animals—albeit with doses much greater than the usual range of human experience. In some cases, these effects result from inhibition of or interference with specific enzymes in the heme synthesis pathway; however, some of these effects can be produced by mechanisms that do not involve an abnormality in the heme-forming system. Regardless of the causative mechanism, these chemical-related changes have potential utility as biological indicators of chemical exposures that might have significant pathologic effects. There is a need for further research, including trial field applications, of such porphyrin biomarkers.

Clinicians should maintain a reasonable degree of suspicion for the possibility of a disturbance in heme synthesis underlying any unexplained syndrome characterized by nonspecific symptoms if even mildly reminiscent of porphyria, whether or not environmental chemical exposures are suspected as causative or contributing agents. However, heme-precursor measurements obtained when a patient is symptomatic should be interpreted cautiously and/or repeated if not substantially elevated (i.e., at least 2-fold greater) relative to the respective value reported for the upper limit of normal.

With the noteworthy exceptions of lead intoxication and the porphyrias in clinically active states, the porphyrin excess associated with porphyrinurias has no known clinically detectable consequences of its own. Symptoms associated with such secondary porphyrinurias are attributed by most reviewers to the condition or agent causing the porphyrinuria or to an unrelated cause and not to a disturbance in heme synthesis. Such porphyrinuria may be relevant as a nonspecific laboratory indicator of some other operative pathophysiologic mechanism, much as an elevated sedimentation rate can provide contributory but nonspecific evidence of disease; however, it is most appropriate for further diagnostic attention to focus on the primary condition or exposure of concern, not necessarily on a mild disturbance of heme synthesis.

A variety of chemical-associated illnesses with unknown causative mechanisms, notably MCS syndrome, have been reported

in association with abnormal results of porphyria-related tests. It is hypothesized that these test values reflect pathophysiologic disturbances of heme synthesis that are directly involved in the manifestation of those illnesses. Proponents of these hypotheses point out that many MCS symptoms resemble the neurologic and cutaneous manifestations of porphyrias. Yet, cutaneous symptoms are usually not a major feature of MCS syndrome, and when present, they differ substantially from the cutaneous manifestations of the inherited and acquired porphyrias. The noncutaneous symptoms of MCS syndrome are much less intense and less discretely episodic than the common neurologic manifestations of porphyrias. The clinical course of porphyrias with neurologic manifestations can, as with MCS syndrome, occur in a chronic or indolent pattern, and does not occur exclusively as acute symptom attacks separated by symptom-free intervals; however, in the neurologic porphyrias, in contrast to MCS syndrome, the chronic pattern occurs much less frequently than the pattern of acute symptom attacks.

Proponents of porphyric hypotheses for MCS syndrome point out another similarity with the porphyrias—the propensity for symptoms to be caused or triggered by exogenous chemical exposures. However, the very low levels of exposure to which MCS patients are characteristically sensitive are much lower than the pharmacological doses of drugs that are known to trigger neurologic symptoms in genetically predisposed individuals. These levels are also much lower than the chemical exposures reported to date in association with PCT or subclinical porphyrinurias in humans, where situations generally have involved chronic industrial exposures, industrial accidents, or environmental exposures that were much higher than normally encountered, and with the exception of lead intoxication, have not been reported to involve low-level environmental exposures.

It is conceptually difficult to reconcile why MCS syndrome, if mediated substantially through a disturbance in heme synthesis, would have a markedly greater degree of chemical intolerance and greater frequency of chronic functional limitations than recognized forms of porphyria but would never manifest symptoms or signs as severe as can occur with porphyrias, and would have markedly lower or even absent elevations of heme-precursor excretion when symptomatic compared to porphyria

patients when symptomatic. One contention is that circumscribed disturbances of heme synthesis (i.e., limited to a small number of cells) can cause symptoms without producing measurable or substantial increases in heme precursors and that heme-synthesis enzyme measurements, therefore, are the more critical, if not the only necessary, diagnostic measure. Although it is well recognized that heme synthesis can be disproportionately affected in different organ systems with any one type of porphyria, it is contrary to other clinical evidence to date (with rare exceptions) that a disturbance of heme synthesis is so profound as to produce symptoms without also producing substantial elevations of heme precursors. Similarly, there is no other evidence to date that a heme-synthesis enzyme with low activity, particularly when marginal activity values are overinterpreted as low, can be linked to symptoms without an accompanying substantial increase in heme precursors.

It is sometimes contended in individual cases that the timing of specimen collection was not sufficiently close in time to a symptom episode to depict the full degree of heme-precursor abnormalities associated with that individual's symptoms. Although the biochemical abnormalities of some porphyrias may return to normal ranges during periods of clinical remission, such normalization generally requires at least several days or weeks, if it occurs at all. Given the typical frequency of symptom episodes in individuals with MCS syndrome, and if a disturbance of heme synthesis is an operative pathophysiologic mechanism, the timing of specimen collection should not be an issue.

To date, the efforts to relate MCS syndrome and other unexplained chemical-associated illnesses to disturbances of heme synthesis have all involved selected case series with no consistent or stated case definitions, using only laboratory reference ranges for comparisons, and with little or no attention to the methodologic and interpretive limitations of measures of heme synthesis. Based on the limited existing data, it is reasonable at least to consider the possibility that there might be a higher than previously recognized prevalence of abnormalities in measures of heme synthesis among patients with MCS syndrome. However, to date there is no convincing evidence that there is—or is not—any such increased prevalence of abnormal measures of heme synthesis associated with MCS syndrome. Given the current paucity

of evidence in favor of disturbances of heme synthesis being operative in MCS syndrome and other unexplained chemical-associated illnesses, and unless supportive evidence can be provided by well designed and controlled studies, the proposed relationships with disturbances of heme

synthesis should, at most, be considered speculative and unestablished. In cases involving these otherwise unexplained illnesses, and when there is no demonstrable substantial accumulation or increased excretion of heme precursors, it is premature or unfounded either to apply a diagnosis of

porphyria (or any less defined pathologic disturbance of heme synthesis) or to use such diagnoses as specific justification for lifestyle alterations or therapeutic interventions; porphyria-specific interventions, such as intravenous hematin, should not be recommended in such instances.

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