The porphyrias: a review

G. H. ELDER, C. H. GRAY, AND D. C. NICHOLSON

From the Department of Chemical Pathology, King's College Hospital Medical School, Denmark Hill, London

The porphyrias are disorders of the biosynthesis of protohaem, the ferrous iron complex of protoporphyrin IX, in which characteristic clinical features are accompanied by specific patterns of porphyrin and porphyrin precursor overproduction, accumulation, and excretion, each pattern defining a particular form of porphyria.

All those forms of porphyria in which there is overproduction of porphyrins have one clinical feature in common—sensitivity of the skin to sunlight—although the nature of the lesions produced differs between diseases. The photosensitivity is due to the photodynamic action of the porphyrins that accumulate in the skin when the plasma porphyrin concentration is increased (Rimington, Magnus, Ryan, and Cripps, 1967) and which probably act as sensitizers for singlet-oxygen-mediated destructive processes, for example, the peroxidation of lipids in the membranes of lysosomes (Allison, Magnus, and Young, 1966; Magnus, 1972).

The other main clinical feature of the porphyrias—neurological lesions typically causing severe abdominal pain, peripheral neuropathy, and often mental disturbance, and frequently precipitated by drugs such as the barbiturates—is associated with increased excretion of the porphyrin precursors, porphobilinogen (PBG) and 5-aminolaevulinic acid (ALA) and does not occur in those forms of porphyria in which excretion of these precursors is always normal.

In some patients only the biochemical features are apparent. Such clinically latent porphyria may occur either as a phase in an episodic illness or as the only manifestation throughout life. Proper treatment of patients with porphyria depends upon accurate diagnosis, which in turn depends entirely upon the accurate interpretation of proper laboratory investigations and proper enquiry into the family history. Discussion of the prevention and management of porphyria is beyond the scope of this review and is summarized by Goldberg (1971).

Chemistry and Biochemistry of the Porphyrins

The general pathway of biosynthesis of haem for haem protein formation is well known except for the details of very early stages and the later stages in uroporphyrinogen and coproporphyrinogen synthesis. Until the formation of protoporphyrin IX this pathway involves not the porphyrins but porphyrinogens, the hexahydro derivatives of porphyrins. Succinyl co-enzyme A, derived from acetate via the Krebs's cycle and α -oxoglutarate, is condensed by the enzyme 5-aminolaevulinic acid synthetase (ALA-S) with glycine, pyridoxal phosphate participating, to form α -amino- β -ketoadipic acid which rapidly loses CO₂ non-enzymically to form ALA. Under the influence of the sulphydryl enzyme ALA dehydratase, two molecules of ALA condense to form one molecule of the monopyrrole PBG with the structure of 2-aminomethyl-3-carboxymethyl-4-carboxyethyl pyrrole (Fig. 1a). The enzyme uroporphyrinogen synthetase (Bogorad, 1958) causes four molecules of porphobilinogen to condense to give uroporphyrinogen I in which 4-carboxymethyl and 4-carboxyethyl groups are arranged alternately around the hexahydroporphyrin ring. When uroporphyrinogen synthetase acts in the presence of uroporphyrinogen III co-synthetase, which may be a separate enzyme (Bogorad, 1963), or, as in mammalian liver, part of a complex containing the synthetase (Bogorad, 1958), uroporphyrinogen III is formed; in this the carboxymethyl and carboxyethyl groups attached to pyrrole ring D are reversed (Fig. 1b). Two other isomeric porphyrinogens are theoretically possible but are not found in nature. In haem-synthesizing tissues, four carboxymethyl groups are presumed to be successively decarboxylated to methyl groups to give hepta-, hexa-, and pentacarboxyl porphyrinogens and finally the tetracarboxyl coproporphyrinogens I and III. Coproporphyrinogen I is not further metabolized but coproporphyrinogen III undergoes a dehydrogenation-decarboxylation reaction converting the carboxyethyl groups on rings A and B to vinyl groups

Fig. 1b The biosynthetic pathway of haem formation (conversion of porphobilinogen to porphyrins and haem)

yielding protoporphyrinogen which is readily dehydrogenated to provide protoporphyrin (Sano and Granick, 1961). In the presence of iron and the enzyme ferrochelatase (Labbe, Hubbard, and Caughey, 1963), this is converted to haem for haem protein synthesis (Figure 1b). At all stages in the biosynthetic pathway the porphyrinogens are readily converted to the corresponding porphyrins which, with the exception of protoporphyrin, cannot be further metabolized.

There are several points of this pathway that are in need of further elucidation. The precise source of succinyl coenzyme A used for ALA synthesis is

unknown and there may well be a separate pool of this compound not directly formed via the Krebs cycle. Secondly, the mechanism of formation of uroporphyrinogens I and III from porphobilinogen remains obscure although numerous theories have been proposed (Llambías and Batlle, 1970). There is need for this to be established as there are almost certainly enzymic deficiencies of this pathway other than that of uroporphyrinogen III co-synthetase in congenital erythropoietic porphyria. Thus there is evidence that abnormality or deficiency of uroporphyrinogen synthetase itself may be the primary abnormality in acute intermittent porphyria (Strand,

Felsher, Redeker, and Marver, 1970; Miyagi, Cardinal, Bossenmaier, and Watson, 1971). The third area which requires elucidation, particularly in mammalian liver, is the decarboxylation of uroporphyrinogen III to coproporphyrinogen III which is usually accepted as occurring sequentially. Recently Dowdle, Goldswain, Spong, and Eales (1970), studying that form of porphyria known as symptomatic porphyria, attribute a paradoxical distribution of isomers I and III in conversion of uroporphyrinogen through hepta- and pentacarboxyl porphyrinogens to two distinct metabolic pathways in the liver. In one, ALA is converted sequentially to haem via PBG, uroporphyrinogens I and III, coproporphyrinogen III and protoporphyrinogen, while in the other, which operates under conditions of ALA overload, uroporphyrinogen III gives rise only to hexa- and heptacarboxyl porphyrinogens which accumulate and are excreted as the respective porphyrins. Isotope experiments have suggested that there may be two distinct metabolic pools of uroporphyrinogen (Goldswain, Dowdle, Spong, and Eales, 1970).

More recently, detailed studies of urinary and faecal and biliary porphyrins have shown that in one form of porphyria—variegate porphyria—there is excretion of a peptide conjugate of a porphyrin (Rimington, Lockwood, and Belcher, 1968). In symptomatic cutaneous hepatic porphyria a vinyl tricarboxyethyl carboxymethyl trimethyl porphyrin occurs (Elder, 1972) (Fig. 2, IV) which is excreted in the bile with a hydroxy derivative in which the elements of water have been added to the vinyl group and is partially converted to ethyl and hydryl (deutero) derivatives by intestinal microorganisms (Elder, 1972). It is not known whether this reflects exaggeration of a normal pathway by an enzyme block in symptomatic cutaneous hepatic porphyria or the presence of a unique metabolic pathway in this condition.

Aminolaevulinic acid synthetase (ALA-S) is the rate-limiting enzyme in the biosynthesis of haem (Granick and Urata, 1963) and normally its amount and activity are adjusted so precisely that only traces of haem precursors are present in the normal organism. In most forms of porphyria, the activity of the enzyme is increased (Dowdle, Mustard, Spong, and Eales, 1967). This may result from increased substrate availability or by diminution of end product inhibition or repression. In the first mechanism an increased formation of succinyl CoA or of glycine might activate the enzyme but there is no evidence that this plays a significant part in increasing ALA synthetase activity in the porphyrias. In end product inhibition, the end product changes the allosteric conformation of the enzyme (Monod,

Fig. 2 New porphyrins recently isolated from faeces (see text).

Isocoproporphyrin (I $R = CH_2 \cdot CH_3$)

Deethylisocoproporphyrin (II $R = -H$)

Hydroxyisocoproporphyrin (III $R = -CH(OH)CH_3$)

Dehydroisocoproporphyrin (IV $R = -CH=CH_2$)

In I, II, III, IV, $R' = CH_2COOH$ (Elder, 1971, 1972)

Acrylic analogue of coproporphyrin-III isomer

(V $R = -CH=CH_2$, $R' = CH_3$)

(French, Nicholson, and Rimington, 1970)

The situation of the acrylic group shown here in position 2 of structure V still requires final proof.

Changeux, and Jacob, 1963), thus inhibiting its activity. End product repression usually involves combination of the end product with an 'aporepressor' molecule synthesized by the activity of a regulator gene (Jacob and Monod, 1961). Normally the aporepressor molecule combined with the end product acting as a co-repressor represses the activity of an operator gene directing the synthesis of the m-RNA controlling the synthesis of the enzyme. If the end product is deficient, or has to compete for the binding sites with compounds which can act as porphyrinogenic agents, the aporepressor molecule no longer represses the operator gene which leads to increased synthesis and induction of the enzyme thus providing a feedback control of the biosynthetic pathway. There is evidence that end-product inhibition and repression of ALA synthetase by the end product haem takes place in *Rhodospseudomonas spheroides* (Burnham and Lascelles, 1963) and repression by haem occurs in rabbit reticulocytes and in embryo chicken liver cell cultures (Granick, 1966) (Fig. 3). The action of compounds which cause experimental porphyria is outside the scope of this review but has been surveyed by Tschudy and Bonkowski (1972).

Classification and Nomenclature

There have been numerous classifications of the porphyrias based on consideration of clinical and

Fig. 3 Possible mechanisms for the control of haemoglobin synthesis.

biochemical features and patterns of inheritance (Günther, 1911; Waldenström, 1937; Watson, 1951; Goldberg and Rimington, 1962; Conference Discussion, 1963; Gray, 1970; Tschudy, 1969; Marver and Schmid, 1972). The system of classification followed in this review is shown in Table I. The porphyrias are usually divided into two main groups according to the site of accumulation and, by inference, of overproduction of haem precursors within the body (Schmid, Schwartz, and Watson, 1954; Watson, Lowry, Schmid, Hawkinson, and Schwartz, 1951).

Erythropoietic

- 1 Congenital erythropoietic porphyria
- 2 Erythropoietic coproporphyria

Erythrohepatic

- 1 Protoporphyria

Hepatic

- 1 Hepatic porphyrias inherited as autosomal dominants
 - i Acute intermittent porphyria
 - ii Variegate porphyria
 - iii Hereditary coproporphyria
- 2 Symptomatic cutaneous hepatic porphyria (symptomatic porphyria)
 - i Those with no family history of the disease
 - (a) associated with alcoholism, liver disease, iron overload, oestrogen therapy
 - (b) due to hexachlorobenzene poisoning
 - ii Those with family history of the disease
- 3 Cutaneous porphyria due to hepatic tumours

Table I Classification of the porphyrias

In the erythropoietic porphyrias the metabolic abnormality is believed to be confined to the bone marrow and in the hepatic porphyrias to the liver. In recent years it has become apparent that in erythropoietic protoporphyria, which was first identified by Magnus, Jarrett, Prankerd, and Rimington in 1961, porphyrins are produced in excess in both erythropoietic and hepatic cells (Gray, Kulczycka, Nicholson, Magnus, and Rimington, 1964), and Scholnick, Marver, and Schmid (1971) have proposed that the condition should be renamed erythrohepatic protoporphyria.

Overproduction of porphyrins in erythropoietic tissues leads to accumulation of porphyrins within erythrocytes. Erythropoietic porphyrias can thus be distinguished from the hepatic porphyrias by measurement of erythrocyte porphyrin levels, for in the purely hepatic porphyrias overproduction of porphyrins is not accompanied by an increase in erythrocyte porphyrin levels.

There is general agreement that the three forms of hepatic porphyria—acute intermittent and variegate porphyria and hereditary coproporphyria—which are transmitted by separate autosomal dominant genes (Waldenström and Haeger-Aronsen, 1967) are distinct disease entities: but there is less agreement about the classification of the remaining patients with hepatic porphyria. The majority of these have certain clinical and biochemical features in common

which differentiate them from the other forms of hepatic porphyria and, although aetiologically diverse, have been classified as a single group: symptomatic cutaneous hepatic porphyria (symptomatic porphyria). Only two patients with acquired porphyria due to overproduction of porphyrins within an hepatoma have been described and will be discussed separately (Tio, Leijnse, Jarrett, and Rimington, 1957; Thompson, Nicholson, Farnan, Whitmore, and Williams, 1970).

No system for the classification of the hepatic porphyrias is satisfactory. Some patients cannot be classified even after extensive investigation (Watson, 1960; Piñol-Aguadé, Castells, Indacochea, and Rodés, 1969) and a more satisfactory classification will have to await greater understanding of the fundamental abnormalities in this group of disorders.

The use of the term 'porphyria cutanea tarda' in respect of a specific cutaneous porphyria of slow or late onset has caused much confusion. Waldenström introduced the term to describe a group of patients developing a cutaneous form of porphyria after puberty (Waldenström, 1937), clearly differentiated from congenital erythropoietic porphyria which was present from birth and from acute intermittent porphyria in which cutaneous symptoms do not occur. Although Waldenström recognized that attacks of abdominal pain were occasionally a feature of 'porphyria cutanea tarda', subjects with cutaneous symptoms were never found amongst families with typical acute intermittent porphyria. Subsequent reports established that 'porphyria cutanea tarda' was predominantly a disease of men over the age of 40, who frequently gave a history of alcoholism and showed evidence of liver dysfunction and that both attacks of acute porphyria and a family history of porphyria were uncommon in this group (Szodoray and Sümegi, 1944; Brunsting, Mason, and Aldrich, 1951; Brunsting, 1954). However, there were also accounts of a less common cutaneous porphyria in which both photosensitivity and acute attacks occurred (Gray, Rimington, and Thomson, 1948; Watson, 1951; MacGregor, Nicholas, and Rimington, 1952; Rimington, 1952; Calvert and Rimington, 1953; Discombe and Treip, 1953; Wells and Rimington, 1953; Holti, Rimington, Tate, and Thomas, 1958). In general these patients were younger than the former group and in some there was evidence of porphyria in other members of the family although there was no difference between the skin lesions in the two groups. In Europe these patients were also referred to as cases of 'porphyria cutanea tarda' (Rimington, 1952; 1958a) though in the United States Watson (1954) described them as 'mixed porphyria' and reserved 'porphyria cutanea tarda'

for the purely cutaneous 'middle-aged alcoholic' type of porphyria. In 1957, Waldenström proposed that 'porphyria cutanea tarda' was in fact a mixed group of at least two diseases and modified his classification accordingly. The patients with both cutaneous lesions and acute attacks were re-classified as 'porphyria cutanea tarda hereditaria' or, to take into account the characteristic faecal abnormality, 'protocoproporphyria'. This group of patients is now classified under variegate porphyria. The remaining patients in whom the disease appeared to be acquired later in life were classified as 'porphyria cutanea tarda symptomatica'. In this category Waldenström placed both the European and American patients with alcoholic liver disease—and the occasional non-alcoholic patient—and the Bantu porphyria of South Africa, a purely cutaneous form of non-familial porphyria associated with liver disease, malnutrition, and alcoholism common amongst the Bantu peoples. Subsequently porphyrin excretion patterns of variegate porphyria and 'porphyria cutanea tarda symptomatica' were shown to be quite distinct, largely as the result of extensive comparisons in South Africa (Eales, Levey, and Sweeney, 1966b). Thus the existence of a purely cutaneous form of hepatic porphyria that could be distinguished both from the erythropoietic porphyrias and from variegate porphyria and hereditary coproporphyria became established. This condition is referred to as 'symptomatic cutaneous hepatic porphyria' (or 'symptomatic porphyria') (Eales, 1963; Eales and Dowdle, 1968). The term 'porphyria cutanea tarda' without qualification has been used for the same condition, especially in the United States (Taddeini and Watson, 1968; Tschudy, 1969), but should be avoided since it has also been used to describe variegate porphyria.

The Erythropoietic Porphyrias

CONGENITAL ERYTHROPOIETIC PORPHYRIA

This disease (Günther's disease) (Günther, 1911; 1922) is a very rare condition characterized by onset at or soon after birth of variable, but usually severe and mutilating, skin lesions due to photosensitivity and often accompanied by haemolytic anaemia (Gray and Neuberger, 1950; Goldberg, 1966; Taddeini and Watson, 1968).

Porphyrins of isomer series I accumulate within the body often discolouring the bones, teeth, and skin and are excreted in large amounts in the urine and faeces. Urine from patients with congenital erythropoietic porphyria not only contains uroporphyrin I and coproporphyrin with eight and four carboxyl groups respectively but also small quantities

of at least one other octacarboxyl porphyrin, perhaps uroporphyrin III as well as porphyrins containing seven, six, and five carboxyl groups (Rimington and Miles, 1951). The faeces contain large amounts of coproporphyrin I.

The underlying enzyme defect which is apparently restricted to the erythroid cells (Schmid, Schwartz, and Sundberg, 1955; Watson, 1968) is a deficiency of uroporphyrinogen III cosynthetase relative to uroporphyrinogen synthetase. This leads to the formation of a greater than normal proportion of uroporphyrinogen I from porphobilinogen, while the overall production of series III porphyrinogens is normal or may even be increased (Taddeini and Watson, 1968). Uroporphyrinogen I cannot be used for haem synthesis and is deposited in the tissues and converted to uroporphyrin I, partly excreted in the urine and partly converted to coproporphyrinogen I and coproporphyrin I for excretion mainly but not entirely in the faeces. The abnormality is inherited as an autosomal recessive gene (Marver and Schmid, 1972). Abnormalities of porphyrin metabolism (Heilmeyer, Clotten, Kerp, Merker, Parra, and Wetzel, 1963) and a slightly decreased co-synthetase in red cells and cultured fibroblasts (Romeo, Glen, and Levin, 1970; Romeo, Kaback, and Levin, 1970) have been described in clinically normal heterozygotes.

Heilmeyer and Clotten (1964) have described a mild form of cutaneous photosensitivity associated with an increase in erythrocyte coproporphyrin but no further patients with this condition have been encountered.

ERYTHROHEPATIC PROTOPORPHYRIA

This condition, although an uncommon cause of photosensitivity (Prentice, Goldberg, and Thompson, 1969), is the commonest form of cutaneous porphyria associated with increased erythrocyte porphyrin. The condition is inherited as an autosomal dominant gene (Waldenström and Haeger-Aronsen, 1967) and is usually associated with mild photosensitivity (Rimington *et al*, 1967) usually beginning in childhood. Exposure to sunlight is rapidly followed by a tingling or burning sensation, irritation, erythema, urticaria, sometimes associated with oedema but rarely with blisters; the lesions usually heal without severe scarring (Rimington *et al*, 1967). Gallstones are a complication (Cripps and Scheuer, 1965). The condition was regarded as benign but in recent years a number of patients have been reported who have died of liver failure (Barnes, Hurworth, and Miller, 1968; Donaldson, McCall, Magnus, Simpson, Caldwell, and Hargreaves, 1971).

Erythrohepatic protoporphyria is characterized biochemically by the presence of excessive free

protoporphyrin in the erythrocytes and is distinguished from Günther's disease in which the erythrocytes contain predominantly uroporphyrin. Chapel, Stewart, and Webster (1972) and Cripps and MacEachern (1971) showed that only variable percentages of the red cells in erythropoietic protoporphyria fluoresced in ultraviolet light, and Clark and Nicholson (1971) found the excess porphyrin to be in the young rather than the old cells. Faecal protoporphyrin and coproporphyrin are increased, especially the former. In contrast to the other forms of cutaneous porphyria the urinary porphyrins are usually normal, and, unlike variegate porphyria and acute intermittent porphyria, there is no excessive urinary excretion of porphyrin precursors.

The source of the excess porphyrins in erythrohepatic protoporphyria was originally assumed to be mainly the bone marrow and the condition was called erythropoietic protoporphyria (Rimington *et al*, 1967). However, the incorporation of ^{15}N glycine (Gray *et al*, 1964) and of ^{14}C ALA (Nicholson, Cowger, Kalivas, Thompson, and Gray, 1973) into erythrocytes, plasma and faecal porphyrins, and stercobilin showed a pattern of labelling suggesting disturbances of porphyrin biosynthesis in both the liver and erythropoietic tissues and that the early peak of labelling of bile pigment was increased, probably due to an increased turnover of non-erythropoietic haems. Moreover, Miyagi (1967) has shown increased ALA synthetase activity in both erythroid and hepatic tissue. Scholnick *et al* (1971) concluded that in erythropoietic protoporphyria some of the excess hepatic protoporphyrin refluxes into the blood contributing by uptake from the plasma to erythrocyte protoporphyrin. They have therefore renamed the condition 'erythrohepatic porphyria'. A different interpretation of labelling experiments was made by Schwartz, Johnson, Stevenson, Anderson, Edmondson, and Fusaro (1971) who consider both faecal and erythrocyte porphyrins to be mainly of erythropoietic origin, the latter pool being complex and the secondary source of early labelled bile pigment.

The enzyme block in erythrohepatic porphyria responsible for the increased synthesis of ALA synthetase is not known with certainty, but is probably a deficient synthesis from protoporphyrin of haem required for a specific haem protein important in the feedback mechanism. This may affect both the liver and the bone marrow. The excess protoporphyrin in the liver is then partially excreted in the bile and faeces and partly transferred via the blood plasma to the circulating red cells which may take up protoporphyrin passively, thus augmenting the protoporphyrin accumulating because of the defect in the erythropoietic tissue.

The Hepatic Porphyrrias

HEPATIC PORPHYRIAS OF AUTOSOMAL DOMINANT INHERITANCE

The acute porphyric attack

Acute intermittent porphyria, variegate porphyria, and hereditary coproporphyria have one important clinical feature in common: patients with any one of these disorders may develop clinically identical attacks of acute porphyria. Main clinical features which may occur separately or in combination are severe colicky abdominal pain, vomiting and constipation, a neuropathy which is mainly motor in type but often associated with severe pain in the extremities, and psychiatric disturbances; respiratory paralysis is not uncommon. All these features probably have a neurological basis (Goldberg, 1959; Ridley, 1969). Attacks are frequently precipitated by drugs (Table II), especially barbiturates, but may

Antipyrine	Isopropyl dipyrone
Barbiturates	Methyldopa
Chlordiazepoxide	Meprobamate
Chlorpropamide	Oestrogens (natural and synthetic)
Diazepam	
Dichloral phenazone	Pentazocine (Fortral)
Ergot preparations	Progestogens
Glutethimide	Sedormid
Griseofulvin	Succinimides
Hydantoins	Sulpha drugs

Table II Some of the more important drugs known or reported to precipitate attacks of acute intermittent porphyria

be provoked by fasting, alcohol, infections, or occur spontaneously (Eales, 1971). They occur most commonly between the second and fourth decades and are commoner in women. In both sexes they are rare before puberty; in some women their onset may be related to a particular phase of the menstrual cycle. Acute attacks during pregnancy are uncommon but there is an increased incidence during the postpartum period (Stein and Tschudy, 1970). The severity of the attacks is variable and slow recovery from the neuropathy may entail prolonged morbidity (Sørensen and With, 1971). The overall mortality in variegate porphyria has recently been estimated as about 20% (Eales, 1971), while in a recent series of 46 patients with acute intermittent porphyria (Stein and Tschudy, 1970) two males and two females died.

Disturbances of salt and water metabolism are common during the acute attack (Eales, Dowdle, and Sweeney, 1971). Hyponatraemia, often accompanied by hypovolaemia, is the commonest disturbance, but hypokalaemia and hypomagnesaemia may occur

(Nielsen and Thorn, 1965). The hyponatraemia is usually associated with vomiting and inadequate salt and water intake, but renal factors (Eales *et al*, 1971) and inappropriate secretion of antidiuretic hormone, perhaps due to a hypothalamic lesion (Hellman, Tschudy, and Bartter, 1962; Nielsen and Thorn, 1965; Perlroth, Tschudy, Marver, Berard, Ziegel, Rechcigl, and Collins, 1966), may also be important in some patients. Additional evidence for involvement of the hypothalamus comes from reports of failure of growth hormone (Perlroth, Tschudy, Waxman, and Odell, 1967) and ACTH release in response to appropriate stimuli (Waxman, Berk, Schalch, and Tschudy, 1969).

Biochemically the attacks are characterized by increased excretion of the porphyrin precursor, PBG, and to a lesser extent ALA. They do not occur in those types of porphyria in which the excretion of PBG is always within normal limits.

The three hereditary hepatic porphyrias can be distinguished on the basis of their clinical and biochemical features.

Acute intermittent porphyria

Patients with this condition suffer only variable and periodic acute attacks of the type described above, there being an associated overproduction of porphobilinogen (Goldberg, 1959; Stein and Tschudy, 1970). Skin lesions due to photosensitivity do not occur. In some the disease may be clinically latent throughout life, while in others the frequency and severity of attacks varies widely.

The conventional tests of liver function are usually normal in acute intermittent porphyria, except for retention of bromsulphthalein (BSP) (Stein and Tschudy, 1970) due to decreased excretion of BSP by the liver cell (Stein, Bloomer, Berk, Corcoran, and Tschudy, 1970). The presence of this defect as well as an increase in serum protein-bound iodine (PBI) and, in females, thyroxine-binding globulin (Hollander, Scott, Tschudy, Perlroth, Waxman, and Sterling, 1967) without hyperthyroidism has led to the suggestion that an 'oestrogen effect' operates in this disease (Stein *et al*, 1970).

Other metabolic abnormalities that have been described include raised plasma iron, hypercholesterolaemia (Stein and Tschudy, 1970) with hyper- β -lipoproteinaemia (Lees, Song, Levere, and Kappas, 1970), and abnormalities of glucose metabolism (Stein and Tschudy, 1970).

The characteristic biochemical abnormality of both the manifest and the latent disease is a persistently increased excretion of PBG (Waldenström, 1937; Watson and Schwartz, 1941) and ALA in the urine, the level of PBG exceeding that of ALA (Haeger-Aronsen, 1958; Waldenström and Haeger-

Aronsen, 1963). Although the amount of PBG excreted may reach a peak during acute attacks the increase persists during remission in the majority of patients (Taddeini and Watson, 1968) but the level varies widely from patient to patient and from day to day and does not correlate with the likelihood or severity of an acute attack (Ackner, Cooper, Gray, Kelly, and Nicholson, 1961). It is probable that the excretion of PBG is only rarely within normal limits in subjects carrying the gene for acute intermittent porphyria, for Wetterberg (1967) found that the urine of 55% of 197 siblings of patients contained abnormally increased amounts of PBG, which is close to the theoretical gene distribution of 50%. However, normal excretion of precursors has been reported in a few patients during the latent phase of acute intermittent porphyria (With, 1961; 1963) and such subjects have been shown to carry the gene by loading tests with ALA and appropriate enzyme estimations (Meyer, Strand, Doss, Reese, and Marver, 1972).

Although the condition is essentially one of overproduction of porphyrin precursors rather than of porphyrins, there are probably small increases in porphyrin excretion. Interpretation of urinary porphyrin analyses is difficult owing to the large amounts of uroporphyrin formed in the urine by non-enzymic polymerization of PBG, but there may be some increase in coproporphyrin III excretion at least during acute attacks (Waldenström and Haeger-Aronsen, 1963). Faecal protoporphyrin and coproporphyrin concentrations are often normal but may be slightly increased in some patients (Waldenström and Haeger-Aronsen, 1963; Wetterberg, Haeger-Aronsen, and Stathers, 1968). More frequently there are small increases in the ether-insoluble faecal porphyrin fraction which may contain both uroporphyrin (Watson, 1960; Taddeini and Watson, 1968; Rimington *et al*, 1968) and small amounts of porphyrin-peptide conjugates (Rimington *et al*, 1968; Moore, Thompson, and Goldberg, 1972).

Variegate porphyria

In this form of porphyria, which is particularly common in the white population of South Africa, both acute attacks and cutaneous lesions occur though not necessarily in the same patient at the same time. In a series of 133 South African patients 50% presented with an acute attack, accompanied by cutaneous lesions, 34% had cutaneous lesions alone, while in 15% an acute attack occurred alone (Eales, 1963). The incidence of variegate porphyria in the white population of South Africa has been estimated as 3 per 1000 (Dean, 1963). Elsewhere the disease is much rarer but similar patients have been described in Europe (Waldenström, 1957; Holti *et al*,

1958) and from the United States (Watson, 1960).

As might be expected from the clinical features, overproduction and excretion of both porphyrin precursors and porphyrins are found in variegate porphyria. The most characteristic biochemical abnormality is a marked increase in the concentration of porphyrin in the faeces with the levels of protoporphyrin exceeding those of coproporphyrin (Waldenström, 1957; Barnes, 1958; Holti *et al*, 1958; Dean and Barnes, 1959; Eales, Dowdle, Saunders, and Sweeney, 1963; Eales *et al*, 1966b). In the majority of South African variegate porphyrics, the faecal porphyrin concentration, although greatly increased in all phases of the disease including latency (total ether-soluble porphyrin > 500 µg/g dry wt in 92% of patients, Eales *et al*, 1966b), is particularly high during acute attacks (Eales, Dowdle, Levey, and Sweeney, 1966a). The concentration of ether-insoluble porphyrin in the faeces is also increased (Watson, 1960; Sweeney, 1963; Rimington *et al*, 1968; Eales, Grosser, and Sano, 1971). Rimington *et al* (1968) found that much of this fraction consists of porphyrin-peptide conjugates ('X porphyrins') and suggested that the excretion of increased amounts of these conjugates in the faeces, and in the urine during acute attacks, is a prominent biochemical feature of variegate porphyria. In the past this hydrophilic material may have been described mistakenly as uroporphyrin.

The porphyrins of bile are similarly abnormal (Smith, Belcher, Mahler, and Yudkin, 1968; Belcher, Smith, and Mahler, 1969).

During the acute attack the concentration of PBG in the urine is increased, often to levels similar to those of acute intermittent porphyria, and there is an increase in urinary coproporphyrin III excretion. However, in contrast to acute intermittent porphyria, during remission the amount of PBG excreted in the urine falls rapidly over a few weeks, usually returning to normal within a few weeks (Eales *et al*, 1966a). Urinary coproporphyrin III concentration is variable but may remain increased during remission (Eales *et al*, 1963). The level of coproporphyrin always exceeds that of uroporphyrin except occasionally during the acute attack when a large amount of uroporphyrin is formed by non-enzymic polymerization of porphobilinogen.

It is possible that each form of dominantly inherited hepatic porphyria although clinically and biochemically distinct is genetically heterogeneous. Thus With (1969) has emphasized small differences that occur between the same disease in different families and has suggested that the condition seen in each family is the expression of a genotype peculiar to that family. In this respect it is interesting to note the remarkable uniformity of the South

African patients with variegate porphyria when compared with those from other parts of the world, since all the South African patients are believed to be descended from one of the original free burghers who married at Cape Town in 1688 (Dean, 1963). Some points of difference reported in families from outside South Africa, not all of which are likely to be due to environmental factors, are the larger number of latent porphyrics, the lower incidence of cutaneous manifestations, and the greater variability of faecal porphyrin levels (Watson, 1960; Hamnström, Haeger-Aronsen, Waldenström, Hysing, and Molander, 1967; Cochrane and Goldberg, 1968; Rimington *et al*, 1968). In particular Rimington and his coworkers have reported a number of British patients in whom impaired hepato-biliary function during the acute attack led to diversion of porphyrins from the bile to the urine (Gray *et al*, 1948; MacGregor *et al*, 1952; Wells and Rimington, 1953). Such reciprocity of porphyrin excretion is apparently not a feature of the disease in South Africa (Eales, 1963) or the United States (Watson, 1960) except when complicated by intercurrent cholestatic jaundice (Eales *et al*, 1966b). The existence of these differences suggests that the biochemical diagnostic criteria established by study of the plentiful South African patients may not always be applicable elsewhere.

Hereditary coproporphyrria

This is probably the least common of the genetic hepatic porphyrias, although accurate assessment of its incidence is difficult since it is frequently asymptomatic (Watson, Schwartz, Schulze, Jacobsen, and Zagaria, 1949; Berger and Goldberg, 1955; Goldberg, Rimington, and Lochhead, 1967; Haeger-Aronsen, Stathers, and Swahn, 1968; Lomholt and With, 1969).

The condition is characterized by the excretion of large amounts of coproporphyrin III, mainly in the faeces. Porphobilinogen and ALA excretion is normal except during an acute attack, which is the most frequent clinical feature. Photosensitivity is uncommon and in four patients, in which it has been reported has been accompanied by hepatic insufficiency (Goldberg *et al*, 1967; Connon and Turkington, 1968; Hunter, Khan, Hope, Beattie, Beveridge, Smith, and Goldberg, 1971).

THE NATURE OF THE METABOLIC ABNORMALITY IN THE INHERITED HEPATIC PORPHYRIAS

Current theories of the pathogenesis of the hepatic porphyrias depend on the concept that increased production of ALA, the first committed precursor of protohaem, in the liver is an underlying abnormality. In 1965, Tschudy, Perlroth, Marver, Collins,

Hunter, and Rechcigl reported marked increase in the activity of ALA-S, the rate-limiting enzyme of hepatic protohaem synthesis, in the liver of a patient who had died from acute intermittent porphyria. This finding, since confirmed in other patients using liver tissue obtained by open surgical or needle biopsy (Nakao, Wada, Kitamura, Uono, and Urata, 1966; Dowdle, Mustard, and Eales, 1967; Masuya, 1969; Strand *et al*, 1970), provided the first direct evidence in support of the suggestion of Watson, Runge, Taddeini, Bossenmaier, and Cardinal (1964) that acute intermittent porphyria is an 'overproduction' disease in which excessive amounts of porphyrin precursors are produced due to an inherited defect in the regulation of ALA-S activity. An increase in the activity of this enzyme in the liver has since been found in variegate porphyria (Dowdle *et al*, 1967; Strand *et al*, 1970) and in hereditary coproporphyrria (Kaufman and Marver, 1970; McIntyre, Pearson, Allan, Craske, West, Moore, Paxton, Beattie, and Goldberg, 1971.)

An increase in ALA-S activity in the liver in these conditions could be either a primary effect of the genetic lesion or could be secondary to interruption of the feedback control of ALA-S activity by a partial block in haem synthesis, by increased catabolism of haem to bilirubin or other catabolites (Landaw, Callahan, and Schmid, 1970), or by increased incorporation into haemoproteins.

In recent years, evidence has accumulated that, at least in acute intermittent porphyria, the increase in ALA-S activity is not the primary defect in haem biosynthesis in these conditions. Thus if overproduction of ALA in the liver were the only lesion in haem biosynthesis one would expect the pattern of porphyrin excretion to be the same in each disease and to resemble that produced by administering ALA to a normal person (Berlin, Neuberger, and Scott, 1956) whereas in fact the different forms of porphyria are characterized by specific inherited patterns of porphyrin excretion (Dowdle *et al*, 1968). Since it is unlikely that two separate inherited lesions are present in each type of porphyria—one leading to an increase in the activity of ALA-S and one determining the consequent excretion pattern—Kaufman and Marver (1970) have proposed that the primary inherited defect is a partial block in the biosynthesis of haem at a different, characteristic step for each disease. The increase in ALA-S activity would then come about through the feedback control mechanism operating to increase the synthesis of intermediates to maintain normal levels of hepatic haem in the face of such a block. Strand *et al* (1970) and Miyagi *et al* (1971) have recently reported that the activity of uroporphyrinogen synthetase in the liver is decreased in patients with

acute intermittent porphyria, an observation which neatly explains the observed excretion of large amounts of PBG and ALA, but not porphyrins, in this condition, and Meyer *et al* (1972) have shown that the activity of erythrocyte uroporphyrinogen synthetase and the ability to convert an exogenous load of ALA to porphyrins are both decreased in patients carrying the gene for acute intermittent porphyria whether or not it is clinically manifest. Strand, Manning, and Marver (1971) have predicted that enzyme defects at different sites may similarly underlie hereditary coproporphyria and variegate porphyria.

Indirect evidence from measurement of bilirubin production rates suggests that hepatic haem turnover is normal in acute intermittent porphyria (Dowdle *et al*, 1968; Bloomer, Berk, Bonkowsky, Stein, Berlin, and Tschudy, 1971; Jones, Bloomer, and Berlin, 1971) and in variegate porphyria (Dowdle *et al*, 1968) although it is probable that the methods used were insufficiently sensitive to detect small alterations in bilirubin production rates.

There is some evidence that the activity of ALA-S is further increased during acute attacks. The excretion of PBG and ALA in the urine tends to be higher during acute attacks in all three forms of porphyria (Taddeini and Watson, 1968).

Many of the drugs that provoke acute attacks (Table II) induce hepatic ALA-S and are metabolized in the liver by haem-containing microsomal enzyme systems (Granick, 1966; Marver and Schmid, 1968). The induction of ALA-S is normally short-lived and appropriate to the provision of the extra haemoprotein—particularly cytochrome P₄₅₀—required for metabolism of the inducer, presumably since any further increase in haem levels represses further synthesis of the enzyme. It may be that in patients with inherited hepatic porphyria, regulation of ALA-S induction fails due to an inability to increase haem levels sufficiently in the presence of a partial block in haem synthesis; thus they respond to the administration of such drugs with a sustained increase in ALA-S activity and hence massive overproduction of the intermediates preceding the block in the pathway.

Carbohydrate loading and starvation are known to influence the excretion of PBG and ALA and the activity of hepatic ALA-S in animals with experimental porphyria (Rose, Hellman, and Tschudy, 1961), and also affect the amounts of these precursors excreted by patients with porphyria. Thus increased dietary carbohydrate decreases the excretion of PBG and ALA in acute intermittent and variegate porphyria (Welland, Hellman, Gaddis, Collins, Hunter, and Tschudy, 1964). Conversely starvation may provoke acute attacks.

A number of clinical observations suggest that steroid hormones are important in the pathogenesis of acute attacks in the inherited hepatic porphyrias. Thus acute attacks are commoner in females, are rare before puberty, and in some patients their onset may be related to a particular phase of the menstrual cycle. The administration of oestrogens and/or progestogens increases the excretion of PBG and ALA (Welland *et al*, 1964) and may provoke acute attacks in some patients with acute intermittent porphyria (Welland *et al*, 1964; Wetterburg, 1964) and variegate porphyria (McKenzie and Acharya, 1972). On the other hand inhibition of ovulation by oral contraceptives may prevent acute attacks in those patients who have cyclical attacks related to the menstrual cycle (Perlroth *et al*, 1966). Although oestrogens are weak inducers of ALA-S in chick embryo liver cell cultures and in whole animals (Granick and Kappas, 1967; Tschudy, Waxman, and Collins, 1967), the ALA-S in the chick embryo liver is induced by oral contraceptives due to the progestational component (Rifkind, Gillette, Song, and Kappas, 1970).

There is also some evidence that the metabolism of endogenous steroids may be abnormal in some patients with acute intermittent porphyria. Thus Goldberg, Moore, Beattie, Hall, McCallum, and Grant (1969) have shown that the excretion of various 17-oxosteroids may be increased and that one of these, dehydroepiandrosterone, and its sulphate conjugate produce a significant elevation of hepatic ALA-S when injected intraperitoneally into rats. Gillette, Bradlow, Gallagher, and Kappas (1970) have shown that in some patients metabolism of exogenously administered androgens is diverted from the 5 α -H pathway towards the 5 β -H pathway, thus producing metabolites which are known to be potent inducers of ALA-S (Granick and Kappas, 1967).

If the fundamental inherited defects in these conditions lie in the pathway of haem biosynthesis, the abnormality of haem metabolism must be directly responsible for the neurological disturbance characterized morphologically by axonal degeneration (Ridley, 1969) that underlies the clinical features of the acute attack. In the past the demonstration that ALA and PBG are pharmacologically inert (Goldberg, Paton and Thompson, 1954) has led to suggestions that there is a deficiency of a substance required to protect nerve function, or of pyridoxine (Ridley, 1969), or that the abnormality of porphyrin precursor metabolism is merely a spectacular side effect of a more fundamental metabolic disturbance (Neuberger, 1968) possibly involving oxidation of NADH (Tschudy and Bonkowski, 1972). Recently Becker, Viljoen, and Kramer (1971) have shown

that ALA inhibits brain tissue ATPase and have suggested that in the acute phase of porphyria ALA is taken up by nerve tissue and causes paralysis of conduction by inhibition of the Na^+/K^+ -dependent ATPase, while Feldman, Levere, Lieberman, Cardinal, and Watson (1971) have reported that PBG in concentrations similar to those found in the cerebrospinal fluid during acute attacks, and one of its non-enzymically produced condensation products porphobilin, produce presynaptic neuromuscular inhibition. Although the concentration of PBG in the cerebrospinal fluid is about one quarter that in plasma (Bonkowsky, Tschudy, Collins, Doherty, Bossenmaier, Cardinal, and Watson, 1971), there is some doubt as to whether ALA crosses the blood-brain barrier in more than trace amounts. Nothing is known of the consequences of disordered haem metabolism within nervous tissue.

SYMPTOMATIC CUTANEOUS HEPATIC PORPHYRIA (SYMPTOMATIC PORPHYRIA)

Almost all the patients with hepatic porphyria who do not have one of the three forms of hepatic porphyria described above have a purely cutaneous form of porphyria—symptomatic porphyria—in which skin lesions indistinguishable from those of variegate porphyria (Eales, 1963; Magnus, 1972) are accompanied by a characteristic abnormality of porphyrin metabolism. This is the commonest form of cutaneous porphyria encountered in the United Kingdom.

With rare exceptions there is no evidence of inheritance, and this form of porphyria has been regarded as acquired (Waldenström, 1957; Goldberg and Rimington, 1962), although possibly only by genetically predisposed individuals (Waldenström and Haeger-Aronsen, 1967; Taddeini and Watson, 1968). The condition has been reported in association with liver disease (particularly when due to alcohol), with oestrogen therapy, and with an outbreak of hexachlorobenzene poisoning in Turkey (Cam and Nigogosyan, 1963).

In Europe and N. America the majority of patients are men between the ages of 40 and 60 (Ippen, 1959) but the age and sex incidence depends on the underlying aetiology. The onset of the condition is insidious and spontaneous remission may occur. The skin lesions, which are the most striking clinical feature of symptomatic porphyria, occur mainly in areas of the skin exposed to sunlight, particularly the face and the backs of the hands and forearms. Increased fragility of the skin in response to trivial mechanical or thermal trauma is usually more prominent than photosensitivity. The acute skin lesions are erythema, vesicles and bullae, which may be haemorrhagic or become infected. Later

lesions include erosions, crusts, and scabs which finally heal with scar formation. Sclerodermatous changes may occur and milia are frequent. Both hirsutism and pigmentation, particularly of the face, are common and may be the only clinical features. There are no diagnostic differences between the skin lesions of symptomatic porphyria and the other hepatic porphyrias accompanied by cutaneous symptoms.

Acute attacks of porphyria with abdominal pain and neurological complications do not occur and in this respect the reaction to drugs, such as barbiturates, is normal (Taddeini and Watson, 1968).

The incidence of diabetes mellitus is increased in symptomatic porphyria (Brunsting, 1954; Eales and Dowdle, 1968) and an association with syphilis (Berman and Bielický, 1956; Gheorghui and Forsea, 1968) and connective tissue disorders (Hetherington, Jetton, and Knox, 1970; Rimington, Sears, and Eales, 1972) has been noted.

Biochemically the condition is characterized by a marked increase in the urinary excretion of uroporphyrin usually to between 1.0 and 10.0 mg/day, with a smaller increase in the coproporphyrin fraction, whereas the concentrations of PBG and, usually, ALA in the urine are normal (Eales *et al.*, 1966b; Taddeini and Watson, 1968). Numerous detailed analyses of the urinary porphyrins, employing chromatographic separation of individual porphyrins, have confirmed that uroporphyrin is the main porphyrin excreted in the urine, but large quantities of heptacarboxylic porphyrin with smaller but increased amounts of porphyrins with six, five and four carboxyl groups are also present (Sweeney, 1963; Nacht, San Martin de Viale, and Grinstein, 1970; Dowdle *et al.*, 1970; Doss, Meinhof, Look, Henning, Nawrocki, Dölle, Strohmeier, and Filippini, 1971b).

Alterations in faecal porphyrin concentration are less striking, although probably as much porphyrin is excreted by this route as in the urine (Sweeney, 1963; Herbert, 1966). The faeces usually contain increased amounts of both ether-soluble and ether-insoluble porphyrins (Eales *et al.*, 1966b; Taddeini and Watson, 1968; Eales *et al.*, 1971; Moore, Thompson, and Goldberg, 1972). The total ether-soluble porphyrins are not increased to the same extent as in variegate porphyria and may occasionally be within normal limits. Most of the increase is due to an increase in the coproporphyrin fraction which frequently exceeds the level of protoporphyrin. However, much of the 'coproporphyrin fraction' is not coproporphyrin but a mixture of tetracarboxylic porphyrins—*isocoproporphyrin*, *de-ethylisocoproporphyrin*, and *hydroxyisocoproporphyrin* (respec-

tively I, II, and III, Fig. 2) of which the first two are probably formed from dehydroisocoproporphyrin (IV, Fig. 2), which is a prominent component of the bile in symptomatic porphyria (Elder, 1971, 1972, and unpublished observations), by intestinal microorganisms. Small amounts of penta-, hexa-, and heptacarboxylic porphyrins are also usually present in this fraction.

The concentration of ether-insoluble porphyrin in the faeces is frequently increased (Watson, 1960; Sweeney, 1963; Herbert, 1966; Eales *et al.*, 1971; Elder, 1971; Magnus and Wood, 1971; Moore *et al.*, 1972). When this fraction is estimated by methods using urea-Triton X100-extraction (see below) (Rimington *et al.*, 1968) the quantities of porphyrin found may be as great as in variegate porphyria (Eales *et al.*, 1971; Magnus and Wood, 1971; Moore *et al.*, 1972). Examination of the composition of the porphyrin fraction extracted by urea-Triton has shown that heptacarboxylic porphyrin and uroporphyrin (Elder, 1971; Magnus and Wood, 1971) rather than porphyrin-peptide conjugates (Moore *et al.*, 1972) are the main ether-insoluble porphyrins excreted in this condition confirming earlier studies in which other methods of extraction were used (Watson, 1960; Sweeney, 1963; Herbert, 1966).

The isomer type of the porphyrins excreted in symptomatic porphyria is variable. Uroporphyrin is about 70% type I, coproporphyrin and penta-carboxylic porphyrin are about 50% type I, while hepta- and hexacarboxylic porphyrins are mainly type III (Nacht *et al.*, 1970; Dowdle *et al.*, 1970).

In addition to excreting excess uroporphyrin and heptacarboxylic porphyrin, patients with symptomatic porphyria accumulate large amounts of these porphyrins within the liver so that liver biopsy samples viewed directly in ultraviolet light show an intense red fluorescence. Increased amounts of porphyrin within the liver cell may persist after otherwise complete clinical and biochemical remission (Lundvall and Enerbäck, 1969) and might precede the onset of symptoms (Doss, Look, and Henning, 1971a).

The large amount of porphyrin stored in the liver underlies the unique response of patients with this condition to chloroquine administration, which produces a transient febrile reaction accompanied by massive uroporphyrinuria and biochemical evidence of liver cell damage (Sweeney, Saunders, Dowdle, and Eales, 1965; Felsher and Redeker, 1966). Chloroquine forms a complex with porphyrins and an abnormally high intracellular concentration may account for its hepatotoxic action in these patients (Scholnick and Marver, 1968).

FACTORS ASSOCIATED WITH SYMPTOMATIC CUTANEOUS HEPATIC PORPHYRIA

Liver disease

Liver damage frequently occurs in symptomatic porphyria even in the absence of known causative factors such as alcoholism or hexachlorobenzene poisoning (Taddeini and Watson, 1968). Liver function tests, particularly the bromsulphthalein retention time, show moderate, not usually severe, impairment of liver function (Waldenström and Haeger-Aronsen, 1960). Hepatomegaly is common, the most frequent histological findings, apart from siderosis, being fatty change, periportal fibrosis, sometimes with round cell infiltration, and cirrhosis, especially in those patients with a long clinical history (Uys and Eales, 1963; Lundvall and Weinfeld, 1968). The electron microscopic findings have been described by Jean, Lambertenghi, and Ranzi (1968) and by Timme (1971).

Alcohol

Alcoholism is a frequent but not invariable factor in the development of liver disease in this condition. Withdrawal of alcohol may lead to clinical and biochemical improvement especially if there is improved nutrition. Nevertheless symptomatic porphyria is uncommon in alcoholics suggesting that alcohol may unmask an inherited predisposition to this condition (Waldenström and Haeger-Aronsen, 1967; Taddeini and Watson, 1968; Bénard, Gajdos, and Gajdos-Török, 1958).

Iron metabolism

Hepatic siderosis (increased stainable haemosiderin iron) is very common but not invariable in symptomatic porphyria (Turnbull, 1971). Nevertheless severe iron overload is uncommon and the condition is only rarely associated with haemochromatosis (Sauer, Funk, and Finch, 1966). Some hepatic siderosis with liver cell damage and cirrhosis is common among heavy drinkers of beers and wines with high iron content, eg, home-brewed Bantu beers (Saunders, Williams, and Levey, 1963) and the red wine of Brescia (Perman, 1967). Symptomatic porphyria is unusually common amongst drinkers of these beverages but in the Bantu malnutrition may also be an important factor. Increased iron absorption and mild to moderate hepatic siderosis are not infrequent in patients with cirrhosis (Williams, Williams, Scheuer, Pitcher, Loiseau, and Sherlock, 1967) and may also be due to enhancement of iron absorption by alcohol itself. Although the cause and role of the increased iron stores in some patients with symptomatic porphyria is not clear, depletion of storage iron by repeated venesection (Ippen, 1961; Epstein and Redeker, 1968; Lundvall and Weinfeld,

1968) or by long-term desferrioxamine treatment (Wöhler, 1964) produces a clinical remission in the majority of patients which is reversed by replenishment of iron stores.

Oestrogens

There is a significantly increased incidence of symptomatic porphyria in patients taking oestrogens, eg, men with prostatic carcinoma or women treated for carcinoma of the breast or postmenopausal symptoms (Warin, 1963; Copeman, Cripps, and Summerly, 1966; Felsher and Redeker, 1966; Zimmerman, McMillin, and Watson, 1966; Vail, 1967). The condition (symptomatic porphyria) is rare in women taking oral contraceptives. The rarity of symptomatic porphyria as a complication of oestrogen therapy, the absence of abnormalities of porphyrin excretion in most patients taking similar amounts of oestrogens (Theologides, Kennedy, and Watson, 1964; Roenigk and Gottlob, 1970), and the lack of evidence of a dose-related effect suggests that, as with alcohol, some underlying predisposition is present.

Heredity

Most patients with symptomatic porphyria have no family history of porphyria and porphyrin excretion is usually normal in other members of their families (Hickman, Saunders, and Eales, 1967; Waldenström and Haeger-Aronsen, 1967; Taddeini and Watson, 1968) although a few patients have been described with the clinical and biochemical syndrome and evidence of latent or manifest symptomatic porphyria in their families (Waldenström and Haeger-Aronsen, 1967; Taddeini and Watson, 1968). There is a high incidence of alcoholism and evidence of liver dysfunction in these patients and it is likely that liver damage may unmask porphyria in these families.

NATURE OF THE METABOLIC ABNORMALITY

There is some evidence that, as in the inherited hepatic porphyrias, there is endogenous overproduction of ALA by the liver in symptomatic porphyria. Kaufman and Marver (1970) have pointed out that if as seems probable there is no increase in haem synthesis in this condition (Dowdle *et al*, 1968) the excessive amounts of porphyrins excreted do not require a great increase in ALA production and that the necessary increase in ALA-S activity may be difficult to detect. An increase in hepatic ALA-S activity has been detected in many patients with this condition (Dowdle *et al*, 1967; Zail and Joubert, 1968; Shanley, Zail, and Joubert, 1969; Moore, Turnbull, Bernardo, Beattie, Magnus, and Goldberg, 1972) but in others there is no increase (Zail and Joubert, 1968; Shanley *et al*, 1969) parti-

cularly when more specific assays are used (Kaufman and Marver, 1970; Strand *et al*, 1970). The activity of the enzyme apparently depends on the stage of activity of the condition, for Shanley *et al* (1969) showed that it is increased by alcohol ingestion and Moore *et al* (1972) have reported that remission induced by venesection is accompanied by a decrease in activity.

In this condition, as in the inherited hepatic porphyrias, the increase in ALA production is possibly secondary to a partial block in haem synthesis which determines the characteristic porphyrin excretion pattern. Although the nature of this fundamental disturbance is unknown, the similarity between the porphyrin excretion patterns of most patients with this condition suggest that it is common to all aetiological groups. At present it is not clear why acute attacks and porphobilinogenuria do not occur.

One factor that the majority, if not all, these patients have in common is disturbed liver function and it is possible that ultrastructural damage within the hepatocyte leads to disruption of the normal rigid compartmentalization of haem synthesis. As a consequence, oxidation of porphyrinogens to porphyrins may be enhanced (Heikel, Lockwood, and Rimington, 1958; Rimington, 1963) or alternative metabolic pathways that are quantitatively insignificant under normal conditions may become activated (Dowdle *et al*, 1970; Elder, 1972). The fact that symptomatic porphyria is a rare complication of several common forms of liver disease has led to the suggestion that some patients with this condition have an inherited predisposition to develop the disease in response to liver injury (Waldenström and Haeger-Aronsen, 1967; Taddeini and Watson, 1968), while in others, notably those poisoned with hexachlorobenzene, the disease may be truly acquired. Although iron is probably not a primary aetiological agent (Kalivas, Pathak, and Fitzpatrick, 1969; Shanley, Zail, and Joubert, 1970) it appears to enhance the excessive excretion of porphyrin both in symptomatic porphyria and in hexachlorobenzene poisoning in rats (Taljaard, Shanley, and Joubert, 1971) without affecting the pattern of porphyrins excreted.

PORPHYRIN-PRODUCING HEPATIC TUMOURS

Two patients with a purely cutaneous form of porphyria due to overproduction of porphyrin by a tumour surrounded by normal liver tissue have been described. In one the tumour was a benign hepatic adenoma and the porphyria disappeared after its removal (Tio *et al*, 1957); the other was due to a malignant primary hepatoma in an otherwise normal liver (Thompson *et al*, 1970). The porphyrin ex-

cretion pattern in both these patients differed from that usually found in symptomatic porphyria and they probably constitute a separate form of acquired cutaneous hepatic porphyria that must be considered in the differential diagnosis of symptomatic porphyria. Such a distinction is tentative and as more patients are described some may be found indistinguishable from symptomatic porphyria. Indeed it is mandatory that all patients with symptomatic porphyria should be carefully examined for the presence of an hepatic tumour.

Laboratory Investigation of the Porphyrins

Table III shows the abnormalities which may be found in the various forms of porphyria and makes clear the importance of examining urine, faeces, and erythrocytes if a correct diagnosis is to be established.

PRESERVATION OF SAMPLES

Porphobilinogen is unstable in urine particularly under acidic conditions; if specimens cannot be estimated immediately after collection, the pH should be adjusted to neutrality and the sample stored at -20° for up to one month (Bossenmaier and Cardinal, 1968). Urinary porphyrins are also stable under these conditions. If faecal porphyrins cannot be estimated within a few hours of collection, the faeces may be preserved for at least a month at -20° . Blood for porphyrin analysis should not be collected unless analysis can be carried out immediately.

NORMAL URINARY, FAECAL, AND ERYTHROCYTE PORPHYRINS

The normal values are summarized in Table IV; all values refer to total porphyrin, ie, including that formed from porphyrinogen by oxidation during analysis. Values for urine are presented in $\mu\text{g/day}$, for faeces in $\mu\text{g/g}$ of dry weight of faeces, and erythrocyte porphyrins in $\mu\text{g}/100$ ml of packed red cells: plasma normally contains no measurable porphyrin.

Urine

Urine contains small amounts of PBG, ALA, and

Porphyrin	Normal Value
<i>Urine</i>	
Porphobilinogen	<1.0 mg/day
Aminolaevulinic acid	<2.5 mg/day
Coproporphyrin	0-160 $\mu\text{g/day}$
Uroporphyrin	5-30 $\mu\text{g/day}$
<i>Faeces ($\mu\text{g/g}$ dry stool)</i>	
Coproporphyrin	0-20
Protoporphyrin	0-76
Ether-insoluble porphyrin	0-21 ^a
<i>Erythrocytes ($\mu\text{g}/100$ ml packed cells)</i>	
Coproporphyrin	0-4
Protoporphyrin	4-52

Table IV Normal values for urinary, faecal, and erythrocyte porphyrins¹

¹Values quoted by Rimington (1971) except where otherwise stated.
²Reported Values for ether-insoluble porphyrins are variable. The 'normal' range shown here is derived from a study of only 13 subjects (Elder, unpublished observations).

	Blood	Urine	Faeces	Isomer Type (I or III)
<i>Erythropoietic</i>				
Congenital erythropoietic (Günther's disease)	Erythrocyte porphyrin increased (mainly uroporphyrin)	PBG and ALA normal Uroporphyrin much increased Coproporphyrin increased	Coproporphyrin increased. Some increase in protoporphyrin and uroporphyrin.	I
Erythrohepatic protoporphyria	Erythrocyte porphyrin increased (mainly protoporphyrin)	Normal usually	Protoporphyrin increased. Coproporphyrin sometimes increased	III
<i>Hepatic</i>				
Acute intermittent porphyria	Normal	PBG and ALA increased Uroporphyrin may be present from non-enzymatic condensation of PBG.	Normal	
Hereditary coproporphyria		Variable. PBG and ALA may be increased. Coproporphyrin increased.	Increase in coproporphyrin	III
Symptomatic porphyria	Normal	PBG and ALA normal. Large increase in porphyrins (mainly uroporphyrin).	Normal to moderate increase of 'copro' and protoporphyrin with 'copro' > proto. Often increased ether-insoluble porphyrin (see text).	Complex (see text)
Variegate porphyria	Normal	PBG and ALA increased, but may be normal during remission. Porphyrins may be increased with the copro > uro.	Large increase in proto- and coproporphyrin with proto > copro. 'X' peptidoporphyrin increased.	Mainly III

Table III Porphyrins and porphyrin precursors occurring in blood, urine, and faeces in the porphyrias

The porphyrias: a review

coproporphyrin, together with traces of uroporphyrin and hepta-, hexa-, and pentacarboxylic porphyrins. The ratio of coproporphyrin I to III is variable, although coproporphyrin I usually predominates.

Faeces

Coproporphyrin of normal faeces is also predominantly isomer type I. Comparison of the amounts of porphyrin excreted in the bile and in the faeces suggests that the dicarboxylic porphyrins of normal faeces are mainly of exogenous origin arising from the activity of microorganisms in the gastrointestinal tract (Table V) (England, Cotton, and French, 1962; French and Thonger, 1966). Protoporphyrin, whether

1	Endogenous
(a)	From the products of intermediary metabolism excreted in the bile
2	Exogenous
(a)	From precursors degraded to porphyrins by intestinal microorganisms
(i)	from chlorophyll
	— in food
(ii)	from haem and haemoproteins
	— in food
	— haemorrhage from walls of alimentary tract
	— desquamation of cells from walls of alimentary tract
(b)	Synthesis by intestinal microorganisms from simple precursors
(c)	Preformed porphyrins in food

Table V Origin of faecal porphyrins

of endogenous or of exogenous origin, is further changed by intestinal microorganisms so that normal faeces contain a variable mixture of proto-, meso-, deuterio-, and pemptoporphyrins (French, England, Lines, and Thonger, 1964). In addition, small quantities of uroporphyrin (Aziz and Watson, 1969) and porphyrin-peptide conjugates ('X porphyrins') (Rimington *et al*, 1968) may be present. Normal bile and meconium contain small amounts of other porphyrins including an acrylic analogue of coproporphyrin (V, Fig. 2) (French and Thonger, 1966; French, Nicholson, and Rimington, 1970) but this has not been detected in normal faeces, perhaps because of its reduction to coproporphyrin by microorganisms.

Erythrocytes

Normal erythrocytes contain only small amounts of protoporphyrin and coproporphyrin; the latter is predominantly of isomer type I (Koskelo and Toivonen, 1968). It is possible that porphyrins are in highest amount in the younger cells as in erythrohepatic protoporphyria (Clark and Nicholson, 1971). Uroporphyrin is not detectable in normal erythrocytes.

Plasma

Coproporphyrin cannot be detected in normal plasma but protoporphyrin is just detectable in amounts which cannot exceed 2-3 µg/dl (Masuya, 1969).

METHODS OF DETERMINATION

PBG and ALA

The widely used screening tests for PBG in which equal volumes of urine and modified Ehrlich's reagent (paradimethylaminobenzaldehyde in HCl) give a red colour are unreliable because of the presence of interfering substances (Table VI),

<p><i>Pyrrhic compounds</i></p> <p>Urobilinogen</p> <p>Phylloerythrinogen</p> <p>Pyrrhic mono- and di-carboxylic acids</p> <p>Indole</p> <p>Indoxyl</p> <p>5:6 Dihydroxyindole (melanogen)</p>	<p>which with paradimethylaminobenzaldehyde also give a red colour</p>
<p><i>Unidentified urinary substances after administration of:</i></p> <p>Levomepromazine</p> <p>Cascara sagrada bark extract</p> <p>Sedormid</p>	
<p>Methyl red</p> <p>Pyridium (phenazopyridinium chloride)</p> <p>Urosein</p>	<p>which become red in the acid of the reagent</p>
<p>Urea</p>	<p>which both inhibits colour due to porphobilinogen and produces a yellow colour</p>
<p>Bilirubin</p>	<p>which produces a green colour</p>
<p>Tryptophan</p>	<p>which produces an orange brown colour</p>

Table VI Substances which may affect the detection of urinary porphobilinogen by the Ehrlich's reagent

and substances, eg, urea, which inhibit colour formation. They are also limited in sensitivity, only reliably detecting concentrations of PBG above about 10 mg/litre. Separation and distinction of the Ehrlich reagent complexes of porphobilinogen and urobilinogen may be affected by extraction of the latter into chloroform after neutralization of acid present in the reagent with sodium acetate. It is essential to allow development of colour for 1-5 minutes before making this neutralization and extraction and a mixture of benzyl and amyl alcohols is a better extractant than chloroform (Rimington, 1958b).

The test is strongly positive in almost every patient presenting acute symptoms but its sensitivity is inadequate for reliable detection of the condition in patients without clinical symptoms. All positive tests should be confirmed by quantitative estimation of ALA and PBG using a method by which por-

phobilinogen is isolated by ion exchange chromatography before determination with Ehrlich's reagent. Such a method is that of Mauzerall and Granick (1956) or some modification of it (eg, Grabecki, Haduch, and Urbanowicz, 1967; Doss and Schmidt, 1971). A convenient kit for these determinations is obtainable from the Biorad Company (New York). These methods have the added convenience of permitting the simultaneous estimation of ALA by condensation with acetyl-acetone to give an Ehrlich-reacting substance which may be similarly estimated.

Porphyrins

Porphyryns, especially protoporphyrins, are unstable and all measurements must be rapidly carried out in subdued light as soon as possible after samples are obtained. It is especially important that all solvents be peroxide-free.

Quantitative estimation of porphyrins is time-consuming. Their intense red fluorescence in near ultraviolet (Wood's) light, however, provides a sensitive method of detection and this forms the basis of widely used screening tests for increased porphyrin concentrations in urine, faeces, and erythrocytes. Suitable tests for faeces and urine have been described by Rimington (1958b) and their use in the diagnosis of the porphyrias is discussed by Eales *et al* (1966b). Similar techniques may be used for the screening of whole blood (Rimington and Cripps, 1965) but in erythropoietic protoporphyria a similar technique may be used for the screening of whole blood but it is more satisfactory to determine the percentage of fluorocytes by examination of the fresh red cells in ultraviolet microscopy using light from an iodine quartz lamp (Chapel *et al*, 1972). The plasma should also be examined for fluorescence in ultraviolet light. Positive screening tests and tests where the interpretation is in doubt should always be confirmed by quantitative estimations, which should also be used for investigation of the families of patients with porphyria.

Most methods for the quantitative determination of porphyrins in urine, faeces, and erythrocytes depend upon preliminary extraction and fractionation by solvent partition followed by spectrophotometric or fluorimetric determination (Schwartz, Berg, Bossenmaier, and Dinsmore, 1960).

The methods most widely used in Britain for the estimation of porphyrins in urine and ether-soluble porphyrins in faeces and erythrocytes have been described in detail in a recent Association of Clinical Pathologists Broadsheet (Rimington, 1971). Much erythrocyte uroporphyrin may be recovered from the aqueous phases left after fractionation of erythrocyte copro- and protoporphyrins, by absorption on alumina and subsequent extraction into 1.5 M

hydrochloric acid. Some uroporphyrin, especially the I isomer, remains in the proteinaceous precipitate formed from the cells during analysis and may be extracted into 10% ammonia. The two aqueous uroporphyrin fractions are then united for spectrophotometry or spectrofluorimetry (Schwartz *et al*, 1960).

Ether-insoluble porphyrins in faeces are best estimated by the method of Rimington *et al* (1968) in which 45% (w/v) urea containing 4% (v/v) Triton X-100 is used as extractant. It is important to ensure that ether-soluble porphyrins have been wholly removed by exhaustive extraction with ether-acetic acid before this method is applied to the faecal residue. Even so the ether-insoluble porphyrin fraction obtained in this way from both normal and porphyric faeces is complex and may yet contain ether-soluble porphyrins, which have escaped extraction by adsorption on solid faecal matter as well as true ether-insoluble porphyrins such as uroporphyrin and other polycarboxylic porphyrins, porphyrin-peptide conjugates ('X porphyrin'), and possibly other porphyrin conjugates. For this reason the ether-insoluble porphyrins extracted by urea-Triton solution should not be referred to as 'X porphyrin' or porphyrin-peptide conjugates until this is confirmed by additional techniques such as reaction with ^{14}C -labelled dinitrofluorobenzene (Rimington *et al*, 1968) or electrophoresis (Rimington *et al*, 1968; Magnus and Wood, 1971). Before this can be done all Triton X-100 must be removed from the sample (Rimington *et al*, 1968) since its presence affects the chromatographic and electrophoretic mobility of porphyrins.

In all methods depending on solvent extraction, porphyrins are divided into fractions according to their solubility properties but are not identified, and it is well recognized that the fractions designated protoporphyrin, coproporphyrin, and uroporphyrin may contain other porphyrins as well. For this reason in recent years increasing use has been made of methods in which porphyrins are separated by electrophoresis (Lockwood and Davies, 1962; Magnus and Wood, 1971) or by thin-layer chromatography after extraction and usually methyl esterification (Doss, 1970). The individual porphyrins are then estimated either spectrophotometrically or fluorimetrically after elution from the plates or by fluorescence scanning *in situ* (Doss, Ulshöfer, and Philipp-Domiston, 1971c). The sensitivity of these methods which have been used, particularly for urine, is increased if the porphyrins are converted to their zinc chelates before separation (Doss, 1971).

DETERMINATION OF ISOMER TYPE

In certain circumstances, determination of isomer

type of porphyrin present may be necessary. This may be carried out in the case of coproporphyrins by chromatography in a lutidine:water:ammonia system (Eriksen, 1958), or in the case of uroporphyrins by decarboxylation to coproporphyrins which may then be characterized in the same way (Edmundson and Schwartz, 1953).

Future Considerations

It will be seen that the porphyrias are an interesting group of metabolic diseases. The diversity of their clinical manifestations and biochemical features has stimulated much useful collaboration between physicians, pathologists, and biochemists. Extensive fundamental knowledge of porphyrin biochemistry and metabolism has accrued from this. Probably the most important problems worthy of further study include the nature of the central nervous system involvement in the inherited hepatic porphyrias, the mode of action of the porphyrinogenic drugs, and the mechanism of the photosensitization in porphyria. There is need also for refinement of the methods whereby the porphyrias are diagnosed and differentiated.

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