

## INVITED REVIEW

### ALCOHOL AND PORPHYRIN METABOLISM

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**Abstract** — Alcohol is a porphyrinogenic agent which may cause disturbances in porphyrin metabolism in healthy persons as well as biochemical and clinical manifestations of acute and chronic hepatic porphyrias. After excessive consumption of alcohol, a temporary, clinically asymptomatic secondary hepatic coproporphyrinuria is observable, which can become persistent in cases of alcohol-induced liver damage. Nowadays, the alcohol–liver–porphyrinuria syndrome is the first to be mentioned in secondary hepatic disturbances of porphyrin metabolism. Acute hepatic porphyrias (acute intermittent porphyria, variegate porphyria and hereditary coproporphyrinuria) are considered to be molecular regulatory diseases, in contrast to non-acute, chronic hepatic porphyria, clinically appearing as porphyria cutanea tarda (PCT). Porphyrins do not accumulate in the liver in acute porphyrias, whereas in chronic hepatic porphyrias they do. Thus, chronic hepatic porphyria is a porphyrin-accumulation disease, whereas acute hepatic porphyrias are haem-pathway-dysregulation diseases, characterized in general by induction of  $\delta$ -aminolevulinic acid synthase in the liver and excessive stimulation of the pathway without storage of porphyrins in the liver. The clinical expression of acute hepatic porphyrias can be triggered by alcohol, because alcohol augments the inducibility of  $\delta$ -aminolevulinic acid synthase. In chronic hepatic porphyrias, however, which are already associated with liver damage, alcohol potentiates the disturbance of the decarboxylation of uro- and heptacarboxyporphyrinogen, which is followed by a hepatic accumulation of uro- and heptacarboxyporphyrin and their sometimes extreme urinary excretion. Especially in persons with a genetic deficiency of uroporphyrinogen decarboxylase, but also in patients with the so-called sporadic variety of PCT, alcohol is able to transform an asymptomatic coproporphyrinuria into PCT. Alcohol has many biochemical and clinical effects on porphyrin and haem synthesis both in humans and laboratory animals. Ethanol suppresses the activity of porphobilinogen synthase (synonym:  $\delta$ -aminolevulinic acid dehydratase), uroporphyrinogen decarboxylase, coproporphyrinogen oxidase and ferrochelatase, whereas it induces the first and rate-limiting enzyme in the pathway,  $\delta$ -aminolevulinic acid synthase and also porphobilinogen deaminase. Therefore, teetotalism is a therapeutically and prophylactically important measure in all types of hepatic porphyrias.

#### INTRODUCTION

'Ethanol should be included in the list of drugs metabolised by hepatic microsomes' (Rubin and Lieber, 1972). Such drugs stimulate their own metabolism, in which haem synthesis is involved, and affect porphyrin synthesis in various ways. Alteration of porphyrin synthesis in an organism without hereditary and acquired toxic disturbances of porphyrin metabolism is an adaptive response. Generally, these adaptive responses caused by alcohol or other drugs in an organism without pre-existent derangements in the haem-biosynthesis sequences do not lead to clinical consequences. But in the genetically determined hereditary disturbance of porphyrin metabolism, alcohol often initiates biochemical and clinical manifestations of the diseases (Doss, 1982).

The association of haem biosynthesis with ethanol ingestion has been studied since 1935 when Franke and Fikentscher reported that ethanol consumption increases urinary porphyrin excretion, which was found to be approximately doubled after ingestion of 1 l of beer or 90 ml of cognac (Franke and Fikentscher, 1935). Later on, alcohol-mediated coproporphyrinuria was studied by Brugsch (1937), and has been the subject of detailed investigations by several groups ever since (Sutherland and Watson, 1951; Orten *et al.*, 1963; Elder, 1976; McEwin, 1976; Doss, 1980; McColl *et al.*, 1980; Sieg *et al.*, 1991; Schoenfeld *et al.*, 1996). Additional research on the interrelationships between alcohol and porphyrin metabolism

has been enforced by clinical observations. On the one hand, alcohol consumption, eruption of cutaneous lesions and increase of uroporphyrin excretion are closely correlated (Shanley *et al.*, 1969; Doss *et al.*, 1971; Habermann *et al.*, 1975), whereas, on the other hand, alcohol is recognized as one of the 'porphyrinogenic' drugs for the exacerbation of acute hepatic porphyrias (Goldberg and Rimington, 1962; Bloomer, 1976; Wetterberg, 1976; Eales, 1979; Moore, 1980).

Indeed, certain drugs and chemicals, heavy metals and alcohol are known to be significant and frequent factors in the induction and maintenance of subclinical and clinical disturbances of porphyrin metabolism, including hepatic porphyrias (Sieg *et al.*, 1991). Especially acute hepatic porphyrias are triggered by drugs and/or alcohol and have therefore been designated as 'pharmacogenetic diseases' (Kalow, 1962). In addition, alcohol is the most frequent manifesting and aggravating factor in chronic hepatic porphyria, including porphyria cutanea tarda.

The clinical–biochemical interactions between alcohol and porphyrin/haem biosynthesis involve three main aspects: (1) inhibition and stimulation of certain enzymes in the haem-biosynthetic pathway; (2) triggering the biochemical and clinical manifestations of acute and chronic hepatic porphyrias; (3) development of symptomatic disturbances in porphyrin metabolism as secondary coproporphyrinuria and secondary protoporphyrinaemia.

#### CLASSIFICATION AND DIAGNOSIS OF PORPHYRIAS

The porphyrias are a heterogeneous group of disorders of haem biosynthesis in which specific patterns of overproduction of haem precursors are associated with characteristic

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Table 1. Classification of the major human porphyrias

Porphyria	Enzyme deficiency	Mode of genetic transmission	Phenotypic expression of the genetic defect	Clinical expression	Biochemical findings <sup>a</sup>		
					Urine	Faeces	Erythrocytes
<b>Erythropoietic</b> Congenital erythropoietic porphyria	Uroporphyrinogen III synthase	Autosomal recessive	Bone marrow	Photosensitivity	Uroporphyrin I, coproporphyrin I	Coproporphyrin I	Uro- and coproporphyrin, Zn-Protoporphyrin
Erythropoietic and erythrohepatic protoporphyria	Ferrochelatase	Autosomal dominant	Bone marrow and liver	Photosensitivity	Coproporphyrin <sup>b</sup>	Protoporphyrin	Protoporphyrin
<b>Hepatic</b> <b>Acute hepatic porphyrias</b> δ-Aminolevulinic acid dehydratase porphyria Acute intermittent porphyria (AIP)	ALA dehydratase PBG deaminase	Autosomal recessive Autosomal dominant	Liver Liver	Viscero-neurological Viscero-neurological	ALA, Coproporphyrin ALA, PBG, Uro- and coproporphyrin	— Protoporphyrin (??)	Zn-Protoporphyrin Absent
Hereditary coproporphyria	Coproporphyrinogen oxidase	Autosomal dominant	Liver	Viscero-neurological, photosensitivity	ALA, PBG, coproporphyrin	Coproporphyrin III	Absent
Variagate porphyria	Protoporphyrinogen oxidase	Autosomal dominant	Liver	Viscero-neurological, photosensitivity	ALA, PBG, coproporphyrin	Protoporphyrin, coproporphyrin III	Absent
<b>Chronic hepatic porphyrias</b> Porphyria cutanea tarda	Uroporphyrinogen decarboxylase	Variable <sup>c</sup>	Liver	Photosensitivity	Uro- and hepta-carboxyporphyrin	Isocoporphyrin	Absent
Hepatoerythropoietic porphyria (HEP)	Uroporphyrinogen decarboxylase	Autosomal recessive	Liver and bone marrow	Photosensitivity, viscero-neurological	Uro- and hepta-carboxyporphyrin	Isocoporphyrin	Zn-Protoporphyrin

<sup>a</sup>Only major diagnostic findings are listed.<sup>b</sup>In hepatobiliary course.<sup>c</sup>Autosomal dominant inheritance has been documented in some families but not in others.  
ALA, δ-aminolevulinic acid; PBG, porphobilinogen.

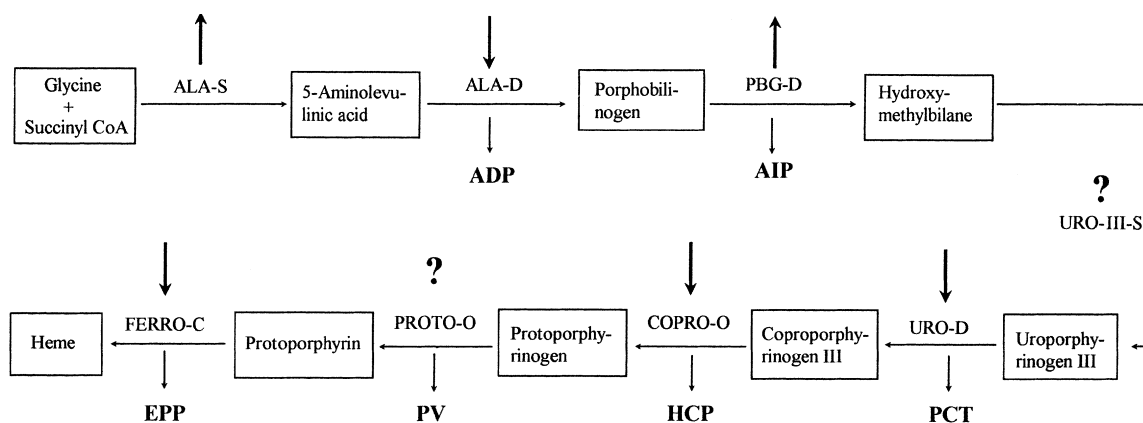


Fig. 1. Alcohol effects on the enzymatic chain of haem biosynthesis.

Abbreviations: (a) enzymes: ALA-S,  $\delta$ -aminolevulinic acid synthase; ALA-D,  $\delta$ -aminolevulinic acid dehydratase; PBG-D, porphobilinogen deaminase; URO-III-S, uroporphyrinogen III synthase; URO-D, uroporphyrinogen decarboxylase; COPRO-O, coproporphyrinogen oxidase; PROTO-O, protoporphyrinogen oxidase; FERRO-C, ferrochelatase; (b) porphyria: ADP,  $\delta$ -aminolevulinic acid dehydratase deficiency porphyria; AIP, acute intermittent porphyria; PCT, porphyria cutanea tarda; HCP, hereditary coproporphyria; PV, porphyria variegata; EPP, erythropoietic protoporphyria.  $\uparrow$ , induction;  $\downarrow$ , inhibition.

clinical features (Nordmann, 1991; Kappas *et al.*, 1995). The molecular cause of each type of porphyria is the result of a specific decrease in the activity of one of the enzymes of porphyrin and haem biosynthesis and is shown in Table 1 (for an outline of the pathway, see Fig. 1). Acquired toxic porphyrias, such as acute lead poisoning and the so-called sporadic, 'non-hereditary' or 'toxic' type of chronic hepatic porphyria, are possible under certain conditions, too (Elder, 1976; Doss, 1982; Doss *et al.*, 1984; De Matteis, 1998).

Generally, the porphyrias can be differentiated into erythropoietic and hepatic porphyrias, depending on the primary organ in which excessive production of porphyrins or porphyrin precursors takes place (Nordmann, 1991; Doss and Sassa, 1994). From the clinical point of view, a classification into acute porphyrias, characterized by acute attacks with abdominal, neurological and cardiovascular symptoms, and non-acute porphyrias, characterized by photosensitivity, is of value (Doss, 1982; Doss and Sassa, 1994; Kappas *et al.*, 1995). A combination of the symptoms may occur in hereditary coproporphyria and variegate porphyria.

Diagnosis of overt porphyria requires metabolic studies. Enzyme tests do not establish that the actual clinical findings are due to an acute or chronic hepatic porphyria. The enzyme deficiency itself is not the factor causing the illness. Decreased enzyme activity reflects only that the patient carries an enzyme mutation. As clinical experience shows, only in a minority of genetically affected family members does the mutation lead to

disease expression. In establishing the diagnosis of porphyria under clinical circumstances, one has to start with metabolite studies (Hindmarsh, 1998). Thus, diagnosis and differential diagnosis of clinically manifest porphyrias can be made by the measurements of porphyrins and their precursors that are excreted into urine or faeces or that are elevated in erythrocytes (Table 1).

For the acute porphyrias there is little doubt that, in the future, DNA analysis will be the method of choice to detect presymptomatic carriers, and consequently to improve the prevention of the acute clinical expression of these porphyrias.

## GENETICS OF PORPHYRIAS

During recent years, cDNA clones have been obtained for the enzymes of haem biosynthesis and the structures of the corresponding genes have been determined. These advances are transforming our knowledge of haem biosynthesis and are certain to improve both our understanding of the pathogenesis of the porphyrias and the methods for identification of carriers of the genes for these disorders (Elder and Nordmann, 1990; Nordmann *et al.*, 1990). The enzymes of the biosynthetic pathway have all been mapped to specific chromosomes (Table 2). The molecular nature of the enzymatic defect is highly heterogeneous, and multiple defects have been demonstrated in each porphyria.

Table 2. Chromosome location and number of all known mutations in porphyrias

Porphyrias	Enzyme defect	Chromosome location	Mutations
ADP	$\delta$ -Aminolevulinic acid dehydratase	9q34	4
Acute intermittent porphyria	Porphobilinogen deaminase	11q24	>140
Congenital erythropoietic porphyria	Uroporphyrinogen III synthase	10q25	>20
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase	1q34	10
Hereditary coproporphyria	Coproporphyrinogen oxidase	3q12	8
Porphyria variegata	Protoporphyrinogen oxidase	1q23	7
Protoporphyrinogen oxidase	Ferrochelatase	18q21	>20

ADP,  $\delta$ -aminolevulinic acid dehydratase deficiency porphyria.

### Acute hepatic porphyrias

**Acute intermittent porphyria.** Acute intermittent porphyria is an autosomal dominant disorder resulting from a partial deficiency of porphobilinogen deaminase activity. The different mutations in acute intermittent porphyria include small deletions and insertions, point mutations and RNA splicing defects, which mostly result in the half-normal activity of both the erythroid-specific and housekeeping isoenzymes (Puy *et al.*, 1997). A subtype of acute intermittent porphyria with normal porphobilinogen deaminase activity in the erythrocytes was first described in Finland (Mustajoki *et al.*, 1992). This variant was also observed in Germany (Groß *et al.*, 1996). An animal model such as a transgenic mouse is available for acute intermittent porphyria (Lindberg *et al.*, 1996).

**Porphyria variegata.** The complete genomic sequence of the protoporphyrinogen oxidase gene has been reported (Puy *et al.*, 1996). Mutations in this gene have been shown in South African and French patients with variegate porphyria (Deybach *et al.*, 1996; Warnich *et al.*, 1996).

**Hereditary coproporphyria.** The sequence and the organization of the gene for the coproporphyrinogen oxidase have been elucidated. It is a 14-kilobase gene with seven exons and six introns (Grandchamp *et al.*, 1995). To date, 14 mutations in the heterozygous case of the coproporphyrinogen oxidase gene have been described (Delfau-Larue *et al.*, 1994; Fujita *et al.*, 1994; Martasek *et al.*, 1994; Lamoril *et al.*, 1995, 1997; Schreiber *et al.*, 1997; Susa *et al.*, 1998; Doss *et al.*, 1999).

**$\delta$ -Aminolevulinic acid dehydratase defect porphyria.** This porphyria is inherited in an autosomal recessive way (Doss *et al.*, 1986). Cloning and sequencing of the mutated genes of one patient showed two different point mutations at each allele of the  $\delta$ -aminolevulinic acid dehydratase gene. The expression of one mutation resulted in a nearly inactive enzyme. The other gene defect produced an unstable protein (Ishida *et al.*, 1992).

### Chronic hepatic porphyria

**Porphyria cutanea tarda (PCT).** The clinical cutaneous stage of chronic hepatic porphyria is PCT. PCT is characterized by a chronic hepatic porphyria disease process. The term PCT is used to indicate a heterogeneous group of diseases, all

characterized biochemically by inhibition of liver uroporphyrinogen decarboxylase and consequent accumulation of uroporphyrin and other highly carboxylated porphyrins; and clinically, by cutaneous symptoms of photosensitivity often associated with evidence of liver damage. Most patients have sporadic (type I) PCT, where decreased uroporphyrinogen decarboxylase activity is restricted to the liver, and a familial occurrence is not observed (Table 3). The liver-specific enzyme defect does not appear to be caused by mutation at the uroporphyrinogen decarboxylase locus (Elder, 1998). However, some cases of PCT that are biochemically indistinguishable from the type I form are clustered in families. At present, it is unclear whether these cases, known as type III PCT, represent a distinct form of the disease or indicate a strong inherited contribution, presumably from outside the uroporphyrinogen decarboxylase locus, to the pathogenesis of type I PCT (Elder, 1998). Table 3 also shows that ~20–50% of patients have familial (type II) PCT, in which the uroporphyrinogen decarboxylase enzyme deficiency is present in all tissues and inherited in an autosomal dominant pattern (Held *et al.*, 1989; Doss *et al.*, 1991; Elder, 1998). Clinical penetrance is low, with <10% of affected individuals developing symptoms. Both catalytic activity and concentration of the immunoreactive enzyme are half normal in erythrocytes, indicating near to total absence of protein product from the mutant allele. This type of PCT is related to the rare disorder hepatoerythropoietic porphyria, where uroporphyrinogen decarboxylase activity is decreased by  $\geq 75\%$  in all tissues, and sustained overproduction of porphyrins is associated with onset during early childhood of skin lesions that are usually severe and persistent (Elder, 1998).

Recently the complete human gene sequence of the uroporphyrinogen decarboxylase has been elucidated (Moran-Jimenez *et al.*, 1996). It has been shown that type I PCT is not due to mutations at the uroporphyrinogen decarboxylase locus. If inherited factors are involved, other loci must be affected (Garey *et al.*, 1993). A splice site mutation has been reported in patients with type II PCT. This mutation causes exon 6 deletion from the corresponding mRNA. It appears to be common, since it was found in five of 22 unrelated pedigrees of type II PCT patients (Garey *et al.*, 1990). In type III PCT (Held *et al.*, 1989), erythrocyte uroporphyrinogen

Table 3. Differentiation of porphyria cutanea tarda (PCT) according to the variations of uroporphyrinogen decarboxylase deficiencies

Type of PCT and mode of inheritance	Uroporphyrinogen decarboxylase activity (% of control mean)		No. of patients (%)	References
	Erythrocytes	Liver		
Sporadic PCT (Type I) (acquired, usually associated with exposure to oestrogens or alcohol)	100	39–57	~50	Doss <i>et al.</i> (1980)
	100	16–47	~78	Elder (1998)
Familial PCT (Type II) (autosomal dominant)	50	ND	~50	Doss <i>et al.</i> (1991)
	41–57	29–58	~22	Elder (1998)
	50	ND	~28	Held <i>et al.</i> (1989)
PCT Type III (familial: inheritance not established)	100	6–15	<5	Elder (1998)
Hepatoerythropoietic porphyria (homozygote or compound heterozygotes, familial PCT, autosomal recessive)	3–28	22	—	
Toxic PCT (acquired or toxogenetic, induced by hexachlorbenzol and TCDD)	50	ND	—	Doss, 1986 <sup>a</sup>

<sup>a</sup>Family study: toxogenetic PCT induced by TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin). ND, not determined.

decarboxylase activity and concentration are normal, but hepatic uroporphyrinogen decarboxylase activity is decreased.

### *Erythropoietic porphyria*

*Erythropoietic protoporphyria.* Erythropoietic protoporphyria is associated with a partial deficiency of ferrochelatase activity. In most cases, it is inherited in an autosomal dominant fashion with a variable degree of penetrance. A *de-novo* mutation, two deletions and two point mutations were detected in Finnish erythropoietic protoporphyria families (Henriksson *et al.*, 1996). A family with autosomal dominant erythropoietic protoporphyria and coinheritance of a low output normal ferrochelatase allele and a mutant allele has been reported from the French porphyria centre (Gouya *et al.*, 1996), suggesting a more complex mode of inheritance. Recently it has been shown that coinheritance of a ferrochelatase gene defect and a wild-type low expressed allele is generally involved in the clinical expression of erythropoietic protoporphyria (Gouya *et al.*, 1999). A compound heterozygous, autosomal recessive form of erythropoietic protoporphyria has been reported (Sarkany *et al.*, 1994). This form of protoporphyria is characterized by severe photosensitivity and cholestatic liver disease in adolescence (Sarkany and Cox, 1995).

*Congenital erythropoietic porphyria.* Congenital erythropoietic porphyria is a rare autosomal recessive disorder of porphyrin metabolism, which can be homozygous or compound heterozygous at the molecular level (Kappas *et al.*, 1995). The uroporphyrinogen III synthase enzyme deficiency leads to an excessive production, accumulation and excretion of predominantly type I porphyrins, which clinically induce severe cutaneous photodermatitis, mostly in association with a haemolytic process (Freesemann *et al.*, 1998). In recent papers, a progress in gene therapy in congenital erythropoietic porphyria is discussed. French authors have been successful in repairing the mutation of the uroporphyrinogen III synthase in bone marrow cells by retroviral vectors (Mazurier *et al.*, 1997).

A mutagenic effect of alcohol on the enzymes of haem biosynthesis is unknown. The action of alcohol on porphyrin biosynthesis is due to an induction and inhibition of several enzymes. The consequence may be a clinical porphyria process with an acute manifestation in the case of a genetic disposition.

## SECONDARY PORPHYRINOPATHIES

The disturbances of porphyrin metabolism are principally differentiated into porphyrias and secondary porphyriopathies, that is, porphyrinurias and porphyriaemias (Doss, 1987). Such secondary porphyrinurias are generally confined to excessive urinary coproporphyrin without uroporphyrinuria,  $\delta$ -aminolevulinic aciduria, or porphobilinogenuria, and secondary porphyriaemias to excessive zinc-protoporphyrin concentration in erythrocytes (Doss, 1987).

Secondary coproporphyrinuria is the most frequent secondary hepatic disturbance of porphyrin metabolism (Doss and Groß, 1997). A reasonable definition and description of porphyrinuria was proposed by Goldberg and Rimington (1962) as follows: 'In the past the term 'porphyria' was reserved for those diseases of porphyrin metabolism caused

Table 4. Various causes and conditions related to secondary coproporphyrinurias

(1)	Intoxications: alcoholism (acute and/or chronic); foreign and environmental chemicals, such as hexachlorobenzene, polyhalogenated biphenyls, dioxins (TCDD), vinyl chloride, carbon tetrachloride, benzene, chloroform; heavy metals such as lead, arsenic, mercury, etc.; drugs
(2)	Liver diseases: e.g. cirrhosis, hepatitis, fatty liver, cholestasis, alcoholic liver syndromes, drug injury, cholangitis, haemochromatosis
(3)	Haematological diseases: e.g. haemolytic, drug-induced, sideroachrestic, sideroblastic, aplastic anaemias; ineffective erythropoiesis (intramedullary haemolysis); pernicious anaemia; thalassaemia; leukaemia, erythroblastosis
(4)	Infectious diseases: acute poliomyelitis
(5)	Diabetes mellitus
(6)	Disturbance of iron metabolism: e.g. haemosiderosis, idiopathic haemochromatosis
(7)	Hereditary hyperbilirubinaemias: e.g. Dubin-Johnson syndrome, Rotor's syndrome
(8)	Malignancies: e.g. hepatocellular tumours, hepatic metastases, pancreatic carcinoma, lymphomatosis and other neoplastic processes
(9)	Myocardial infarction
(10)	Adverse effects of drugs: analgesics, sedatives, hypnotics, antibiotics, sex hormones, i.e. oestrogens and oral contraceptives, and narcosis
(11)	Pregnancy
(12)	Fasting

by an 'inborn error of metabolism', that is, a genetic defect. The term 'porphyrinuria' should be confined to those minor disorders of porphyrin metabolism caused by another disease, or certain drugs or chemicals, in which the clinical features are not directly attributable to the porphyrin abnormality.'

Thus, secondary coproporphyrinuria means that an increased excretion of coproporphyrin occurs as the biochemically dominant symptom of a disturbance in porphyrin and haem metabolism during an intoxication, individual condition, or basic disease (Table 4). Secondary coproporphyrinuria is observed in two-thirds of patients with hepatic damage, especially alcoholic liver disease. The alcohol-liver-porphyrinuria syndrome is the first to be mentioned in secondary hepatic disturbances of porphyrin metabolism (Doss and Sieg, 1995).

Treatment of these subclinical disturbances of porphyrin metabolism is not indicated (with the exception of subclinical lead poisoning); instead, the underlying associated disease has to be diagnosed and treated. From the clinical point of view, the overinterpretation of secondary coproporphyrinuria as being hepatic porphyria is the most frequent reason for errors in diagnosis and therapy in this field (Doss, 1987).

## THE INFLUENCE OF ALCOHOL ON ENZYMES OF HAEM BIOSYNTHESIS

The metabolic effect of alcohol on enzymes of haem biosynthesis is very complex (Fig. 1) and has been the subject of numerous studies over many years. In this section, clinical and experimental studies on the influence of alcohol on enzymes in hepatic and non-hepatic tissues will be reviewed.

*δ-Aminolevulinic acid synthase*

$\delta$ -Aminolevulinic acid synthase (ALAS) is the first and rate-limiting enzyme in the haem biosynthetic pathway. It catalyses the condensation of glycine and succinyl CoA to form  $\delta$ -aminolevulinic acid. There are two tissue-specific iso-enzymes, i.e. the non-specific isoform (ALAS-N) expressed ubiquitously and the erythroid-specific isoform (ALAS-E) expressed exclusively in erythroid cells (Doss and Sassa, 1994; Kappas *et al.*, 1995; Harigae *et al.*, 1998). The human ALAS-N gene has been assigned to chromosome 3p21, the ALAS-E gene to a distal subregion of band X p11.2 (Harigae *et al.*, 1998). Biosynthesis of haem in the liver is controlled largely by the production rate of the hepatic isoform of  $\delta$ -aminolevulinic acid synthase. The activity of this enzyme is rate-limiting, and enzyme synthesis is feedback-regulated by the intracellular concentration of haem. In contrast, the regulation of haem synthesis in erythroid cells is not controlled by the erythroid-specific  $\delta$ -aminolevulinic acid synthase isoform alone.

In accord with the differing control of erythroid-specific  $\delta$ -aminolevulinic acid synthase and the hepatic isoform of  $\delta$ -aminolevulinic acid synthase, iron-responsive elements (IRE) have been identified on the mRNA for the erythroid-specific  $\delta$ -aminolevulinic acid synthase. This IRE phenomenon is probably functional, and it implies that the IRE and the IRE-binding protein system is involved in the control of haem biosynthesis during erythroid differentiation (Cox *et al.*, 1991; Dandekar *et al.*, 1991).

*Studies in animals and cell cultures.* The effect of alcohol on the hepatic  $\delta$ -aminolevulinic acid synthase on both animals and cell cultures has been studied over many years. Shanley *et al.* (1968), Beattie *et al.* (1973), Moore (1974) and Held (1977) reported that ethanol is a well known porphyrinogen, and it is induced by ~3-fold hepatic  $\delta$ -aminolevulinic acid synthase activity in laboratory animals as well as in cultured rat hepatocytes (Lane and Stewart, 1983). Held (1977) showed that after acute alcohol ingestion (a single dose of 2 g alcohol/kg body weight) the activity of  $\delta$ -aminolevulinic acid synthase in the liver of starved rats increased 2–3-fold within 3 h, and by ~4-fold after chronic alcohol consumption (drinking an 8% alcohol solution instead of water for 16–21 and 56 days). Consequently, porphyrin synthesis was stimulated. A previous injection of pyrazole could abolish the inducing effect of alcohol on the  $\delta$ -aminolevulinic acid synthase. The inducing effect of alcohol was increased by a previous injection of phenobarbital (Held, 1977). The activity of hepatic  $\delta$ -aminolevulinic acid dehydratase, the concentration of cytochrome *P*-450 and the activity of the microsomal monooxygenase were not altered in these animals (Held, 1977), in contrast to a decrease in hepatocytes, where ethanol treatment also caused a transient decrease in intracellular haem (Lane and Stewart, 1983). Teschke *et al.* (1987) studied the effect of prolonged alcohol consumption on hepatic haem and porphyrin metabolism. Female Wistar rats were fed for 60 days a nutritionally adequate liquid diet containing 36% of total calories as ethanol, whereas the control diet was isocaloric and contained no alcohol. The administration of the alcohol diet resulted in an increased hepatic activity of  $\delta$ -aminolevulinic acid synthase by 223% ( $112.3 \pm 19.6$  versus  $362.8 \pm 42.5$  nmol/h/100 g body weight;  $P < 0.01$ ), an enhanced urinary excretion of  $\delta$ -aminolevulinic acid by 101% ( $64.8 \pm 11.8$  versus

$130.8 \pm 22.4$  nmol/day;  $P < 0.05$ ), and an augmented urinary output of total porphyrins by 142% ( $1.2 \pm 0.2$  versus  $2.9 \pm 0.5$  nmol/day;  $P < 0.05$ ). Concomitantly, the hepatic content of cytochrome *P*-450 was significantly enhanced and that of hepatic catalase activity marginally increased.

The mechanisms by which ethanol induces the activity of  $\delta$ -aminolevulinic acid synthase are not yet completely elucidated. Different possibilities were discussed. (1)  $\delta$ -Aminolevulinic acid synthase is under negative feedback control by free haem, and the increased activity of this enzyme may indicate reduced intra-mitochondrial concentrations of free haem as a result of either reduced synthesis or increased utilization. (2) The alcohol-related depression of the intermediate enzymes in the pathway might reduce haem synthesis. (3) Increased haem requirement in the liver cells may occur as ethanol enhances the synthesis of the haemoprotein cytochrome *P*-450 (Pennington *et al.*, 1978; Teschke *et al.*, 1987). (4) The porphyrinogenic effect of ethanol may not be caused by alcohol itself, but either by its metabolites acetaldehyde or acetate or by changes of the liver metabolism caused by the effect of alcohol on the redox state of the NAD couple (Shanley *et al.*, 1968; Labbe *et al.*, 1970; Beattie *et al.*, 1973; Held, 1977), because the hepatic metabolism of ethanol alters the intra-mitochondrial redox state.

*Studies in humans.* The effect of alcohol on hepatic  $\delta$ -aminolevulinic acid synthase in humans is comparable to that in animals. In the study of Shanley *et al.* (1969), evidence was presented that, when patients with symptomatic porphyria were challenged with alcohol, liver  $\delta$ -aminolevulinic acid synthase activity is raised, leading to increased urinary porphyrin excretion. Conversely, enforced abstinence from alcohol may induce remission of various degrees. An increased hepatic  $\delta$ -aminolevulinic acid synthase activity was also observed in cirrhotic patients (Bonkowsky and Pomeroy, 1977; Kodama *et al.*, 1983). But this increase does not seem to reach levels expected in acute intermittent porphyria and is not followed by an elevation of urinary  $\delta$ -aminolevulinic acid and porphobilinogen excretion, in contrast to acute hepatic porphyrias (Doss *et al.*, 1972; Bonkowsky and Pomeroy, 1977).

The effect of alcohol on  $\delta$ -aminolevulinic acid synthase in humans is not confined to hepatic tissue. Brodie *et al.* (1979) and McColl *et al.* (1980, 1981) monitored the effects of acute and chronic ethanol ingestion on the activities of the enzymes of haem biosynthesis in peripheral blood cells. Their results demonstrated that ethanol consumption causes an increased activity of leukocyte  $\delta$ -aminolevulinic acid synthase after acute (McColl *et al.*, 1980) as well as chronic (Brodie *et al.*, 1979; McColl *et al.*, 1981) ethanol ingestion. In addition, McColl *et al.* (1980, 1981) investigated the changes in the activity of  $\delta$ -aminolevulinic acid synthase in leukocytes after acute alcohol ingestion in healthy subjects as well as in chronic alcoholics. In healthy non-porphyrin subjects, the ingestion of 200 ml of 69.5% vodka ( $1 \times 316$  mol ethanol) raised the activity of leukocyte  $\delta$ -aminolevulinic acid synthase on average by 110% (McColl *et al.*, 1980) and similar disturbances of haem biosynthesis were observed in chronic alcoholics (McColl *et al.*, 1981).

In the acute reversible sideroblastic anaemia of alcoholics, increased bone marrow  $\delta$ -aminolevulinic acid synthase activity was noted (Fraser and Schacter, 1980). In contrast, patients with sideroblastic anaemia showed diminished  $\delta$ -aminolevulinic acid synthase activity (Bottomley *et al.*, 1973).

The mechanisms by which ethanol induces the activity of  $\delta$ -aminolevulinic acid synthase in humans are similar to those discussed in animal and cell culture studies.

#### *$\delta$ -Aminolevulinic acid dehydratase*

Among those enzymes of porphyrin metabolism which are affected by alcohol,  $\delta$ -aminolevulinic acid dehydratase is the second in the haem-biosynthetic pathway and catalyses the condensation of two  $\delta$ -aminolevulinic acid molecules to porphobilinogen, the immediate precursor of porphyrinogens. Biochemically, this enzyme consists of eight identical subunits and contains sulphhydryl groups and zinc, which are essential for its full activity (Shemin, 1976; Anderson and Desnick, 1979; Sieg *et al.*, 1991).

*Studies in animals and cell cultures.* Regarding the effect of alcohol on  $\delta$ -aminolevulinic acid dehydratase activity in the rat, Moore *et al.* (1971) showed that the maximum alcohol concentration in the blood, reached within 2 h of alcohol intake, corresponded to a decrease in  $\delta$ -aminolevulinic acid dehydratase activity. The effect of ethanol intoxication on  $\delta$ -aminolevulinic acid dehydratase activity has also been measured in other tissues. A significant depression of enzyme activity in blood, liver and kidney, but not in heart or spleen, was shown by Moore *et al.* (1971). These authors concluded that effects of ethanol on  $\delta$ -aminolevulinic acid dehydratase will be localized to those organs known to metabolize or handle ethanol, since it is only in those organs that the NAD redox potential will be sufficiently elevated to repress  $\delta$ -aminolevulinic acid dehydratase activity. These observations confirm the data reported by Abdulla *et al.* (1976), where a considerable decrease in the activity of the liver enzyme in rats after ethanol loading was recognized.

*Studies in humans.* In humans, the effect of alcohol has been well studied for  $\delta$ -aminolevulinic acid dehydratase, where acute ingestion of alcohol has a marked and direct effect on its activity. It was first shown by Moore *et al.* (1971) that the  $\delta$ -aminolevulinic acid dehydratase activity was depressed by ethanol in human blood cells; an effect closely related to the increase in blood alcohol concentration. A more differentiated investigation by Sieg *et al.* (1991) showed differences in the response of this enzyme after cessation of alcohol consumption in normal subjects compared to chronic exposure in alcoholics. Following the administration of alcohol to a group of six normal subjects,  $\delta$ -aminolevulinic acid dehydratase activity was diminished by 44%.  $\delta$ -Aminolevulinic acid dehydratase activity in a group of alcoholic subjects was found to be even more strongly inhibited, by 57% on average. These findings are in agreement with experiments *in vivo* by other authors (Krasner *et al.*, 1974; McColl *et al.*, 1980, 1981; Kondo *et al.*, 1983; Doss, 1985).

An increase of the intracellular NAD redox potential by alcohol and its effects on the redox balance of other compounds within the cell has been regarded as a direct cause of the enzyme inhibition. The augmented redox potential may influence the equilibrium between oxidized and reduced forms of sulphhydryl compounds (Moore *et al.*, 1971; Sieg *et al.*, 1991). On the other hand, the toxicity of acetaldehyde (interaction with amino acids and binding to cystein and/or glutathione) may decrease glutathione concentration in the liver (Lieber, 1980; Sieg *et al.*, 1991). Glutathione depletion may contribute to the change in  $\delta$ -aminolevulinic acid

dehydratase activity. In addition, it may promote peroxidative damage of cellular membranes, which is one pathogenetic factor for the chronic hepatic porphyria (Koszo *et al.*, 1982).

More than 20 years ago, Abdulla *et al.* (1976) reported that the induction of  $\delta$ -aminolevulinic dehydratase by zinc counteracts the inhibitory effect of orally administered ethanol in rats. Later on, *in vitro* assays on human erythrocytes after acute and chronic alcohol ingestion *in vivo* showed that reactivation by dithiothreitol was better than that by zinc ions. The most potent effect on restoration of  $\delta$ -aminolevulinic dehydratase activity occurred under the combined addition of zinc and dithiothreitol (Sieg *et al.*, 1991). The easily performed reactivation of this enzyme *in vitro* is an additional help in recognizing and excluding irreversible inhibitory effects, such as those caused by styrene (Fujita *et al.*, 1987) or hereditary enzyme deficiency (Doss *et al.*, 1984; Sieg *et al.*, 1991). Additionally, the extent of lead intoxication, which also induces a reversible  $\delta$ -aminolevulinic dehydratase inhibition, may be diagnosed (Polo *et al.*, 1995).

Erythrocyte  $\delta$ -aminolevulinic dehydratase activity was also assessed for outpatient detection of alcoholic liver disease and compared with  $\gamma$ -glutamyltransferase and casual blood ethanol (Hamlyn *et al.*, 1979). These authors found that the highest specificity for alcoholism was achieved by  $\delta$ -aminolevulinic dehydratase; the best overall performance, with highest sensitivity and specificity, was, however,  $\gamma$ -glutamyltransferase. Erythrocyte  $\delta$ -aminolevulinic dehydratase activity may also be regarded as a sensitive biochemical indicator for chronic alcohol consumption, as has been proposed by Krasner *et al.* (1974), and also for acute alcohol intake (Sieg *et al.*, 1991). This suggestion differed from the result reported by Aubin *et al.* (1997), where  $\delta$ -aminolevulinic dehydratase showed no advantage over  $\gamma$ -glutamyltransferase as a marker for recent alcohol intake.

The effect of alcohol on  $\delta$ -aminolevulinic dehydratase activity in the liver has not been studied so far. However, there is evidence for a similar reaction of liver and erythrocyte  $\delta$ -aminolevulinic dehydratase after alcohol uptake (Sieg *et al.*, 1991).

#### *Porphobilinogen deaminase*

*Studies in humans.* Acute alcohol ingestion in healthy subjects results in an increased activity of porphobilinogen deaminase in erythrocytes (McColl *et al.*, 1980). An increased activity was also observed in chronic alcoholics (McColl *et al.*, 1981). More recently, the activity of porphobilinogen deaminase in erythrocytes of alcoholics was assayed and compared to those in controls and abstaining alcoholics (Wright *et al.*, 1990). Confirming the results obtained by McColl *et al.* (1981), the porphobilinogen deaminase activity in alcoholics was found to be significantly higher than that of controls and significant correlations between enzyme activity and mean corpuscular volume (MCV) could also be observed. Alcoholics abstaining from alcohol over a 9-month period showed a return to normal enzyme activity paralleling the normalization of their MCV (Wright *et al.*, 1990).

It is not known if the results obtained in peripheral blood cells are applicable to the liver and consequently have impact on the regulatory disturbance in porphyria gene carriers and patients.

### *Uroporphyrinogen decarboxylase*

*Studies in animals and humans.* Uroporphyrinogen decarboxylase is reduced in experimental chronic hepatic porphyria (Elder *et al.*, 1976; von Tiepermann *et al.*, 1980) and in human chronic hepatic porphyria, clinically appearing as PCT (Doss *et al.*, 1980; Doss, 1982, Doss and Sassa, 1994). In order to find out if alcohol can be the exogenous toxic cause of chronic hepatic porphyria, via an inhibition of uroporphyrinogen decarboxylase, Doss *et al.* (1980, 1981) examined the influence of alcohol consumption on the enzyme activity in hepatic and non-hepatic tissues of female Sprague–Dawley rats. Chronic alcohol intake inhibited uroporphyrinogen decarboxylase activity in liver and spleen, associated with a pathological porphyrinuria (Doss *et al.*, 1980, 1981). McColl *et al.* (1980, 1981) showed that, in humans, acute and chronic alcohol consumption inhibits uroporphyrinogen decarboxylase activity in erythrocytes.

Alcohol is an important manifestation factor in all types of PCT (see Table 3). One can assume that persons possessing the genetic variant of uroporphyrinogen decarboxylase deficiency may be more sensitive (Doss, 1985; Hift and Kirsch, 1995). Alcohol omission leads to a decline in porphyrin excretion (Topi *et al.*, 1984).

The mechanisms whereby alcohol and other causes of hepatocyte damage, such as hepatitis C (HCV) infection and oestrogens, provoke PCT in susceptible individuals have not been established. Recent studies show that hepatotoxins such as alcohol, HCV and other infections may release stored iron from ferritin in a form that catalyses the formation of reactive oxygen species and thus switch on the inactivation. Iron inhibits uroporphyrinogen decarboxylase: (a) by direct interaction with the essential sulphhydryl groups of the enzyme; (b) indirectly, due to generation of free radicals in the presence of oxygen and an electron donor such as cysteine (Mukerji *et al.*, 1984; Hift and Kirsch, 1995; Elder, 1998). These radicals might interact directly with the enzyme and/or oxidize the porphyrinogen substrates to non-metabolizable porphyrins, which accumulate in porphyric patients (Mukerji *et al.*, 1984, 1997; De Matteis, 1998).

### *Coproporphyrinogen oxidase*

*Studies in humans and animals.* Coproporphyrinogen oxidase is the sixth enzyme of the haem-biosynthetic pathway. This soluble protein is localized in the intermembrane space of mitochondria and catalyses the conversion of two propionate groups at positions 2 and 4 of coproporphyrinogen III to two vinyl groups of protoporphyrinogen IX (Doss and Sassa, 1994; Martasek *et al.*, 1994). The effects of chronic and acute ethanol treatments on the enzyme seem to be different, as the coproporphyrinogen oxidase activities in leukocytes were found unchanged in chronic alcoholics (McColl *et al.*, 1981) and depressed in healthy humans after acute ethanol ingestion (McColl *et al.*, 1980). In a study performed by Li *et al.* (1989) the specific and total activity of coproporphyrinogen oxidase have been assayed in human liver from healthy control subjects and patients with alcoholic liver disease. Statistical analysis showed no significant differences in enzyme specific activity or total activity between the chronic alcoholics with fatty liver and control subjects. Later, Li *et al.* (1990) studied the influence of alcohol on coproporphyrinogen oxidase in

chronically and acutely ethanol-treated rats. In contrast to the results reported by McColl *et al.* (1980), both chronic ethanol feeding and acute ethanol injection (fasting or non-fasting) showed no effects on the activity of coproporphyrinogen oxidase.

It seems probable that the molecular injury to mitochondria produced by ethanol and acetaldehyde changes the activity of this hepatic enzyme, resulting in a pathological coproporphyrinuria; alcohol interferes with functions of the inner mitochondrial membrane (Cederbaum and Rubin, 1975). The change in the redox state of the cell with an increase in the hepatic [NADH]/[NAD<sup>+</sup>] ratio therefore cannot serve as an explanation of coproporphyrinuria, as NAD<sup>+</sup> is not a cofactor of mammalian coproporphyrinogen oxidase (Doss, 1985). On the other hand, coproporphyrinogen oxidase is highly sensitive to changes in oxygen concentrations (Keithly and Nudler, 1983). Factors affecting the metabolism of coproporphyrinogen III are intriguing (Elder, 1976): a decrease in the amount of the active enzyme as well as competitive inhibition has been discussed in connection with an increased oxidation and decreased uptake of coproporphyrinogen by the inner membrane of the mitochondrion.

### *Protoporphyrinogen oxidase*

Protoporphyrinogen oxidase activity in both acutely and chronically ethanol-treated rats was not significantly different from controls (Li *et al.*, 1990). Also, in patients with alcoholic fatty liver the specific and total activities of protoporphyrinogen oxidase in the liver seem to be unchanged (Li *et al.*, 1989).

### *Ferrochelatase*

*Studies in animals and cell cultures.* Ferrochelatase, the enzyme that inserts iron into protoporphyrin to form haem, shows a marked depression after administration of ethanol. Decreased ferrochelatase activity was found in acutely ethanol-dosed rats (Moore, 1974, 1998; Moore *et al.*, 1984). In chronically ethanol-fed rats normal ferrochelatase activity was found (Li *et al.*, 1990). The activity in acutely ethanol-treated rats was not measured in the last-named study.

*Studies in humans.* A significant suppression of leukocyte ferrochelatase activity in healthy humans after acute ethanol ingestion was reported by McColl *et al.* (1980). A depression of the activity of ferrochelatase was also found in hepatic tissue. In chronic alcoholics, however, ferrochelatase was reported normal (McColl *et al.*, 1981). This agrees with the results reported by Li *et al.* (1989) that show that, in chronic alcoholic patients who consumed between 80 and 180 g of ethanol per day, the ferrochelatase activity was not significantly different from controls.

## ALCOHOL AS INDUCING FACTOR OF HEREDITARY PORPHYRIAS

Toxic strains on the organism caused by certain chemicals, heavy metals, drugs or alcohol affect the haem pathway functionally. The extent of the toxic effect on porphyrin and haem biosynthesis in the liver depends not only on the quantity and duration of the precipitating factor but also on the type of cell that is influenced (a healthy cell, one that is already damaged by other processes or a cell with hereditary enzyme



Table 5. Effects of alcohol on the biochemical and clinical expression of hepatic porphyrias

Phase	Acute/chronic hepatic porphyrias	Alcohol
A	Genetic disposition	
↓		↓
B	Latency compensated	↓↓
↓		↓↓↓
C	Latency decompensated	↓↓↓
↓		↓↓↓
D	Clinical expression	

defects of haem biosynthesis). Since alcohol affects haem biosynthesis even in healthy organisms, its effect on an already genetically damaged synthesis must be stronger than on intact control mechanisms of the molecular feedback of haem/ $\delta$ -aminolevulinic acid synthase in the liver (Goldberg *et al.*, 1981). In the following sections, the influence of alcohol on the clinical manifestation of the porphyrias based on hereditary defects of porphyrin metabolism will be described.

#### Acute hepatic porphyrias

Acute hepatic porphyrias are 'regulatory diseases' with pharmacogenetic implications due to the enhanced inducibility of  $\delta$ -aminolevulinic acid synthase (Doss, 1982). A variety of factors may precipitate the clinical attacks in patients with the genetic defect in each of the acute porphyrias. The most important precipitating factors are drugs, hormones, calorie-reduced diet and alcohol (Nordmann, 1991; Doss and Sassa, 1994; Groß *et al.*, 1995; Kappas *et al.*, 1995; Moore, 1998). In our previous aetiological study of 100 patients with acute hepatic porphyrias, alcohol was in second position as a causative factor (16%). Drugs, including oestrogens, were responsible for about half of the clinical exacerbations. Fasting together with alcohol led to manifestations of the acute phase in 5% of the cases.

In the biochemical and clinical manifestation of hepatic porphyrias from disorder to disease triggered by alcohol, four phases can be differentiated (Table 5). A single consumption of alcohol (>80 g) or chronic alcohol abuse may shift acute hepatic porphyrias from the compensated latent phase into the decompensated latent phase (with remarkable increase of porphyrin precursor and porphyrin excretion without overt disease) and from the decompensated latent phase into the clinically manifest stage with abdominal, neurological, cardiovascular and psychiatric syndromes. Doss *et al.* (1982) reported that patients in the decompensated latent phase with high excretory profiles of haem precursors are especially endangered. Alcohol intake of >60 g/day can increase urinary  $\delta$ -aminolevulinic acid, porphobilinogen, and porphyrin excretion up to 3–6-fold (Table 6), leading to an acute exacerbation (Doss *et al.*, 1982). Recently, a patient was reported suffering from  $\delta$ -aminolevulinic acid dehydratase deficiency in the acute crisis that was associated with an excessive intake of alcohol (300 g in one day). Urinary excretion of  $\delta$ -aminolevulinic acid, porphobilinogen, and total porphyrins have been 2.6 mmol, 29 and 10.7  $\mu$ mol per day, respectively (Groß *et al.*, 1998).

Agents in alcoholic beverages other than ethanol play important roles in precipitating the porphyric symptoms.

Table 6. Response to alcohol intake in acute porphyrias

Patients, no., sex, age (porphyria)	Day	Alcohol intake (g)	24-h urinary excretion of			
			ALA ( $\mu$ mol)	PBG ( $\mu$ mol)	Uro (nmol)	Copro (nmol)
1, M, 24 (AIP)	1		107	210	627	1067
	2	60	287	430	1073	1376
	3 <sup>a</sup>	100	379	619	2394	2129
			2 weeks after alcohol withdrawal:			
	18	80	94	187	748	232
	19 <sup>a</sup>		418	443	1760	1553
2, F, 16 (AIP)	1	20	260	416	903	689
	2 <sup>a</sup>		397	720	1233	1166
3, M, 23 (ADP)	1	110	84	2	5	2512
	2 <sup>a</sup>		196	3	17	4028
4, M, 25 (ADP)	1	100	150	2	73	2577
	2 <sup>a</sup>		277	7	80	3874
5, F, 35 (AIP)	1		30	61	489	1092
	9		23	23	484	511
6, M, 34 (VP)	1		12	9	102	1092
	11		10	3	63	511 <sup>b</sup>
Normal levels			<49	<8	<30	<120

M, male; F, female; AIP, acute intermittent porphyria; VP, variegate porphyria; ADP,  $\delta$ -aminolevulinic acid dehydratase deficiency porphyria; ALA,  $\delta$ -aminolevulinic acid; PBG, porphobilinogen; Uro, uroporphyrin; Copro, coproporphyrin.

<sup>a</sup>Expression of acute symptoms.

<sup>b</sup>In addition, fecal protoporphyrin decreased from 970 to 360 nmol/g (normal <150 nmol/g dry weight).

Polyphenolic compounds and 3- to 5-carbon chain hydrophobic alcohols (for example present in whisky and red wine) may be responsible for the induction of clinical symptoms in acute porphyrias. Furthermore, combination of alcohols at proportions present in brandy and American light beer cause higher induction of  $\delta$ -aminolevulinic acid synthase than does ethanol alone (Thunell *et al.*, 1992).

Alcohol increases the absorption of drugs like phenobarbital and inhibits the rates of disappearance of the porphyrinogenic drugs phenobarbital, tolbutamide and meprobamate *in vivo* in man and animals (Mezey, 1976). Inhibition of drug metabolism results from direct interference with alcohol. Acceleration of drug metabolism is due to the induction of microsomal enzymes by ethanol and can increase toxicity (Mezey, 1976). Both actions provide a synergistic basis for the expression of acute porphyrias as a pharmacogenetic disease. Alcohol increases the induction of  $\delta$ -aminolevulinic acid synthase in rats pre-treated with phenobarbital (Held, 1977). A sensitization of hepatic haem biosynthesis by the drug to the porphyrinogenic effect of alcohol is suggested. Drugs potentiate the porphyrinogenic effect of alcohol and vice versa in patients with the genetic enzyme defects of acute hepatic porphyria.

#### Porphyria cutanea tarda

Porphyria cutanea tarda is the most frequent porphyria, and is regularly associated with liver lesions such as fatty liver, chronic hepatitis, fibrosis, cirrhosis or siderosis (Lefkowitz and Grossmann, 1983; Cripps, 1987; Köstler and Doss, 1993; Freeseemann *et al.*, 1995). The disease-promoting site of the pathological porphyrin metabolism is the liver, therefore PCT

is described as chronic hepatic porphyria (Doss, 1990). Hepatic uroporphyrinogen decarboxylase activity is decreased in all patients, however the activity of this enzyme in blood cells and in other tissues is decreased only in the genetically determined type (Doss *et al.*, 1980). Skin symptoms characterize the biochemically and clinically manifest stage. Latent or subclinical phases in which urinary porphyrin constellations change from mild to severe stages can be recognized before cutaneous lesions arise. The development of chronic hepatic porphyria is outlined in Figure 2. In a course of gene-environment interaction (alcohol, oestrogens and hormonal contraceptives, polyhalogenated organocompounds etc.), the chronic hepatic porphyria disease process is initiated (Doss *et al.*, 1991). Alcohol is the factor most commonly associated with the development of porphyria cutanea tarda in humans (Habermann *et al.*, 1975; Grossmann *et al.*, 1979; Hift and Kirsch, 1995; De Matteis, 1998). According to our own investigations, alcohol was the causative factor in about two-thirds of the cases (Doss, 1982). Alcohol consumption correlates positively with total porphyrin excretion and with the excretion of urocarboxyporphyrin and heptacarboxyporphyrin (Doss *et al.*, 1971). The strong association of alcohol with PCT could be explained by the inhibiting effect alcohol has on uroporphyrinogen decarboxylase and by inducing

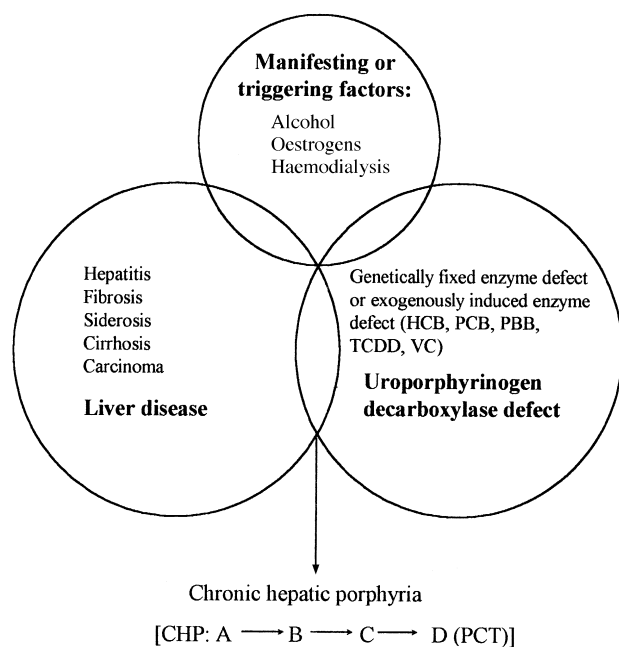


Fig. 2. Pathobiochemical development of the chronic hepatic porphyria (CHP) disease process.

The diagram includes the coincidence of endogenous, especially genetic, and exogenous, especially toxic, factors. Hepatic uroporphyrinogen decarboxylase activity is decreased in all conditions; however, the activity of this enzyme in blood cells and other tissues is decreased only in the genetically determined type of chronic hepatic porphyria or porphyria cutanea tarda (PCT). Abbreviations: HCB, hexachlorobenzene; PCB, polychlorinated biphenyls; PBB, polybromated biphenyls; TCDD, tetrachlorodibenzo-*p*-dioxin; VC, vinyl chloride.

$\delta$ -aminolevulinic acid synthase (Doss, 1985; Hift and Kirsch, 1995). However, recent studies show that its contribution to the development of PCT may be indirect, perhaps by precipitating PCT by an iron-dependent mechanism (Hift and Kirsch, 1995; De Matteis, 1998).

In addition to alcohol, the disease may easily be induced — in both humans and animals — by polyhalogenated hydrocarbons, such as hexachlorobenzene, polychlorinated and polybrominated biphenyls and dioxins (Elder *et al.*, 1976; Strik and Doss, 1978; von Tiepermann *et al.*, 1980; Doss *et al.*, 1984; Doss, 1985; Jones and Chelsky, 1986; Cripps, 1987; Calvert *et al.*, 1994; Hift and Kirsch, 1995; Hahn and Bonkovsky, 1997). These agents can cause liver damage as well as an uroporphyrinogen decarboxylase defect, the extent of which depends on the period of intoxication (Köstler and Doss, 1993; Hift and Kirsch, 1995). Alcohol, with respect to its toxic potential in the pathobiochemical development and clinical manifestation of a chronic hepatic porphyria, is less porphyrinogenic than hexachlorobenzene. This was shown by comparing the findings in metabolite and enzymatic changes after chronic alcohol ingestion (Doss *et al.*, 1981) with those found in hexachlorobenzene porphyria in rats (Elder *et al.*, 1976; von Tiepermann *et al.*, 1980). With hexachlorobenzene-induced porphyria in animals, a concentration of 5% alcohol in the diet initially enhances the porphyrin excretion (Doss, 1985). Spin-labelling investigations have shown that both hexachlorobenzene and alcohol increase the fluidity of the hepatocyte lipid membranes (Koszo *et al.*, 1982). This mechanism and the augmentation of the hepatic microsomal ethanol-oxidizing system (Teschke *et al.*, 1983) may be an important pathogenetic factor in human chronic hepatic porphyria, including PCT, which is regularly associated with liver injury and designated a 'membrane disease' (Doss, 1982).

Additional exacerbating or precipitating factors that have been identified more recently and which appear to play a dominant role in most cases of sporadic PCT are viral infections. Among these, the first viral infection found to be associated with PCT was the one caused by hepatitis B virus (HBV) (Bel and Girard, 1980). A similar effect of human immunodeficiency virus infection has been reported by Wissel *et al.* (1987), Lobato and Berger (1988), Blauvelt *et al.* (1992) and Soriano *et al.* (1993). However, the viral infection that is described most frequently in connection with PCT is the HCV. Approximately 60–90% of patients from Italy, Spain, and France who had PCT were HCV-seropositive (Fargion *et al.*, 1992; Ferri *et al.*, 1993; Lacour *et al.*, 1993; Cribier *et al.*, 1995; Navas *et al.*, 1995; Blauvelt, 1996; Martinez *et al.*, 1996; Quecedo *et al.*, 1996). However, studies in Germany (Stölzel *et al.*, 1995) and New Zealand (Salmon, 1996) showed less correlation between positive anti-HCV antibodies and PCT than those in southern European countries.

#### *Erythropoietic (hepatic) protoporphyria*

Hepatobiliary complications in erythropoietic protoporphyria are not caused by alcohol, but more likely by a toxic effect of protoporphyrin deposited in the liver and the bile duct system (Bloomer, 1982). As alcohol also inhibits hepatic ferrochelatase — which is genetically defective in this disease, and produces liver damage — its ingestion presents a pathogenetic factor for the expression and/or aggravation of the potential hepatobiliary involvement.

## ALCOHOL AND SECONDARY HEPATIC COPROPORPHYRINURIA

The relation between coproporphyrinuria, alcohol and alcoholic liver disease has been known for a long time (Brugsch, 1937; Orten *et al.*, 1963; Doss *et al.*, 1972; Ostrowski *et al.*, 1984). More recently Sieg *et al.* (1991) and Schoenfeld *et al.* (1996) measured the increase in renal coproporphyrin excretion after alcohol intake by healthy patients, as well as in alcoholics with and without liver defects. In the study of Sieg *et al.* (1991), determination of urinary  $\delta$ -aminolevulinic acid, porphobilinogen and coproporphyrin showed increased levels for all non-alcoholics after acute alcohol ingestion (160–230 g/4 h alcohol). Among the group of chronic alcoholics,  $\delta$ -aminolevulinic acid and porphobilinogen excretion remain within the normal range, although the values for coproporphyrin were found to be distinctly or even strongly increased. The study by Schoenfeld *et al.* (1996), on the influence of alcohol on patients with and without liver damage, showed that the extent of the coproporphyrinuria in alcoholics increases with the severity of liver damage. This study also measured the distribution of coproporphyrin isomers I and III in the urine of the participants. Healthy patients showed a preponderance of coproporphyrin isomer III, whereas alcoholics excrete more coproporphyrin isomer I. Among alcoholics with no evidence of hepatocellular damage, the proportion of coproporphyrin isomer I was 55%; in alcoholics with moderate (i.e. fatty liver and impaired function of liver enzymes) and severe liver disease (i.e. liver cirrhosis), this proportion was 80%. The increased coproporphyrin excretion in all three groups (healthy patients and alcoholics with and without liver damage) demonstrates the effect of alcohol on haem metabolism. But the difference in the distribution of coproporphyrin isomers I and III, as well as in the excretion of the porphyrin precursors ( $\delta$ -aminolevulinic acid and porphobilinogen) enabled us to differentiate between the direct primary effect of alcohol on the haem-biosynthetic pathway and the secondary indirect effect, which is probably related to liver damage that follows alcohol consumption (Doss, 1987). Thus,

coproporphyrinuria in patients without liver damage after acute alcohol ingestion is mainly due to a direct inhibition of coproporphyrinogen oxidase in the liver counter-regulated by an increase of  $\delta$ -aminolevulinic acid synthase. On the other hand, the coproporphyrinuria observed in chronic alcoholic liver disease is due to the secondary effect of alcohol. In this case an impairment in the postulated common hepatic carrier transport mechanism caused a reduction in total coproporphyrin transport into bile with consequent diversion of more of the type I than type III isomer compound from biliary to the urinary route of elimination (Kaplowitz *et al.*, 1972; Doss, 1987). Therefore, excessive coproporphyrin isomer I in the urine is a useful sign of acquired intrahepatic cholestasis, especially in alcoholic liver cirrhosis, and also indicates an inherited derangement of bilirubin transport mechanisms.

In chronic liver damage, particularly when alcohol-related, the transition of a secondary coproporphyrinuria to a chronic hepatic porphyria is not rare and is characterized by special urinary porphyrin constellations with high coproporphyrin components (Table 7). Besides, the absolute and especially the relative increase in heptacarboxyporphyrin within the entire porphyrin pathway shows a transition to chronic hepatic porphyria caused by an incipient lack of uroporphyrinogen decarboxylase. The following urinary and hepatic porphyrin ratios are biochemical indicators for the transition of secondary coproporphyrinuria to chronic hepatic porphyria: the urinary uroporphyrin/coproporphyrin ratio as well as the hepatic uroporphyrin/protoporphyrin ratio. Both ratios are <1 in secondary coproporphyrinuria; a progression into chronic hepatic porphyria can be documented by the inversion of these ratios.

Two factors are necessary for the transition of a secondary coproporphyrinuria to chronic hepatic porphyria: (1) genetic disposition of a dominantly inherited autosomal defect in uroporphyrinogen decarboxylase, which becomes subclinically or clinically relevant only through alcohol or oestrogens (including oral contraceptives) in conjunction with liver injury; (2) toxic inhibition or inactivation of uroporphyrinogen decarboxylase in the liver caused by polyhalogenated hydrocarbons in

Table 7. Progression of chronic hepatic porphyria

Condition	Pattern of urinary porphyrins	Total porphyrins ( $\mu\text{mol}/24 \text{ h}$ )	U (%)	7 (%)	C (%)	Porphyrin accumulation in liver (U, 7)	Liver damage	Cutaneous symptoms
Normals	C >>> U >> 7 = 5 > 6 > 3	<0.2	<30	<5	>60	–	–	–
Secondary coproporphyrinuria	C >> U > 5 > 7 > 6 > 3	<1.0	<20	<3	>80	–	+	–
Transition								
CHP A	C > U > 7 > 5 > 6 > 3	<1.0	<40	>5	>40	(+)	+	–
↓			↓	↓	↓			
CHP B	U > C > 7 > 5 > 6 > 3	<1.5	>40	<20	<40	+	+	–
↓			↓	↓	↓			
CHP C	U > 7 > C > 5 > 6 > 3	<2.0	>50	>20	>10	++	+	–/(+)
↓				↓	↓			
CHP D (PCT)	U > 7 >> C > 5 > 6 > 3	>2.5	>50	>20	<10	+++	+	+/++

The progression from latent phases (CHP A, B and C) to clinical porphyria cutanea tarda (PCT) is indicated by the arrows. The transition of secondary coproporphyrinuria to chronic hepatic porphyria (CHP) is induced by alcohol in most cases.

C, coproporphyrin; U, uroporphyrin; 7, heptacarboxyporphyrin; 6, hexacarboxyporphyrin; 5, pentacarboxyporphyrin; 3, tricarboxyporphyrin. ↓, decrease; –, absent; (+), +, ++, +++, present, from a slight to great extent.

combination with liver damage, already present, or caused by one of these agents (Doss, 1980; 1987). Therefore, a coproporphyrinuria should be checked regularly, in order not to miss this transition phase for therapeutic and prophylactic reasons.

### ALCOHOL AND PROTOPORPHYRINAEMIA

Protoporphyrinaemia is of various origins (Labbe and Nielsen, 1976). In alcoholics it can be explained by ferrochelatase inhibition if blood lead levels are normal, since lead intoxication is implicated in the development of protoporphyrinaemia. Protoporphyrin is present as a zinc chelate both in alcohol- and lead-induced protoporphyrinaemia. The alcohol-mediated inhibition of ferrochelatase will lead to alcohol-induced anaemia in chronic alcoholism. Alcohol reduces concentrations of pyridoxal 5-phosphate, which has been postulated as an essential cofactor for ferrochelatase (Labbe and Nielsen, 1976). Probably, the depressed ferrochelatase and coproporphyrinogen oxidase activities in alcoholics are causally related to the increased bone marrow  $\delta$ -aminolevulinic acid synthase in patients with the acute reversible sideroblastic anaemia of alcoholics (Fraser and Schacter, 1980) in contrast to idiopathic sideroblastic anaemia (Bottomley *et al.*, 1973).

### INTERFERENCE BETWEEN ALCOHOL- AND LEAD-INDUCED ENZYME INHIBITION AND CORRESPONDING HEREDITARY ENZYME DEFICIENCIES IN THE HAEM PATHWAY

Acute lead poisoning represents an exogenous toxic porphyria on the basis of clinical and biochemical analogies to hereditary hepatic acute intermittent porphyria and coproporphyrinuria. In both acute hepatic porphyrias and acute lead poisoning, abdominal pain, constipation, and peripheral neuropathy predominate (Doss, 1986, 1987). Pathobiochemically, in acute lead poisoning an extremely high excretion of  $\delta$ -aminolevulinic acid and coproporphyrin (~90% isomer III) in urine, as well as an increase of protoporphyrin (mainly as zinc protoporphyrin) in the erythrocytes, was shown. This accumulation of porphyrins and their precursors is due to the inhibition of the haem-biosynthetic enzymes by lead, at the  $\delta$ -aminolevulinic acid dehydratase, coproporphyrinogen oxidase, and ferrochelatase steps.

Regarding the enzyme defects caused by lead, an interference with alcohol ingestion is possible; because the metabolic effects of lead and alcohol on haem-biosynthetic sequences catalysed by  $\delta$ -aminolevulinic acid synthase,  $\delta$ -aminolevulinic acid dehydratase, coproporphyrinogen oxidase, and ferrochelatase are similar in principle (Table 8). This means that alcohol can potentiate the rapid enzyme inhibition by lead, as well as prolong the hepatic and erythropoietic phase of lead intoxication (Doss, 1982).

The frequent clinically reported synergism of lead toxicity and chronic ethanol consumption is not related to increased lead absorption or diminished lead excretion but rather to nutritional deficiencies and increased lead exposure among some alcoholics (Barton and Conrad, 1978). The additive

Table 8. Interference between alcohol- and lead-induced enzyme inhibition ( $\downarrow$ ) and corresponding hereditary enzyme deficiencies in the haem pathway: toxic porphyria versus genetic porphyria

Enzyme	Lead	Ethanol	Genetic deficiency/disorder
$\delta$ -Aminolevulinic acid dehydratase	$\downarrow\downarrow$	$\downarrow$	Heterozygotes without clinical symptoms but with increased sensitivity to lead. Homozygotes with acute intermittent porphyria syndrome
Coproporphyrinogen oxidase	$\downarrow\downarrow$	$\downarrow$	Hereditary coproporphyrinuria
Ferrochelatase	$\downarrow\downarrow$	$\downarrow$	Erythrohepatic (erythropoietic) protoporphyria

effects of combined chronic lead exposure and acute ethanol ingestion are not limited to the metabolic effects of lead and alcohol on the haem-biosynthetic sequences. Recent investigations on lead-exposed adult rats *in vivo* show that acute  $Pb^{2+}$  exposure and ethanol ingestion also exert many similar neurotoxic actions. For example, both neurotoxins inhibit *N*-methyl-D-aspartate receptor mediated activity (Alkondon *et al.*, 1990; Grover *et al.*, 1994, 1997) and synaptic plasticity (Blitzer *et al.*, 1990; Altmann *et al.*, 1991).

### TREATMENT

Treatment of acute porphyrias includes the omission of precipitating factors, symptomatic measures according to the drug lists and regulatory treatment by glucose or haem (Mustajoki and Nordmann, 1993; Kaupinen *et al.*, 1994). Both glucose and haem have been shown to normalize the excessive production of haem precursors, as well as to lead to a clinical improvement (Doss, 1982; Doss *et al.*, 1985; Kappas *et al.*, 1995; Schoenfeld *et al.*, 1995). Low-dose chloroquine therapy is a safe and highly effective treatment of extrahepatic manifestations of chronic hepatic porphyria, due to mobilization and elimination of high-carboxylated porphyrins from the liver cells and body tissue (Köstler and Doss, 1993; Drago *et al.*, 1995; Freeseemann *et al.*, 1995; Kappas *et al.*, 1995). Phlebotomy is also effective, but contraindicated in patients with liver cirrhosis, because of the high protein loss.

Apart from these therapeutic measures used during an acute phase of the porphyria disease process, prevention of recurrent attacks is of special interest for the treatment of acute and chronic hepatic porphyrias in the latent or subclinical phase. This includes the omission (avoidance) of alcohol, fasting (Goldberg *et al.*, 1981; Doss *et al.*, 1982; Doss, 1985; Thunell *et al.*, 1992), porphyrinogenic drugs (Wetterberg, 1976; Kappas *et al.*, 1995; Doss *et al.*, 1997) and oestrogenic steroids. Also gene carriers should avoid alcohol consumption for prophylactic reasons. Besides, individual alcohol tolerance seems to be modulated in porphyria patients by nutritional conditions and other, not clearly elucidated, metabolic factors. Different types of alcoholic beverages seem to have different influences on the clinical outbreak of latent porphyria. Red wine and whisky seem to produce porphyria symptoms more often, whereas white wine and beer are better tolerated (Thunell *et al.*, 1992).

## GENERAL CONCLUSIONS AND COMMENTS

Alcohol is an important precipitating and aggravating factor in human hereditary porphyrias: it is most important for PCT, including latent phases of chronic hepatic porphyria, but it is also important for the expression of the polysymptomatic acute hepatic porphyrias. In addition, alcohol ingestion will present a pathogenetic factor for the expression and/or aggravation of the potential hepatobiliary involvement in erythropoietic protoporphyria. In healthy subjects and patients with alcoholic liver syndromes, alcohol can produce a secondary coproporphyrinuria which is not of clinical relevance and does not lead to clinical symptoms because of this porphyrin abnormality. In patients with liver injury, however, alcohol could trigger the transition of a secondary coproporphyrinuria into a chronic hepatic porphyria.

The main reasons for these effects of alcohol are the inhibition of uroporphyrinogen decarboxylase and the induction of  $\delta$ -aminolevulinic acid synthase in the liver. Alcohol interferes with a pre-existing uroporphyrinogen decarboxylase defect in chronic hepatic porphyria (membrane disease) and augments the inducibility of  $\delta$ -aminolevulinic acid synthase in the pharmacogenetic acute hepatic porphyria (regulatory disease). Along with this mechanism, synergistic forces between the primary enzyme deficiency, the depression of several haem biosynthetic enzymes by alcohol (inhibition of  $\delta$ -aminolevulinic acid dehydratase, coproporphyrinogen oxidase, and ferrochelatase and induction of uroporphyrinogen synthase), and an altered drug metabolism may be responsible for the potentiation of the porphyrinogenic effect of alcohol.

Abstinence from alcohol is a therapeutically and prophylactically important measure in all types of symptomatic hepatic porphyrias as well as in erythropoietic protoporphyria with hepatobiliary involvement. Also in gene carriers and patients in the subclinical latent phase of the disease process, alcohol consumption should be avoided, for prophylactic reasons.

From clinical experience, it follows that in cases with chronic consumption of alcohol, fatty liver, alcohol-induced hepatitis and liver cirrhosis, porphyrin studies in urine should be made to diagnose a hepatic porphyria in the latent phase. When dealing with abdominal and cutaneous symptoms in the clinical context with consumption of alcohol, one has to exclude a hepatic porphyria by differential diagnosis.

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