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THE ACUTE HEPATIC PORPHYRIAS

by

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Thesis presented to the University of Glasgow
for the degree of Doctor of Medicine

from the

University Department of Medicine
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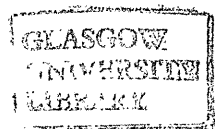
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PREFACE

Over the past 4 years, I have had the privilege of working with Professor Abraham Goldberg in the fascinating field of the porphyrias. His clinical and scientific advice has been invaluable and his personal interest in my career most encouraging. My research in the porphyrias has taught me important principles of human disease processes as well as giving me an insight into the effects of such diseases on the personal lives and families of afflicted patients.

Some of the work has been published and reprints of each publication are submitted with the thesis. Collaboration with a number of colleagues has been necessary as described in the formal acknowledgements. Except where indicated, the work presented has been carried out by myself.

The writing of this thesis is entirely my own work.

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SUMMARY

The acute hepatic porphyrias are the result of hereditary partial deficiencies of individual enzymes in the pathway of haem biosynthesis. Seven enzymes are known to be involved in the pathway, converting glycine and succinyl CoA first to porphyrin precursors and then to porphyrins and finally to haem. The rate of the process is regulated by the initial enzyme, delta-aminolaevulinic acid (ALA) synthase, which is under negative feedback control by haem. The partial block in this pathway, in each of the acute porphyrias, results in compensatory increased activity of ALA synthase and, consequently, overproduction of porphyrins and precursors formed prior to the deficient enzyme. Patients with acute porphyria generally enjoy good health, but exposure to certain recognised factors, namely specific drugs, alcohol, hormones and fasting, may precipitate severe clinical attacks characterized by gastro-intestinal symptoms, psychiatric disturbances and neuropathy. All of the systemic manifestations of the attack are thought to be explained by neurological dysfunction, but the mechanism by which the abnormal haem biosynthesis causes the neuropathy is not known. Following a general description of haem biosynthesis and the porphyrias, this thesis concentrates on the management of acute hepatic porphyria studying, in turn, the detection of subjects with the genetic trait, the factors which may precipitate attacks in these subjects and the treatment of patients in established attack.

Much of the work presented is based upon the ability to measure the activities of the individual enzymes of haem biosynthesis in human peripheral blood cells. The mitochondrial enzymes, ALA synthase, coproporphyrinogen oxidase and ferrochelatase, are measured in leucocytes and the cytosolic enzymes, ALA dehydratase, uroporphyrinogen-1-synthase (URO synthase) and uroporphyrinogen decarboxylase, in erythrocytes.

The measurement in peripheral blood cells of the activities of both the rate-controlling enzyme, ALA synthase, and the appropriate genetically deficient intermediate enzyme, is shown to be a sensitive and specific means of detecting latent cases of acute porphyria, the majority of whom have normal excretion of porphyrins and precursors. Enzymatic screening of affected families confirms that the genetic trait is inherited in an autosomal dominant sex-independent manner. The family studies also demonstrate that the vast majority of subjects with the trait remain clinically latent, and the reason why the minority of patients experience clinical manifestations is examined. The activity of the partially deficient enzyme is similar in latent and manifest cases, but ALA synthase activity is higher in patients who have experienced an attack and this may reflect higher circulating levels of endogenous inducing agents.

Drugs are an important exogenous precipitating factor for acute porphyria, and an important aspect of the prevention of attacks is the identification of

potentially porphyrinogenic drugs. The testing of 26 commonly prescribed drugs, by assessing their effect on hepatic ALA synthase activity in rats, is described. The mechanism by which drugs stimulate ALA synthase activity is examined with particular reference to the role of induction of the mixed function oxidase enzyme (MFO) system. A close correlation is noted between induction of the MFO system, as reflected in increased hepatic cytochrome P450 content, and increased activity of ALA synthase, though not the converse. Sequential studies following commencement of phenytoin administration to rats show that the rise in ALA synthase activity is transitory and coincides with the rise in hepatic cytochrome P450 content which is maintained. These observations indicate that induction of the MFO system demands increased ALA synthase activity to provide the additional haem for synthesis of the haemoprotein, cytochrome P450.

The ability to measure the activities of the enzymes of haem biosynthesis in peripheral blood cells is used to examine the effects of the porphyrinogenic factors, drugs, alcohol, hormones and fasting, on the pathway in non-porphyric human subjects. Each of these factors, except fasting, increases ALA synthase activity, confirming the mechanism by which they precipitate biochemical relapse in porphyric subjects. Sequential studies of leucocyte ALA synthase activity following commencement of the porphyrinogenic drug phenytoin, in non-porphyric subjects, show that the pattern of rise in enzyme activity is similar to that observed in animal hepatic tissue, indicating that the drug is inducing a haem-requiring enzyme system in leucocytes as well as in the liver. The acute ingestion of a moderate amount of alcohol (200 mls Vodka)

in healthy subjects, results in increased activity of ALA synthase and URO synthase, and depression of the activities of ALA dehydratase, uroporphyrinogen decarboxylase, coproporphyrinogen oxidase and ferrochelatase. In chronic alcoholic subjects, ALA synthase activity is increased, and ALA dehydratase and uroporphyrinogen decarboxylase activities depressed. This stimulation of ALA synthase and depression of uroporphyrinogen decarboxylase, explains the biochemical mechanism of alcohol-related porphyria cutanea tarda.

In two subjects with alcohol-induced anaemia, marked depression of coproporphyrinogen oxidase and ferrochelatase activities is observed and these enzymatic changes in blood cells are relevant to the mechanism by which alcohol causes bone marrow depression. With respect to hormones and haem biosynthesis, a marked fluctuation in leucocyte ALA synthase activity is observed during the female menstrual cycle. This hormonal effect on blood ALA synthase activity may be relevant to the mechanism of the hormonal regulation of erythropoiesis.

The two currently available means of treating attacks of acute porphyria are assessed. The administration of intravenous laevulose reduces the duration and severity of the attack but in patients suffering frequent attacks venous access soon becomes compromised. Intravenous haematin therapy results in biochemical improvement, shown by repression of leucocyte ALA synthase activity and reduced urinary excretion of porphyrin precursors. The clinical response to haematin is less consistent, definite improvement

accompanying only half of the courses. The only adverse effect noted following haematin is localized thrombophlebitis. In spite of the administration of both laevulose and haematin and full intensive care supportive therapy, two of our patients died in attack, illustrating that current therapy remains inadequate. Advancement in the treatment of acute porphyria is hindered by lack of understanding of the relationship between the biochemical and clinical manifestation of the disease, and it is concluded that further studies of neurological aspects of abnormal haem biosynthesis are needed.

ABBREVIATIONSAbbreviations used in certain parts of Text

ALA	Delta-aminolaevulinic acid
A. I. A.	Allylisopropylacetamide
AIP	Acute intermittent porphyria
Cyt. P450	Cytochrome P450
COPRO oxidase	Coproporphyrinogen oxidase
DHA	Dehydroepiandrosterone
GABA	Gamma aminobutyric acid
M. F. O. system	Mixed function oxidase enzyme system
PBG	Porphobilinogen
URO synthase	Uroporphyrinogen - 1 - synthase

Abbreviations used in certain figures and tables

L. ALA. S	Leucocyte delta-aminolaevulinic acid synthase
L. COPRO. O	Leucocyte coproporphyrinogen oxidase
L. FERR	Leucocyte ferrochelataase
E. ALA. D	Erythrocyte delta-aminolaevulinic acid dehydratase
E. URO. S	Erythrocyte uroporphyrinogen - 1 - synthase
E. URO. D	Erythrocyte uroporphyrinogen decarboxylase

CHAPTER 1

HAEM METABOLISM

1. HAEM METABOLISM

1.1. INTRODUCTION

Haem (iron - protoporphyrin - IX) is the most ubiquitous metalloporphyrin chelate of the animal kingdom and catalyses the basic energy reactions upon which life depends (Figure 1). It is synthesized in all metabolically active cells in the human body, a normal adult producing about 0.45 mmol haem every 24 hours (Elder, 1976). Haem forms the basic structure of oxygen-binding haemoproteins such as haemoglobin and myoglobin, cellular enzymes such as catalases and peroxidases which decompose hydrogen peroxide, cellular respiratory pigments such as mitochondrial cytochrome b's, monooxygenases such as mitochondrial cytochrome P450, dioxygenases such as tryptophan pyrrolase, and microsomal cytochrome b5 which is important in fatty acid desaturation reactions (Maines, 1979). Eighty percent of the body's daily haem production occurs in the bone marrow where it is required for haemoglobin formation, and most extra-medullary production occurs in the liver where it is required for the many haemoprotein enzyme systems.

1.2. HAEM BIOSYNTHESIS

Eight enzymes are known to be involved in the biosynthesis of haem, four being present within the mitochondrion and four in the cytoplasm (Figure 2). The process starts within the mitochondrion with the condensation of succinyl CoA and glycine to form delta-aminolaevulinic acid (ALA). This

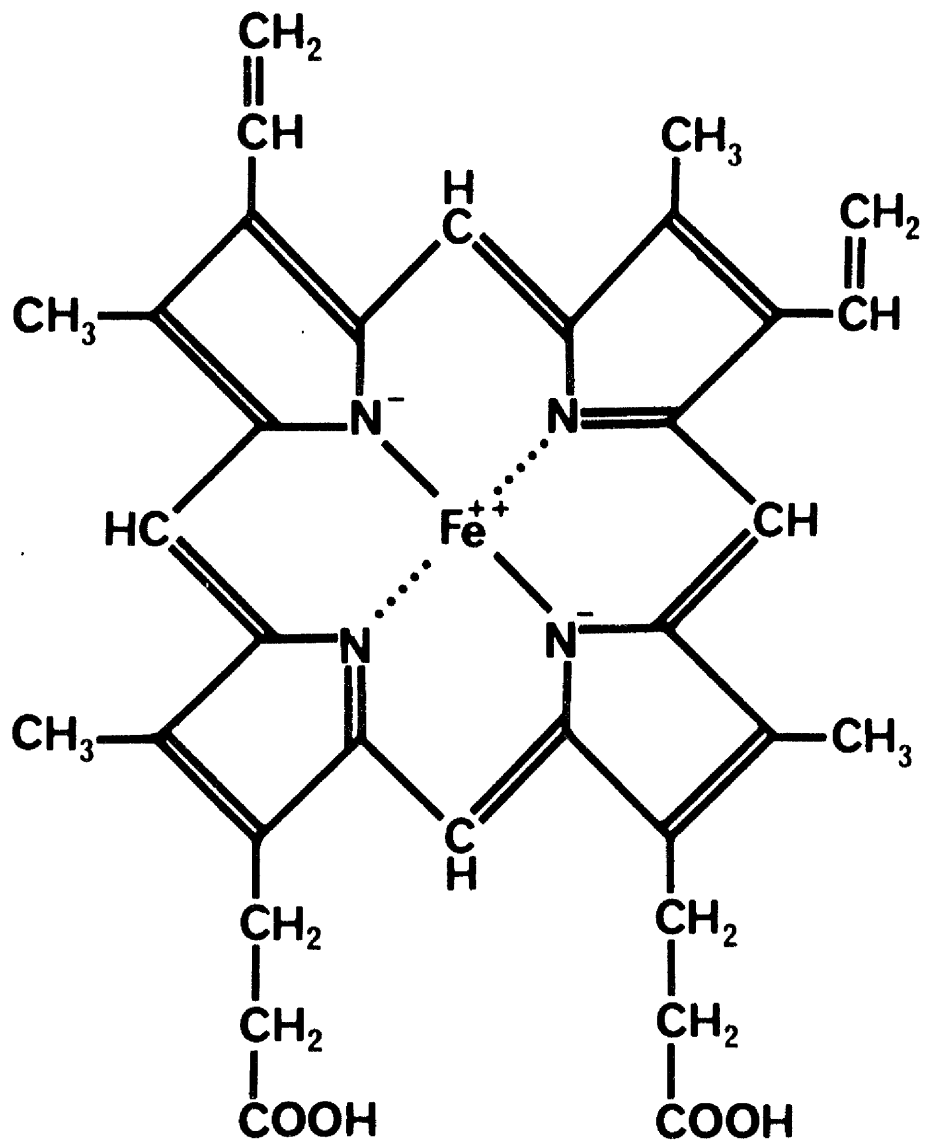


Figure 1

The Structure of Haem.

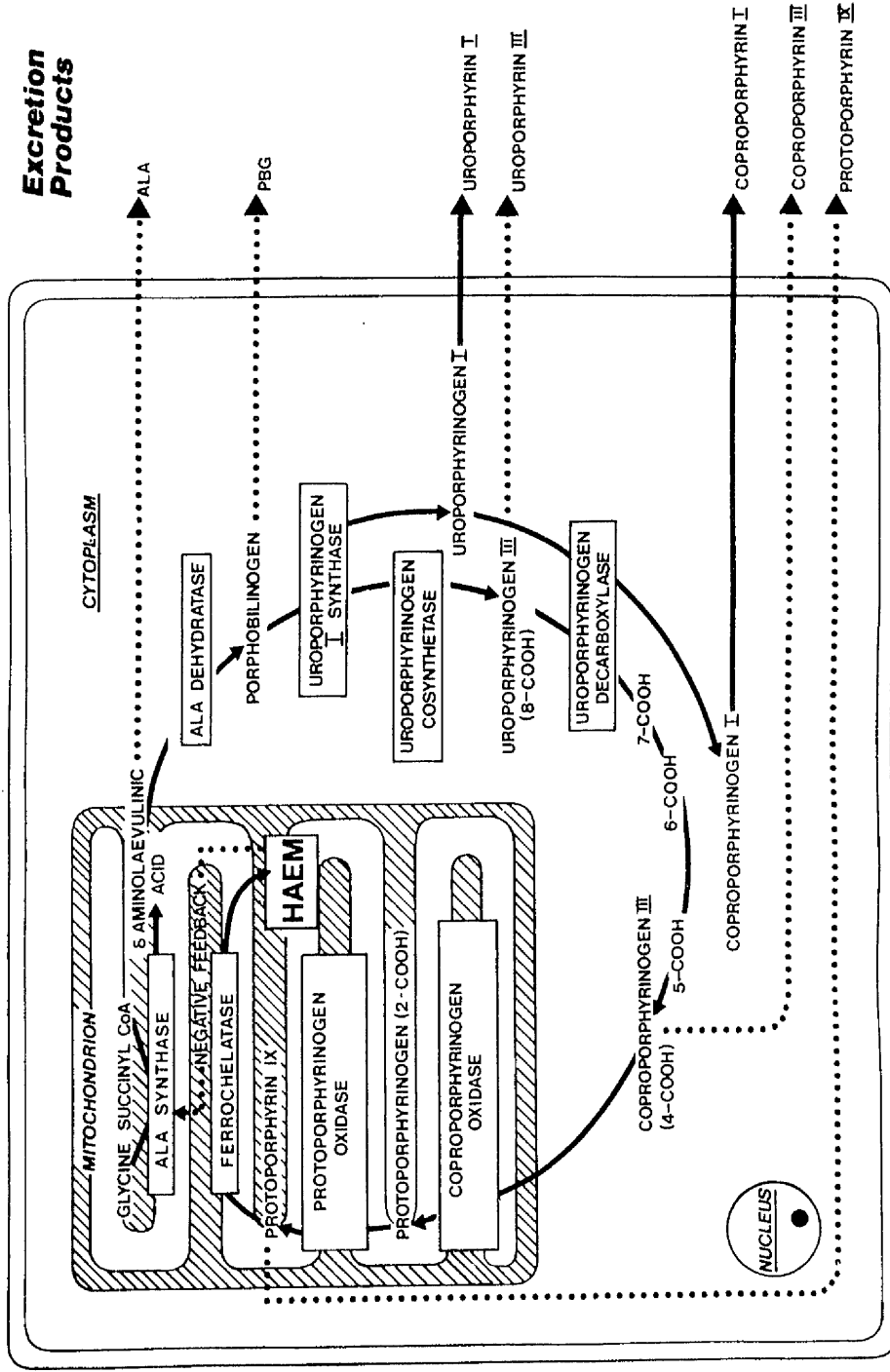


Figure 2
The Pathway of Haem Biosynthesis.

reaction is catalysed by the initial and rate-controlling enzyme of the pathway, ALA synthase. Pyridoxal phosphate is required as a cofactor for ALA synthase. The synthesized ALA passes out of the mitochondrion and in the cytoplasm two molecules condense under the catalytic control of ALA dehydratase to form the monopyrrole porphobilinogen (PBG). The next step requires the concerted action of two enzymes, uroporphyrinogen-I-synthase and uroporphyrinogen-III-synthase, which control the condensation of four molecules of PBG to form the tetrapyrrole uroporphyrinogen III. The combined action of these two enzymes is necessary to form the uroporphyrinogen III isomer. Uroporphyrinogen-I-synthase acting alone, only produces the symmetrical uroporphyrinogen-I-isomer which cannot be formed into haem and is excreted as coproporphyrin I. The next step of the reaction is controlled by the enzyme uroporphyrinogen decarboxylase which catalyses the sequential decarboxylation of the octacarboxylic uroporphyrinogen to form the tetracarboxylic coproporphyrinogen. Uroporphyrinogen decarboxylase acts on both uroporphyrin I and III forming both isomers of coproporphyrinogen. Coproporphyrinogen III then enters the mitochondrion where coproporphyrinogen oxidase catalyses the combined oxidation and decarboxylation of two propionyl residues on rings A and B of the porphyrin nucleus to form protoporphyrinogen. Coproporphyrinogen oxidase will not act on the coproporphyrinogen I isomer which, unable to proceed any further in the pathway, is excreted. Within the mitochondrion the protoporphyrinogen is oxidized to protoporphyrin by the oxygen-dependent enzyme, protoporphyrinogen oxidase. The final enzyme

of the pathway is ferrochelatase which catalyses the insertion of ferrous iron into protoporphyrin to form haem.

1.3. REGULATION OF THE RATE OF HAEM SYNTHESIS

The rate of haem biosynthesis is regulated by the initial enzyme of the pathway, ALA synthase, which is under negative feedback control by haem (Marver & Schmid, 1972). ALA synthase is particularly suited to this role being present in the lowest concentration and having the shortest half-life of any of the enzymes of the pathway and being readily inducible (Moore, 1980). It is postulated that within the mitochondrion there exists a pool of "free" or "regulatory" haem which controls the activity of ALA synthase (Bonkowsky et al, 1979). Any depletion of this free haem pool, by increased destruction, reduced synthesis or increased utilization, results in increased activity of ALA synthase. Likewise, supplementation of the free haem pool depresses ALA synthase activity (Whiting & Granick, 1976). Haem probably reduces ALA synthase activity by repression and inhibition at both transcriptional and translational cellular levels (Granick & Sassa, 1971).

The only other enzyme, apart from ALA synthase, with a markedly lower activity than the other enzymes of the pathway is uroporphyrinogen-1-synthase. It has been suggested that this enzyme may play a secondary rate-controlling role in the pathway becoming important when ALA synthase is derepressed (Brodie et al, 1977).

1.4. EXCRETION OF HAEM PRECURSORS

Porphyrins and porphyrin precursors which leave the cell are very rapidly excreted and therefore plasma concentrations are normally very low. The quantities of haem precursors which leave the cell and are excreted are trivial compared with the amounts used for haem synthesis (Elder, 1976). The hydrophilic porphyrin precursors ALA and PBG are excreted exclusively in the urine whereas the lipophilic formed porphyrins are excreted mainly in the bile. In normal subjects considerable renal tubular reabsorption of the porphyrin precursors occurs; when glomerular filtration rate is estimated from endogenous creatinine clearance, 90-95 per cent of the filtered load of ALA and PBG is reabsorbed (Druyan et al, 1965). In the disease situation when the plasma concentrations of ALA and PBG rise their renal tubular reabsorption decreases and may fall to zero. The urinary ALA excretion is slightly increased by water-loading and reduced by water-deprivation (Araki, 1978). Both coproporphyrin and protoporphyrin, which are predominantly excreted in the bile, undergo enterohepatic circulation (Struthers, 1966).

1.5. HAEM CATABOLISM

A small proportion of the haem synthesized by the liver undergoes rapid catabolism, contributing to the "early labelled" bilirubin, but most is incorporated into hepatic haemoproteins, of which microsomal cytochrome P450 quantitatively is the most important (Bonkowsky et al, 1979). The

major route of hepatic haem catabolism involves the microsomal enzyme haem oxygenase which requires molecular oxygen and NADPH-cytochrome C reductase for activity (Tenhunen et al, 1968) (Figure 3). The final products of the reaction are iron, carbon monoxide and biliverdin. Haem oxygenase can be induced by haem (Tenhunen et al, 1970) and certain other substances (Maines & Kappas, 1976). The location and source of the haem which serves as substrate for haem oxygenase is uncertain. Biliverdin is then further reduced to bilirubin by the cytoplasmic enzyme biliverdin reductase. Most of the bilirubin formed is then conjugated by the enzyme bilirubin glucuronidase before being excreted in the bile (Tait, 1978).

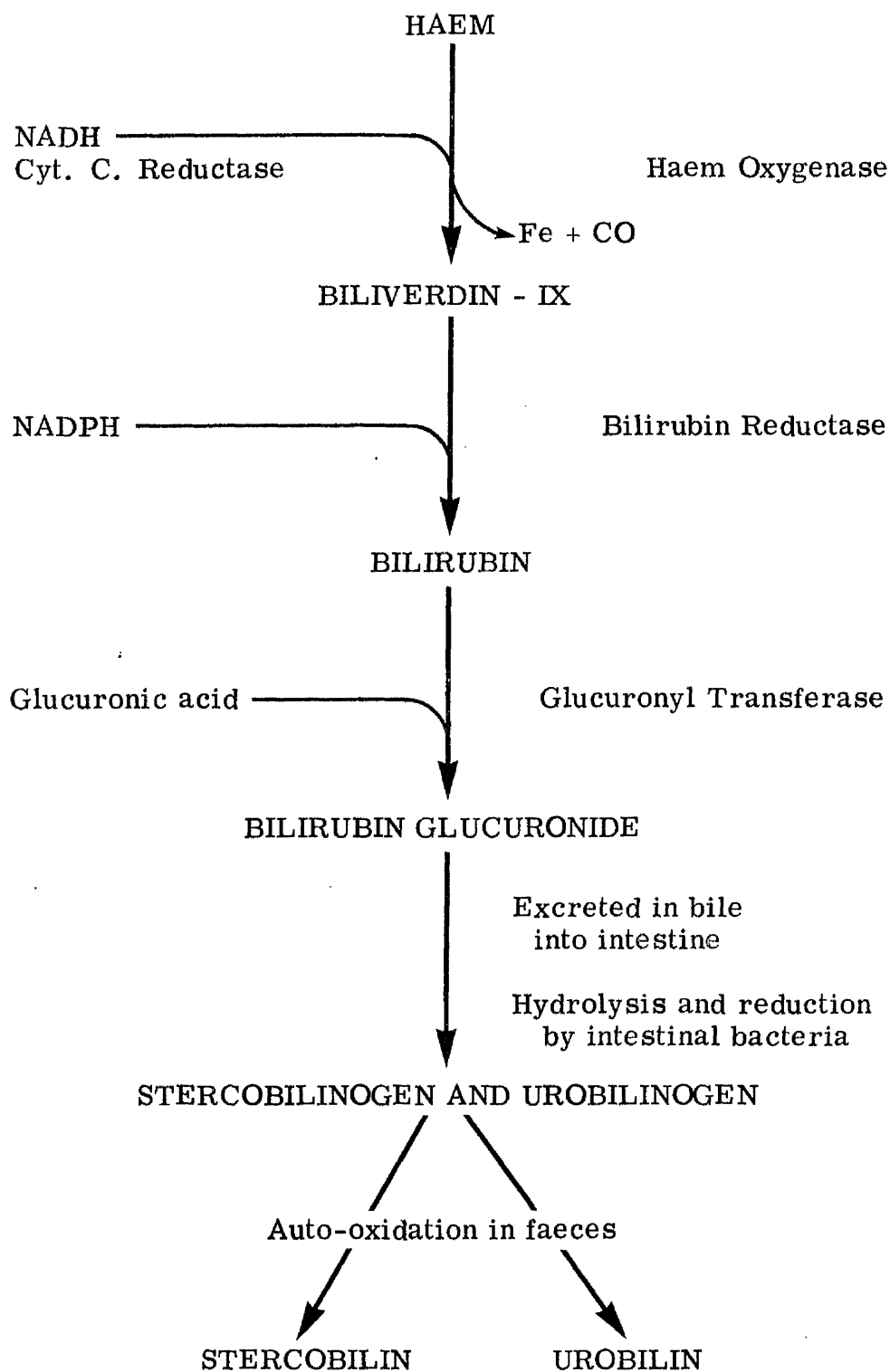


Figure 3 Hepatic Haem Catabolism

CHAPTER 2

THE PORPHYRIAS

2. THE PORPHYRIAS

2.1. INTRODUCTION

The porphyrias are a group of diseases resulting from hereditary abnormalities in the pathway of haem biosynthesis, each being characterized by a genetic partial deficiency of a different intermediate enzyme of the pathway. The name porphyria is derived from the Greek word "porphoros" which means purple and was probably adopted on account of the dark-coloured porphyrin-laden urine which patients pass during an acute attack of the condition. The association with the colour purple may have a further connotation as there is some evidence that several members of royal lineage suffered from the disease (McAlpine, 1968). It is thought to have explained the attacks of madness which plagued George III and some also suggest that the disease was handed down through Mary Queen of Scots and James VI (Figure 4). The evidence, for porphyria being a "Royal Malady" is not, however, totally convincing.

2.2. CLASSIFICATION OF THE PORPHYRIAS

2.2.1. Clinical Classification

The classification of the porphyrias has always been problematic and depends whether one is regarding them from a clinical or biochemical point of view. From the clinician's viewpoint, there are the acute hepatic porphyrias - acute intermittent porphyria, hereditary coproporphyria and variegate porphyria and the non-acute porphyrias - cutaneous hepatic



Figure 4

James VI of Scotland.

porphyria, erythropoietic protoporphyria and congenital porphyria (Figure 5). The acute hepatic porphyrias present with intermittent attacks of systemic illness characterized by abdominal pain and often with accompanying neuropathy and a neuropsychiatric syndrome. Patients with variegate porphyria or hereditary coproporphyria may, in addition, develop solar photo-sensitivity skin lesions. The non-acute porphyrias present solely with cutaneous manifestations. In the acute porphyrias there is increased excretion of the porphyrin precursors, ALA and PBG, and porphyrins, whereas, in the non-acute porphyrias, there is only increased excretion of porphyrins. The photosensitive skin lesions are seen where there is accumulation of formed porphyrins, while acute attacks are seen in association with accumulation of porphyrin precursors.

2.2.2. Biochemical Classification

From the biochemist's viewpoint, the different forms of porphyria are characterized by partial deficiencies of different enzymes in the pathway of haem biosynthesis (Brodie et al, 1977). As a result of the enzyme deficiency there is reduction in haem synthesis and therefore by negative feedback control increased activity of the rate-controlling enzyme of the pathway, ALA synthase. The combination of increased activity of the initial enzyme, ALA synthase, and partial enzymatic block later in the pathway, results in an accumulation of porphyrins and porphyrin precursors formed proximal to the block and these may appear in blood, urine and faeces. Each of the different types of porphyria, therefore, has a characteristic pattern of overproduction of porphyrins and precursors

THE ACUTE HEPATIC PORPHYRIAS

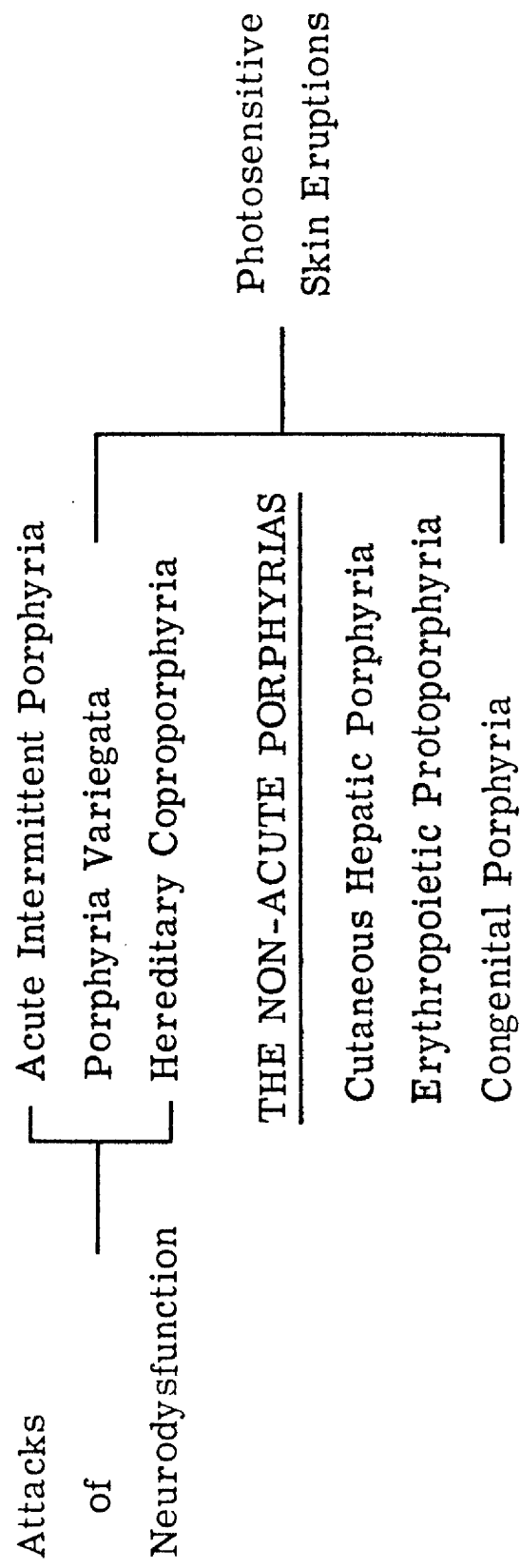


Figure 5

Clinical Classification of the Porphyrias.

determined by the site of the enzymatic block. The enzyme deficiency in acute intermittent porphyria is uroporphyrinogen-1-synthase, in hereditary coproporphyria, coproporphyrinogen oxidase, in porphyria variegata, protoporphyrinogen oxidase, in cutaneous hepatic porphyria, uroporphyrinogen decarboxylase, in erythropoietic protoporphyria, ferrochelatase and in congenital porphyria, uroporphyrinogen cosynthetase. It has been reported that a further form of acute porphyria may exist which, in common with lead poisoning, presents with depressed activity of ALA dehydratase (Doss et al, 1980). The activity of the enzyme uroporphyrinogen-1-synthase is particularly important in determining the biochemical and clinical presentation of the porphyrias. In acute intermittent porphyria the activity of this enzyme is reduced, but in the other two acute porphyrias, it is normal. In each of the non-acute porphyrias it is increased. This enzyme is at the point in the pathway where porphyrin precursors are converted into porphyrins. It is probably the increased activity of uroporphyrinogen-1-synthase which prevents the accumulation of porphyrin precursors in the non-acute porphyrias (Brodie et al, 1977). Further details of the biochemical disorder will be given under the discussion of the individual types of porphyria.

2.3. ACUTE INTERMITTENT PORPHYRIA

2.3.1. Introduction

This is the commonest and most severe form of acute porphyria. It is inherited in an autosomal dominant fashion. Estimates of the prevalence of symptomatic acute intermittent porphyria vary considerably from country to country. The highest rate is probably in Lapland, estimated as 1/1000. In Western Australia, on the other hand, the prevalence (including latent cases) is only 3/100,000 (Tschudy, 1974). Though the genetic trait is equally distributed between sexes the clinical disease is more commonly seen in females. This is probably due to the hormonal fluctuations in females increasing the incidence of clinical attacks.

Acute intermittent porphyria presents with attacks of neurological dysfunction, the patient usually enjoying good health between attacks. It is the one form of porphyria not associated with photosensitive skin lesions. The frequency and severity of attacks varies considerably from patient to patient. In a proportion the disease remains latent throughout life, even in the presence of precipitating factors. Other patients experience frequent and sometimes life-endangering attacks even in the absence of extrinsic precipitating factors. In spite of recent therapeutic advances, an acute attack of porphyria with severe neurological involvement may have a fatal outcome.

2.3.2. Underlying Biochemical Disorder

The basic defect in acute intermittent porphyria appears to be partial deficiency of the enzyme uroporphyrinogen-I-synthase. This has been demonstrated in erythrocytes (Meyer et al, 1972), skin fibroblast cultures (Bonkowsky et al, 1975) and amniotic cells (Sassa et al, 1975). As a result, there is excess formation and urinary excretion of the porphyrin precursors ALA and PBG (Tschudy, 1965). There is also increased urinary, and to a lesser extent faecal, excretion of uroporphyrin. The excess uroporphyrin is probably not due to hepatic overproduction but merely the result of spontaneous polymerization of the excess PBG. An attack is always associated with increased urinary excretion of ALA and PBG though there is no close correlation between the magnitude of this and the severity of the attack. Most patients excrete excess porphyrin precursors during asymptomatic periods between attacks, but some have completely normal excretion profiles between attacks.

Patients with acute intermittent porphyria are also known to produce excess steroids of the 5 Beta-H configuration (Goldberg et al, 1969). This is due to deficiency of hepatic 5 alpha reductase (Kappas et al, 1971). Some of the 5 Beta-H steroids which are found in excess can induce ALA synthase in rat hepatic tissue and they may play a role in the precipitation or exacerbation of the acute attacks (Moore et al, 1973).

2.3.3. Features of the Acute Attack

The acute attacks of neurological dysfunction seen in acute intermittent porphyria are similar to those seen in hereditary coproporphyria and porphyria variegata. Attacks are rarely seen before puberty. The highest incidence of onset of symptoms is between puberty and 30 years of age and attacks are most common in the decade from 20-30 years (Waldenstrom et al, 1963). It should be emphasized, however, that serious attacks may occur for the first time at any age. In one third of reported cases there is no family history either prospectively or retrospectively, the condition presumably having remained latent for several generations (Beattie & Goldberg, 1973).

Abdominal pain is the most frequent complaint, occurring in 95% of cases. The pain is often very severe and patients frequently require parenteral narcotic analgesics. It may be either colicky or constant in nature. The pain may be localized to one region of the abdomen but is more usually felt more diffusely over the abdomen. Patients are almost always constipated during an acute attack. About 10%, however, experience diarrhoea. Anorexia usually occurs and there is often associated nausea and vomiting. On abdominal examination there is usually mild generalised tenderness which often appears inappropriately mild for the degree of pain experienced by the patient. Muscle guarding is rarely seen and bowel sounds are normal. Patients presenting with their first attack may be misdiagnosed as having appendicitis, biliary or renal colic or small intestinal obstruction, and if they are subjected to an unnecessary laparotomy their condition may be

considerably aggravated.

Peripheral Neuropathy may be the presenting feature of an acute attack and it complicates more than 50% of porphyric attacks. In most cases the neuropathy begins with motor symptoms consisting of muscle weakness often preceded by cramp-like pain and stiffness. The peripheral flexor muscles are often the first to be affected resulting in foot-drop and wrist-drop. The motor neuropathy may extend, sometimes rapidly, to involve other muscles. The tendon jerks become diminished or absent and, later, muscle wasting becomes evident. Patients may also complain of parasthaesia, and sensory diminution may be found on testing. Varying combinations and degrees of motor and sensory involvement may be seen. Difficulty controlling micturition may be experienced. In severe cases with extensive neuropathy the muscles of ventilation become involved necessitating assisted ventilation (Figure 6). It is often heralded by weakening of the voice. Cranial nerve palsies are occasionally seen. Grand mal convulsions may also occur and are commonest at the height of an attack.

Psychiatric manifestations are common. Waldenstrom (1957) found pronounced mental symptoms in 55% of 233 patients with acute intermittent porphyria. Patients may be misdiagnosed as suffering from a purely psychiatric condition and admitted to psychiatric wards. In one study by Kaebling et al (1961), 2,500 consecutive patients admitted to a mental hospital



Figure 6
Nineteen year old girl completely paralysed with attack of acute intermittent porphyria precipitated by the contraceptive pill. Note the dark coloured porphyrin-laden urine.

were screened for acute porphyria. Of the 35 positive tests obtained, the admitting diagnoses included schizophrenia, psychiatric depression, neuroses, personality disorders, alcoholism and acute and chronic brain syndromes. The psychiatric symptoms occasionally persist between attacks.

Tachycardia and Hypertension are features of the vast majority of attacks.

Sinus tachycardia of up to 160/min may be seen. The hypertension may be severe and result in encephalopathy and cardiac failure. The hypertension tends to be labile. A substantial proportion of patients will be found to have postural hypotension, if the blood pressure is checked with the patient erect as well as supine. A percentage of patients remain hypertensive between attacks (Beattie & Goldberg, 1976).

In some patients a low grade pyrexia is noted during an attack. The urinary output is nearly always low during an attack. The urine itself is dark reddish-brown in colour and this becomes more pronounced if left standing.

2.3.4. Diagnosis of Acute Attack of Porphyria

Any patient presenting with unexplained abdominal pain, neuropathy or psychiatric manifestations should be tested for acute porphyria. All patients in acute attack excrete excess ALA and PBG in the urine. The modified Watson Schwartz test provides a simple and rapid method of demonstrating excess urinary PBG (Watson et al, 1961). 2 ml of fresh

urine is added to a test tube. The addition of a further 2ml of fresh Ehrlich's Aldehyde reagent will result in a red-pink discolouration in the presence of excess PBG or urobilinogen. These can be differentiated by the further addition of 4ml chloroform. If the discolouration is due to excess PBG it will remain only in the upper aqueous layer. In order to confirm the diagnosis and define the type of porphyria, quantitative estimation of porphyrins and precursors should be performed on faeces and 24 hour urine collection.

2.3.5. Other Laboratory Findings in Acute Attack

Haematology - The haemoglobin concentration usually remains normal during the acute attack. This is in some ways surprising in a disease due to a partial block in the pathway of haem biosynthesis. The white cell count and erythrocyte sedimentation rate are also normal or occasionally slightly elevated.

Biochemistry - Electrolyte abnormalities are common in the acute attack (Eales & Dowdle, 1969). Vomiting and inadequate fluid intake may result in hypokalaemia and varying degrees of uraemia. The creatinine clearance is reduced in a proportion of patients in attack (Eales et al, 1971). Severe dilutional hyponatraemia may occur as a result of inappropriate secretion of anti-diuretic hormone possibly due to hypothalamic dysfunction.

The plasma albumin concentration is frequently low in severe or prolonged attacks, but there is little information available on the mechanisms responsible. A proportion of patients in acute attacks have increased serum concentrations of total thyroxine and tri-iodothyronine (Ludwig & Goldberg, 1963). This may be at least partly explained by increased thyroid binding globulin (Hollander et al, 1967). An elevation of serum aspartate and alanine transaminases and creatinine kinase may occur during the acute attack and is probably the result of muscle necrosis. Glucose metabolism is deranged during the acute attack, the glucose tolerance frequently being diabetic in type (Waxman et al, 1967). There is delayed but excessive insulin secretion after a glucose load and this may result in hypoglycaemia several hours after glucose ingestion. Hypercholesterolaemia occurs in about 50% of patients during the acute attack (Taddeini et al, 1964). Examination of C.S.F. is usually normal but slight elevation of the protein is sometimes seen (Stein & Tschudy, 1970).

Electrophysiology - The electroencephalogram is abnormal in the majority of patients experiencing an acute attack and in a proportion this persists during remission (Albers et al, 1978). This usually takes the form of a generalised slowing of the record and in addition focal abnormalities may be evident. Peripheral nerve conduction studies in the presence of clinically established neuropathy show changes consistent with axonal injury predominantly of motor nerves (Cavanagh & Mellick, 1965). Wochnik-Dyjas et al (1978) found nerve conduction abnormalities which were initially reversible and only

sometimes proceeded to axonal degeneration (Wochnik-Dyjas et al, 1978).

2.3.6. Factors which may Precipitate Acute Attack

Precipitating factors can be identified in the majority of patients presenting in acute attack (Figure 7). Drugs are the most common culprits, barbiturates and the oral contraceptive pill heading the league. Alcohol is another common precipitating agent. Hormonal factors are important. Acute attacks rarely occur before puberty and are particularly common during pregnancy and the puerperium. Many women with acute porphyria are troubled with recurrent attacks in the week prior to the onset of menstruation. The hormonal factors undoubtedly explain why attacks are seen more commonly in females, particularly between the ages of 20 and 30 years. Attacks may also be precipitated by dieting or fasting and acute infections. All of these precipitating factors are capable of inducing hepatic ALA synthase and the mechanisms involved will be discussed in detail in Chapters 5-8.

2.3.7. Management of Acute Attack of Porphyria

In patients presenting in an acute attack of porphyria it is important to identify and where possible remove any precipitating factors. Fluid and electrolyte balance should be monitored and corrected appropriately. The maintenance of adequate carbohydrate intake is essential. It is known that calorie restriction can precipitate attacks and also that the maintenance of a good carbohydrate intake reduces the incidence of attacks (Welland et al, 1964; Felscher & Redeker, 1967). Carbohydrates have been shown to block the

Precipitating Factors
for Acute Hepatic Porphyrria

DRUGS

ALCOHOL

HORMONES

FASTING

Figure 7

induction of hepatic ALA synthase and many other enzymes and this has come to be known as the "glucose effect" (Tschudy et al, 1964; Goldberg, 1974). Acute attacks are generally associated with nausea and vomiting and thus reduced caloric intake, which almost certainly aggravates the attack. This cycle must be broken. Adequate caloric intake may be maintained orally using high calorie drinks. Chlorpromazine or promazine may be helpful in alleviating the nausea and vomiting. Improved oral caloric intake may be achieved by slow continuous infusion via a soft thin nasogastric tube. The infusion of 20% laevulose (2 litres per 24 hours) via a central venous line results in biochemical and clinical improvement in most patients (Brodie et al, 1977).

Great care must be taken in order to ensure that the patient is not prescribed any of the contra-indicated drugs. Analgesics are usually required to relieve the abdominal pain. Aspirin, paracetamol, dihydrocodeine, pethidine, morphine or diamorphine may be used according to the severity of the symptoms. The abdominal pain of acute porphyria is sometimes very refractory and some patients require large frequent doses of intramuscular narcotic analgesics. Severe postural hypotension may result from the use of powerful narcotics, especially if combined with any of the phenothiazines. This can be treated by keeping the patient horizontal and if necessary elevating the foot of the bed. A fuller understanding of the mechanism of the abdominal pain might promote more effective treatment.

It has been suggested that it is due to autonomic neuropathy leading to imbalance of the innervation of the gut, with resultant areas of spasm and dilatation (Gibson & Goldberg, 1956). Bowel spasms have been observed in patients with acute porphyria subjected to laparotomy (Mason et al, 1933). The use of ganglion blocking drugs and splanchnicectomy have been tried but with limited success (Wehrmacher, 1952). The hypertension and tachycardia which frequently occur in an acute attack can be controlled by adequate doses of propranolol (Douer et al, 1978). There is also some evidence that propranolol may have a beneficial effect on the abnormal porphyrin metabolism (Blum & Atsmon, 1976). Severe agitation and other forms of psychiatric disturbance which may occur can be controlled with promazine or chlorpromazine. Diazepam may also be used. If long term anti-convulsant therapy is required, sodium valproate may be safely used. Constipation is usually a problem and neostigmine may be required to alleviate this.

The main innovation in the treatment of acute porphyria over the past decade has been the administration of haem in the form of intravenous haematin. The place of intravenous haematin and intravenous laevulose in the treatment of the acute attack of porphyria is examined in detail in Chapter 9.

Preventive medicine has an important role to play in the management of

the acute porphyrias. Patients with the genetic trait must be identified and advised regarding the avoidance of precipitating factors. The screening of patients for latent acute porphyria is examined in detail in Chapter 4.

2.4. PORPHYRIA VARIEGATA

2.4.1. Introduction

Porphyria variegata is so called because it presents with both acute attacks of neurological dysfunction, as seen in acute intermittent porphyria, and with photosensitive skin eruptions. It is inherited as a Mendelian autosomal dominant trait. The disorder was described and named in South Africa by Dean & Barnes (1955) who traced over a thousand patients with porphyria variegata to a Dutch Cape settler who married an orphan girl in 1688. The prevalence of the disorder varies from country to country and appears to be highest in the white population of South Africa where it is estimated to be 3 per 1,000 (Dean, 1971). Porphyria Variegata may present in an identical fashion to acute intermittent porphyria with acute attacks of neurological dysfunction and no cutaneous manifestations (Kramer, 1980). More usually patients in acute attack will show evidence of photosensitive skin eruptions. Patients may also present solely with the cutaneous manifestations. The prominence of the skin lesions is largely determined by the extent of exposure to sunlight.

2.4.2. Underlying Biochemical Disorder

The disease is most probably the result of deficiency of the enzyme

protoporphyrinogen oxidase. In 1979, Brenner and Bloomer demonstrated a 50 per cent reduction of activity of the enzyme in fibroblasts of patients with variegate porphyria. Patients have markedly increased faecal excretion of protoporphyrin and to a lesser extent coproporphyrin (Barnes, 1958). There is also a considerable increase of the porphyrin peptide complex known as porphyrin X in the faeces (Rimington et al, 1968; Eales et al, 1975). There may be increased urinary excretion of coproporphyrin and to a lesser extent uroporphyrin. During an acute attack there is markedly increased urinary excretion of ALA and PBG and also of uroporphyrin; the latter probably being due to non-specific conversion from PBG. In porphyria variegata there is a close correlation between the urinary excretion of ALA and PBG and the manifestations of acute neurological attacks (Kramer et al, 1973).

2.4.3. Cutaneous Manifestations

The skin lesions of porphyria variegata occur on the sun-exposed areas (back of the hands, lower arms, face and neck) as illustrated in Figure 8. The most important feature is increased skin fragility; minimal trauma being sufficient to cause lesions even to unblemished areas of skin. Lesions usually start as erythema progressing to vesicles that become confluent to form bullae. Haemorrhage may occur into the bullae which heal leaving scars. There may be local pitting oedema at the site of lesions. Hyperpigmentation



Figure 8

This 48 year old woman developed a bullous eruption following sunbathing and was found to have porphyria variegata.

may occur and hirsutism may be troublesome in women. Light microscopy examination of the skin shows the presence of an amorphous material around the smaller blood vessels and capillaries which stains strongly with PAS (Findlay et al, 1966; Van der Sar & Den Ouden, 1976). The bullae are commonly subdermal rather than intradermal in type (Magnus, 1968).

The skin lesions in the porphyrias appear to be the result of interaction between porphyrin deposited in the skin and visible light (Magnus, 1980). Monochromator studies demonstrate that light of the same wavelength as that absorbed by the porphyrin molecule (400nm) will cause skin lesions in the porphyric patient. Absorption of a suitable quantum of light is thought to convert the porphyrins into the so-called "triplet state". The porphyrins, in this excited and reactive state, then transfer their energy to oxygen forming a molecule of excited oxygen. The cellular damage is probably the result of the excited oxygen forming oxidized products of biological substrates.

2.4.4. Management

The treatment and prevention of acute neurological attacks of porphyria variegata is the same as for acute intermittent porphyria. There is no specific treatment for the dermatological manifestations. Patients should avoid exposure of skin to sunlight or strong daylight. The wavelength of light which is most damaging to the skin is within the visible spectrum and therefore the ultraviolet barrier creams used to protect against sunburn are

of little use. Creams filtering visible light would of necessity be opaque and therefore unacceptable for cosmetic reasons. There is evidence that Beta-carotene may block the interaction of the light and porphyrin molecule and in this way offer a degree of protection (Magnus, 1980).

2.5. HEREDITARY COPROPORPHYRIA

2.5.1. Introduction

Hereditary coproporphyria is the least common of the acute porphyrias. The disease was named in 1955 by Berger and Goldberg when they reported four cases in a Swiss family. It is inherited as an autosomal dominant and often remains clinically latent (Brodie & Goldberg, 1980). It presents with attacks of neurological dysfunction. These are similar to the attacks seen in acute intermittent porphyria though generally less severe. During an attack patients may develop photosensitive skin eruptions similar to that seen in porphyria variegata. The skin lesions in hereditary coproporphyria are rarely seen outwith an attack (Goldberg et al, 1978).

2.5.2. Underlying Biochemical Disorder

Hereditary coproporphyria is thought to be due to a partial deficiency of the enzyme coproporphyrinogen oxidase. This was demonstrated in circulating leucocytes by Brodie et al in 1977 and later in cultured skin fibroblasts (Elder et al, 1976) and lymphocytes (Grandchamp et al, 1978). Patients with hereditary coproporphyria excrete large amounts of

coproporphyrin of the isomer III type in faeces and, to a lesser extent, in urine (Marver & Schmid, 1972; Goldberg et al, 1967). As in the other forms of acute porphyria, neurological attacks are associated with increased urinary excretion of ALA, PBG and uroporphyrin. As in acute intermittent porphyria, 17 oxosteroids are excreted in excess in this disease (Paxton et al, 1975).

2.5.3. Management

Prevention and treatment of acute neurological attacks is as for acute intermittent porphyria. In addition, patients should be advised to avoid over-exposure to sunlight.

2.6. RELATIONSHIP BETWEEN THE UNDERLYING BIOCHEMICAL DISORDER AND NEUROLOGICAL MANIFESTATIONS IN THE ACUTE HEPATIC PORPHYRIAS

2.6.1. Introduction

The underlying biochemical disorder in the acute hepatic porphyrias is now clearly defined as a partial deficiency of one of the intermediate enzymes of haem biosynthesis. This results in partial haem deficiency and, therefore, derepression of the initial and rate-controlling enzyme, ALA synthase. The main biochemical consequences are a) haem deficiency and b) excess of the haem precursors formed prior to the block. The abnormality of haem biosynthesis in the acute porphyrias is present in most and probably all body tissues (Brodie et al, 1977).

The other generally accepted fact about the acute porphyrias is that all the clinical manifestations of the disease can be explained by neurodysfunction involving the peripheral, central and autonomic nervous systems (Figure 9). There are only a few studies of the morphological changes in nervous tissue in patients with acute porphyria. It is unethical to obtain adequate biopsies of nervous tissue during life and published studies are virtually all from post-mortems of patients dying in acute attack. Studies in such patients have not always shown significant morphological changes and the changes which have been reported do not constitute any pathognomonic lesion. Gibson and Goldberg (1956) noted demyelination and axonal degeneration in peripheral and autonomic nerves and also chromatolysis and vacuolization in the brain and spinal cord. Ten Eyck et al (1961) noted patchy demyelination and axonal degeneration scattered at all levels of the neuraxis. Similar changes were reported by Tschudy et al (1975). All of these neurohistological changes are non-specific and may be seen in a variety of toxic conditions.

The main unanswered question in the acute porphyrias is the relationship between the underlying abnormality in haem biosynthesis and the functional and structural disorder of the nervous system. A number of possibilities have been postulated and are discussed below.

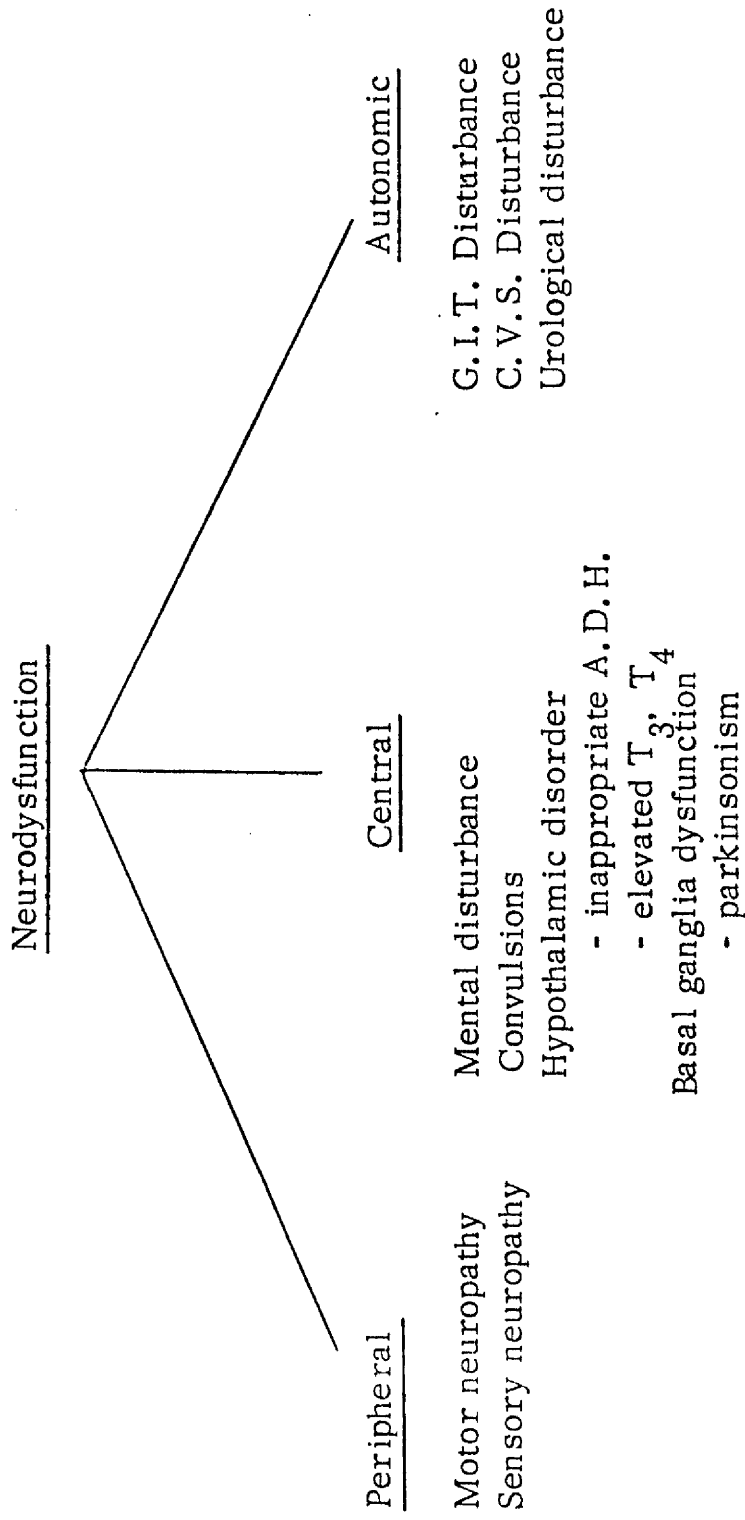


Figure 9

All the manifestations of an attack of acute hepatic porphyria may be explained by dysfunction of the nervous system.

2.6.2. Porphyrin Precursor Neurotoxicity Theory

The simplest explanation is that the excess circulating ALA or PBG or metabolites, which are mainly hepatic in origin, are neurotoxic. This initially appears plausible as excess ALA and PBG are a constant feature of attacks of acute porphyria. Neurodysfunction does not occur in the non-acute porphyrias (cutaneous hepatic porphyria, erythropoietic protoporphyria and congenital porphyria) which are also due to hereditary enzyme deficiencies in the pathway of haem biosynthesis and result in overproduction of porphyrins but not of porphyrin precursors. For the "porphyrin precursor neurotoxicity" hypothesis to be tenable these substances must firstly be able to gain access to the nervous system. The few studies of CSF concentrations in patients in acute attacks of porphyria (Percy & Shanley, 1977; Bonkowsky et al, 1971) have shown that both are present but at concentrations of less than 20 per cent of serum levels. Moore and Meredith (1976) studied, in rats, the tissue concentrations of ^{14}C labelled ALA injected intraperitoneally in a dosage of 330 ug/kg. They found brain concentrations of 5-10 per cent of blood levels. Tenability of the neurotoxicity theory also depends on the ability of these substances to cause neurodysfunction. Goldberg et al (1954) were unable to demonstrate any pharmacological effects of PBG. Marcus et al (1970) administered high doses of ALA (3.3 mmol/kg) to rats and noted no EEG or behavioural changes. Watson et al (1978) reported that the administration of ALA and PBG to nephrectomized rats

resulted in no alteration of blood pressure or nervous behaviour. In allylisopropylacetamide-induced chemical porphyria, in which high blood levels of porphyrin precursors occur, no neurological effects have been observed (Marcus et al, 1970). More recently, however, Cutler et al (1979) reported behavioural changes following the injection of ALA (1.6 mmol/kg) in rats. Several workers have reported a variety of effects of ALA on neuro-muscular function in a variety of in-vitro preparations (McGillion, et al, 1975; Loots et al, 1975; Becker et al, 1975; Cutler et al, 1978, Becker et al, 1971; Becker & Kramer, 1977). No studies in acute porphyria to date, however, have shown CSF levels comparable with those required to produce neurological changes in these in-vitro models (Percy & Shanley, 1977). ALA is structurally similar to the neuro-transmitter gamma aminobutyric acid (GABA) and has been shown to have GABA agonist effects in animals at concentrations similar to those occurring in acute porphyria (Muller & Snyder, 1977). The relevance, however, of these conflicting reports of minor behavioural and neuropharmacological effects of ALA to the severe and widespread functional and structural neuronal changes seen in human acute porphyria must remain in doubt. Another obstacle for acceptance of the neurotoxicity theory is the poor correlation in clinical practice between the urinary excretion of ALA and PBG and the presence and severity of neurodysfunction (Miyagi et al, 1971; Watson et al, 1978). Some patients have very high levels of porphyrin precursors in blood and urine without any clinical disease whereas in others widespread neuropathy may

be seen with only minimally elevated levels. Even within individual patients there is poor correlation of urinary porphyrin precursor excretion and clinical disease activity. Variations in blood brain barrier permeability may be important.

2.6.3. Disordered Haem Synthesis within nervous tissue

The other main theory explaining the neurodysfunction of acute porphyria is that it is the result of disordered haem biosynthesis within nerve cells. This could result in deficiency of essential haemoproteins such as mitochondrial cytochromes required for oxidative phosphorylation or microsomal cytochromes that catalyse mixed function oxidation (Meyer & Schmid, 1974; Maxwell & Meyer, 1974). If the neuropathy of acute porphyria is due to impaired intra-neuronal haem biosynthesis then the precipitating factors of acute attacks must be capable of modifying neuronal haem biosynthesis. Little is known of the regulation of neuronal haem biosynthesis. Paterniti et al, (1978) were unable to induce rat brain ALA synthase with fasting or intraperitoneal injections of alcohol, allylisopropylacetamide or 3, 5 dicarbethoxy-1, 4 dihydrocollidine, Percy & Shanley (1979) were unable to detect any alteration in rat brain ALA synthase activity, total haem or cytochrome P450 levels following intraperitoneal injections of allylisopropylacetamide and/or phenobarbitone. The rate of haem degradation in rat brain is also

unaffected by phenobarbitone, lead and allylisopropylacetamide (Percy & Shanley, 1980). The inability to demonstrate alterations in haem metabolism in nervous tissue by porphyrinogenic agents casts doubt on the above theory.

2.6.4. Other Postulated Mechanisms for Porphyrinic Neuropathy

The abnormality of haem biosynthesis in the liver could affect the nervous system by mechanisms other than the overproduction of haem precursors. Peplinkhuizen et al (1980) propose that the abnormal hepatic haem biosynthesis results in abnormal methylation or cyclisation of monoamines which could have a psychotogenic effect. The mechanism postulated is that the increased demand for glycine will be met by demethylation of serine and that the released methylene group will result in methylation of monoamines. Another possibility is that abnormal hepatic haem biosynthesis may result in deficiency of important haemoproteins required for normal hepatic metabolism. Anderson et al (1976) showed that patients with acute porphyria have impaired hepatic metabolism of administered antipyrine and that this was most marked in those with the most severe clinical history. Deficiency of certain hepatic haemoproteins could result in impaired clearance of endogenously produced neurotoxins or impaired synthesis of factors essential for the integrity of the nervous system.

The biochemical explanation for the neurological dysfunction in acute porphyria remains to be discovered. Its pursuit is important as it is likely to be of

relevance to a wide range of neurological disorders in addition to the porphyrias. Clinical studies of intravenous haematin therapy may be helpful in this respect and this is discussed in detail in Chapter 9.

2.7. THE NON-ACUTE PORPHYRIAS

2.7.1. Introduction

The non-acute porphyrias are so named because they do not cause the attacks of neurological dysfunction characterizing the acute porphyrias. Instead, they present with photosensitive skin eruptions, similar to that described for porphyria variegata and due to increased circulating levels of formed porphyrins. The levels of porphyrin precursors are normal in the non-acute porphyrias, their accumulation being prevented by increased activity of uroporphyrinogen-1-synthase (Brodie et al, 1977).

2.7.2. Cutaneous Hepatic Porphyria (Porphyria Cutanea Tarda)

Cutaneous Hepatic Porphyria is the commonest form of porphyria seen in Europe and North America. It is characterized by accumulation and increased urinary excretion of uroporphyrin which is due to deficiency of hepatic uroporphyrinogen decarboxylase activity (Kushner et al, 1976; Elder et al, 1978). Both hereditary and acquired factors are important in the aetiology of this enzymatic deficiency. A proportion of patients have affected relatives and family studies have shown a reduced activity of uroporphyrinogen decarboxylase in erythrocytes which is inherited in an autosomal dominant fashion (Tiepermann et al, 1978; Verneuil et al, 1978).

In most cases, however, there is no family history and the erythrocyte uroporphyrinogen decarboxylase activity is normal. Acquired factors are important in all cases of cutaneous hepatic porphyria including the familial ones described above. Chronic alcohol abuse resulting in varying degrees of hepatic dysfunction seems to be the commonest precipitating agent (Brunsting, 1954) (Figure 10a and b). The relative contributions of the alcohol, liver damage and the almost constantly associated hepatic siderosis to the biochemical disorder is not known. Oestrogen administration may also precipitate cutaneous hepatic porphyria and there have been reports of cases developing in men receiving oestrogen therapy for prostatic cancer (Roeningk & Gottlab, 1970) and in young women on the contraceptive pill. A number of chemical agents can result in cutaneous hepatic porphyria. There was a famous outbreak in Turkey between 1956 and 1960 when several thousand new cases appeared and this was found to be the result of the bread being contaminated with hexachlorobenzene which had been sprayed onto the wheat as a fungicide (Dean, 1971). Cutaneous hepatic porphyria was also found in a survey of chemical workers in a 2, 4-dichlorophenoxy-acetic acid and 2, 4, 5-trichlorophenoxy acetic acid plant (Poland et al, 1971). Similar effects are likely to be associated with many halogenated hydrocarbons (Elder, 1976).

The management of cutaneous hepatic porphyria involves the identification and withdrawal of precipitating factors and this will result in biochemical

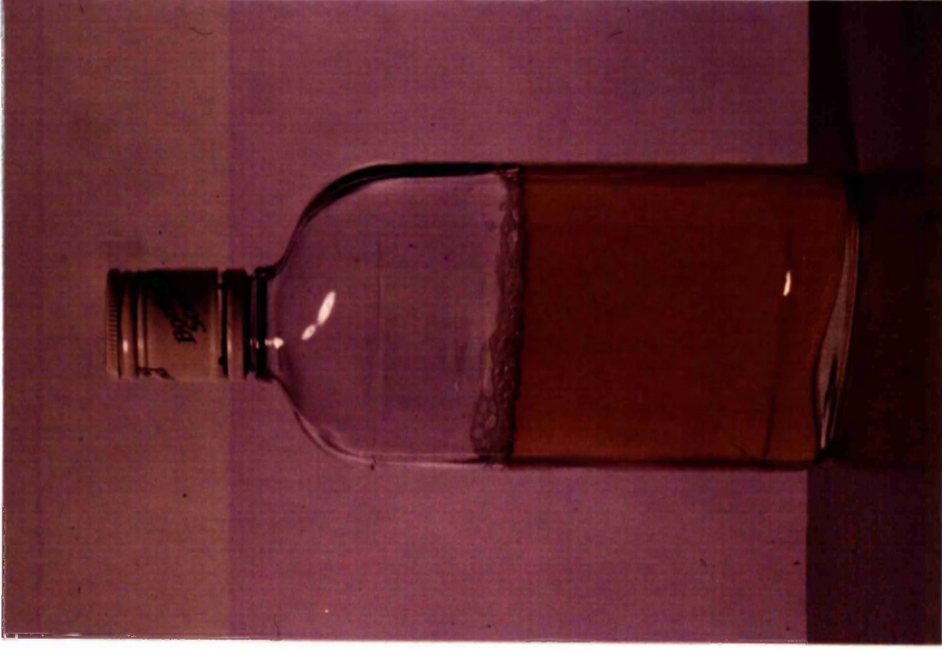


Figure 10a + b

This man presented with skin lesions due to cutaneous hepatic porphyria. No aetiological factors were forthcoming in the history but a clue was provided by the bottle which he used for his urine collection.

and clinical improvement. Most cases are associated with hepatic siderosis and removal of iron by venesection accelerates the improvement (Lundvall & Weinfield, 1968). There are some reports of the use of chloroquine (125mg twice weekly) in the treatment of cutaneous hepatic porphyria (Swanbeck & Wennersten, 1977). This initially results in marked increase of urinary porphyrin excretion followed by biochemical and clinical remission, though the exact mechanism of the chloroquine effect is poorly understood.

2.7.3. Erythropoietic Protoporphyrin

Erythropoietic Protoporphyrin is inherited in a Mendelian autosomal dominant fashion and may present at any age including infancy and childhood (Lynch & Miedler, 1965). It is characterized by overproduction and accumulation of protoporphyrin which is most marked in erythropoietic tissue. This is due to deficiency of the enzyme ferrochelatase which inserts iron into protoporphyrin to form haem. The excess protoporphyrin is excreted solely and in high concentrations in the bile. There may be deposition of protoporphyrin crystals in the biliary system and liver cells and this can result in cholestasis, hepatitis, cirrhosis and liver failure (Bloomer, 1979). Once liver damage is initiated and cholestasis develops the protoporphyrin cannot be excreted and there is consequently a rapid accumulation in the liver. The common history of patients with protoporphyrin who have died in liver failure is that death occurred within a few months of the onset of jaundice. There is also an increased incidence of gall stones

in erythropoietic protoporphyria (Deleo et al, 1976). The oral administration of the chelating agent cholestyramine interrupts the enterohepatic circulation of protoporphyrin and may prevent hepatobiliary complications (Strathers, 1966; Kniffen, 1970). B-carotene may be useful in controlling the cutaneous manifestations (Magnus 1980).

2.7.4. Congenital (Erythropoietic) Porphyria

This is the rarest form of porphyria and is inherited in a Mendelian autosomal recessive fashion (Dean, 1971). It presents usually in early childhood and the photosensitive skin lesions are more severe than in any of the other porphyrias. There is increased urinary and to a lesser extent faecal excretion of uroporphyrin. Erythrocyte and plasma levels of uroporphyrin, coproporphyrin and protoporphyrin are also increased. Decreased activity of the enzyme uroporphyrinogen-III-cosynthase has been demonstrated in erythrocytes (Romeo et al, 1970).

Haematological abnormalities are also a feature of congenital porphyria. Splenomegaly is a consistent finding and there is usually a normochromic, normocytic anaemia with a moderate reticulocytosis. Leucopenia and thrombocytopenia may also occur as part of the picture of hypersplenism. Erythrokinetic studies by Kramer et al (1965) suggested that the anaemia of congenital porphyria is due to ineffective erythropoiesis and shortened red cell survival. Splenectomy improves both the anaemia and the skin manifestations though the long term prognosis remains poor.

CHAPTER 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. PORPHYRINS AND THEIR PRECURSORS

Urinary ALA and PBG were measured by the method of Mauzerall and Granick (1956). Urinary uroporphyrin and coproporphyrin, and erythrocyte and faecal coproporphyrin and protoporphyrin, were measured as described by Rimington (1971). The normal ranges for the above are shown in Table 1.

3.2. ENZYMES OF HAEM BIOSYNTHESIS IN PERIPHERAL BLOOD CELLS

3.2.1. Preparation of Blood

30 ml of venous blood was withdrawn from the subject and placed in 3 x 10 ml lithium heparin containers standing in melting ice. This was delivered to the laboratory within 15 min. 25 ml of the sample was centrifuged at 2,500 g for 30 min at 4°C. During centrifugation the remaining 5 ml of whole blood was used for estimating the packed cell volume and activity of ALA dehydratase. The plasma was removed from the centrifuged blood and the leucocyte layer separated from the erythrocytes using a glass rod. The activities of the mitochondrial enzymes, ALA synthase, coproporphyrinogen oxidase (COPRO oxidase) and ferrochelatase, were measured in leucocytes and the activities of the cytosolic enzymes ALA dehydratase, uroporphyrinogen-1-synthase (URO synthase) and uroporphyrinogen decarboxylase in erythrocytes.

<u>ERYTHROCYTE</u>	}	Protoporphyrin	0 - 657 nmol/l
		Coproporphyrin	0 - 64 nmol/l
<u>FAECES</u>	}	Protoporphyrin	0 - 200 nmol/g dry wt.
		Coproporphyrin	0 - 76 nmol/g dry wt.
<u>URINE</u>	}	Delta-aminolaevulinic acid	0 - 43 umol/l (0 - 40 umol/24h)
		Porphobilinogen	0 - 25 umol/l (0 - 16 umol/24h)
		Uroporphyrin	0 - 22 nmol/l (0 - 49 nmol/24h)
		Coproporphyrin	0 - 227 nmol/l (0 - 432 nmol/24h)

Table 1

Normal ranges for porphyrins and precursors in erythrocytes, urine and faeces of man.

3.2.2. Leucocyte Enzyme Assays

The leucocytes were washed four times in ice-cold isotonic saline solution. The washed cells were lysed by freeze-thawing in liquid nitrogen and sonication, and then split into aliquots for assay of ALA synthase, COPRO oxidase, ferrochelatase and protein. Protein was measured by the method of Lowry et al (1951).

Leucocyte ALA synthase (EC 2.3.1.37)

This was measured by a modification of the method of Freshney & Paul (1970). In this 200 μ l of the white cell homogenate were incubated with 200 μ l of reaction mixture containing 2 (14 C) glycine as substrate (250 μ Ci), and a total glycine concentration of 375 mmol/l. This was incubated for 1 h at 37 $^{\circ}$ C in a shaking water bath. The reaction was stopped by addition of 100 μ l of a solution of 51 μ M delta-aminolaevulinic acid in 1.5 M trichloro-acetic acid. The precipitate was removed by centrifugation and 20 μ l of the clear supernatant solution spotted on to strips of Whatman 3 MM paper for electrophoresis. Delta-aminolaevulinic acid and glycine were separated by running the electrophoretograms for 4 h at 2000V at 4 $^{\circ}$ C in potassium phthalate buffer (0.05 M at pH 4). After electrophoresis, the spots were identified with a ninhydrin solution, cut out, and oxidized in a sample oxidizer (Intertechnique) prior to beta counting in a scintillation counter (Packard tricarb). The results were expressed as nmol ALA produced per g protein/h.

Leucocyte COPRO oxidase (EC 1. 3. 3. 3)

This was assayed according to a modification of the method of Battle et al (1965). Coproporphyrinogen was prepared from coproporphyrin by reduction with 3 per cent (w/w) sodium amalgam under nitrogen in the dark. The coproporphyrinogen was then separated by filtration through a sintered tube into a Buchner flask containing 0.13 ml sodium thioglycollate (2 M) and subsequently brought to pH 7.4 with 40 per cent (w/v) phosphoric acid. 200ul of this solution of coproporphyrinogen were taken with 2.5 ml of tris buffer (0.01 M) pH 7.4 and 400 ul of the leucocyte preparation into a 25 ml Erlenmeyer flask which was closed with a cotton wool stopper. This was incubated with shaking at 37^oC in the dark for one hour. The reaction was stopped with 10 ml 4 : 1 ethyl acetate : acetic acid solution mixed and left in full daylight for 30 min to convert the residue of coproporphyrinogen to coproporphyrin. Protoporphyrin produced by this reaction was extracted and quantitated by the method of Rimington (1971). Results were expressed as nmol protoporphyrin produced per g protein/h.

Leucocyte Ferrochelatase (EC 4. 99. 1. 1)

This was assayed by a modification of the method of Porra (1975). In this 400 ul of the leucocyte preparation was added to 1.5 ml tris buffer (pH 8.2, 0.45 M) containing 1.5 mg reduced glutathione. This was incubated with 0.5 ml

free mesoporphyrin (in 0.13 M sodium bicarbonate) for 10 min at 37°C. 1 ml of iron solution (16 µM Fe Cl₂ in tris buffer pH 8.2) was then added and the incubation continued for 1 h at 37°C in the dark. The reaction was stopped using alkalinized iodoacetamide and pyridine. The solution was then divided into two cells and a small amount of sodium dithionite added to the test cell. The test cell was scanned against the control cell spectrophotometrically from 600-500 nm. The results were expressed as pmol haem per g protein/h.

3.2.3. Erythrocyte Enzyme Assays

Erythrocyte ALA dehydratase (EC 4. 2. 1. 24)

This was assayed using 0.2 ml whole blood by the EEC standard method (Berlin & Schaller, 1974). The results were expressed as µmol ALA formed per litre RBC/min.

Erythrocyte URO synthase (EC 4. 3. 1. 8)

This was assayed by the method of Frydman & Feinstein (1974). In this assay washed red cells were sonicated in an equal volume of phosphate buffer (83 mmol/l) pH 7.65. Prior to sonication an aliquot was taken for packed cell volume determination. 0.2 ml of this sonicated material was taken and incubated with 0.1 ml of porphobilinogen solution (0.82 mmol/l) and 1 ml of a solution of reduced glutathione (3.25 mmol/l) in phosphate buffer (83 mmol/l) pH 7.65. This was incubated in stoppered tubes in the dark for one hour at

37°C and the reaction stopped by the addition of 9 ml of a 2:1 solution of ethyl acetate:acetic acid. This was centrifuged to remove the precipitate and the precipitate re-extracted with 5 ml aliquots of 2:1 ethyl acetate:acetic acid until no more porphyrin fluorescence was observed. The total volume of extract was noted and 0.4 ml mixed with 5 ml 0.5 M HCl. The fluorescence in this sample was measured on a spectrofluorimeter to measure the formation of uroporphyrin. Results were expressed as nmol uroporphyrin formed/litre RBC/h.

Erythrocyte uroporphyrinogen decarboxylase (EC 4. 1. 1. 37)

This method, based on that of Frydman & Feinstein (1974), utilizes two stages. The first, to produce uroporphyrinogen the substrate for the reaction, is based on the previous assay of URO synthase.

For the first stage 0.2 ml of washed RBC haemolysate in phosphate buffer pH 7.65 was incubated as described for URO synthase for one hour at 37°C. At the end of this hour 0.19 ml of a solution of potassium dihydrogen orthophosphate (0.4 M) was added to the reaction mixture and the incubation continued for another hour at 37°C. The reaction was stopped with 9 ml 2:1 ethyl acetate:acetic acid, and extractions continued with this until no further porphyrin fluorescence could be extracted from the precipitate. The combined extract was then back-extracted with a saturated solution of sodium acetate until all uroporphyrin had been extracted into the aqueous phase. The ethyl acetate/acetic acid phase, containing the synthesized

coproporphyrin was then treated as for blood-porphyrin estimations (Rimington, 1971) and the results of the assay expressed as nmol coproporphyrin produced/litre RBC/h.

3. 2. 4. Normal Range for enzymes and effect of age, sex and time of day

The activities of the enzymes of haem biosynthesis in peripheral blood cells were studied in 62 healthy volunteers. Twenty eight were female and 34 male and the mean age was 34 years (range 9-82 years). All had normal haemoglobin, erythrocyte protoporphyrin and blood lead concentrations. None was taking any form of medication and all refrained from alcohol for 48 h prior to testing. None of the females studied were pregnant or taking the contraceptive pill. The activities of each of the 6 enzymes studied in these healthy subjects showed a normal distribution and the mean values, standard deviations and ranges are shown in Table 2.

Neither age or sex had any significant effect on the activities of the enzymes as shown in Tables 3 and 4. In 8 subjects the enzymes were studied at 9a.m. and at 10p.m. on the same day. Erythrocyte ALA dehydratase was significantly increased in the evening (Figure 11) but the other enzymes showed no evidence of diurnal rhythm (Table 5).

3. 3. HEPATIC ALA SYNTHASE AND CYT P450 IN EXPERIMENTAL ANIMALS

The animals (rats and rabbits) were killed by cervical dislocation. The abdominal cavity was opened and the liver perfused with 10 ml isotonic saline (0.15 M) at 30°C. The liver was then excised, chilled on ice and

	Leucocyte ALA. S. (nmol ALA/ g prot. /h)	Erythrocyte ALA. D. (μ mol ALA/ l RBC/min)	Erythrocyte URO. S. (nmol URO/ l RBC/h)	Erythrocyte URO. D. (nmol COPRO /l RBC/h)	Leucocyte COPRO. O. (nmol proto/ g prot. /h)	Leucocyte FERR. (pmol mesohaem /g prot. /h)
MEAN	173	25	33	13	114	4.0
S.D.	\pm 74	\pm 9.6	\pm 7.7	\pm 5.2	\pm 70	\pm 2.3
RANGE	40-372	9-48	16-51	4-28	20-398	0.6-11

Table 2

Activities of Enzymes of Haem Biosynthesis in peripheral blood cells of 62 normal subjects.

Age (years)	L. ALA. S.	E. ALA. D.	E. URO. S.	E. URO. D.	L. COPRO. O.	L. FERR.
9 - 20 (6 studied)	Mean S. D. Range 178 ±87 40-281	19 ±4.9 11-24	30 ±4.4 26-38	14 ±4.8 9-22	93 ±42 38-164	3.9 ±2.5 1.6-8.5
21-30 (26 studied)	Mean S. D. Range 175 ±91 45-372	24 ±9.3 12-48	31 ±6.2 22-51	13 ±5.6 5-28	102 ±50 20-250	3.6 ±2.0 1.1-9.7
31-40 (15 studied)	Mean S. D. Range 163 ±56 87-305	29 ±9.8 12-45	31 ±8.7 16-46	12 ±4.2 4-18	130 ±89 39-398	3.9 ±2.6 1.8-11
41-55 (8 studied)	Mean S. D. Range 161 ±56 79-256	22 ±12 9-45	33 ±5.8 25-37	13 ±5.9 5-26	112 ±81 31-300	2.1 ±1.0 0.6-3.2
56-82 (7 studied)	Mean S. D. Range 189 ±41 127-257	28 ±7.7 21-40	43 ±9.7 28-51	14 ±6.6 5-24	84 ±45 42-155	4.5 ±1.2 2.3-5.9

Table 3

Age and the Activities of Enzymes of Haem Biosynthesis in peripheral blood cells of normal subjects (for units of enzyme activities, see Table 2).

	L. ALA. S.	E. ALA. D.	E. URO. S.	E. URO. D.	L. COPRO. O.	L. FERR.
Females (28 studied)	Mean 166 S.D. ±78 Range 40-300	27 ±9.6 9-48	32 ±8.0 18-51	13 ±6.3 5-28	130 ±80 39-318	3.4 ±2.2 0.6-11
Males (34 studied)	Mean 179 S.D. ±72 Range 52-372	23 ±9.6 10-40	33 ±7.5 16-49	13 ±4.2 4-26	106 ±69 20-398	4.1 ±2.4 1.2-11

Table 4

Activities of Enzymes of Haem Biosynthesis in peripheral blood cells of normal males and females.
(for units of enzyme activities, see Table 2).

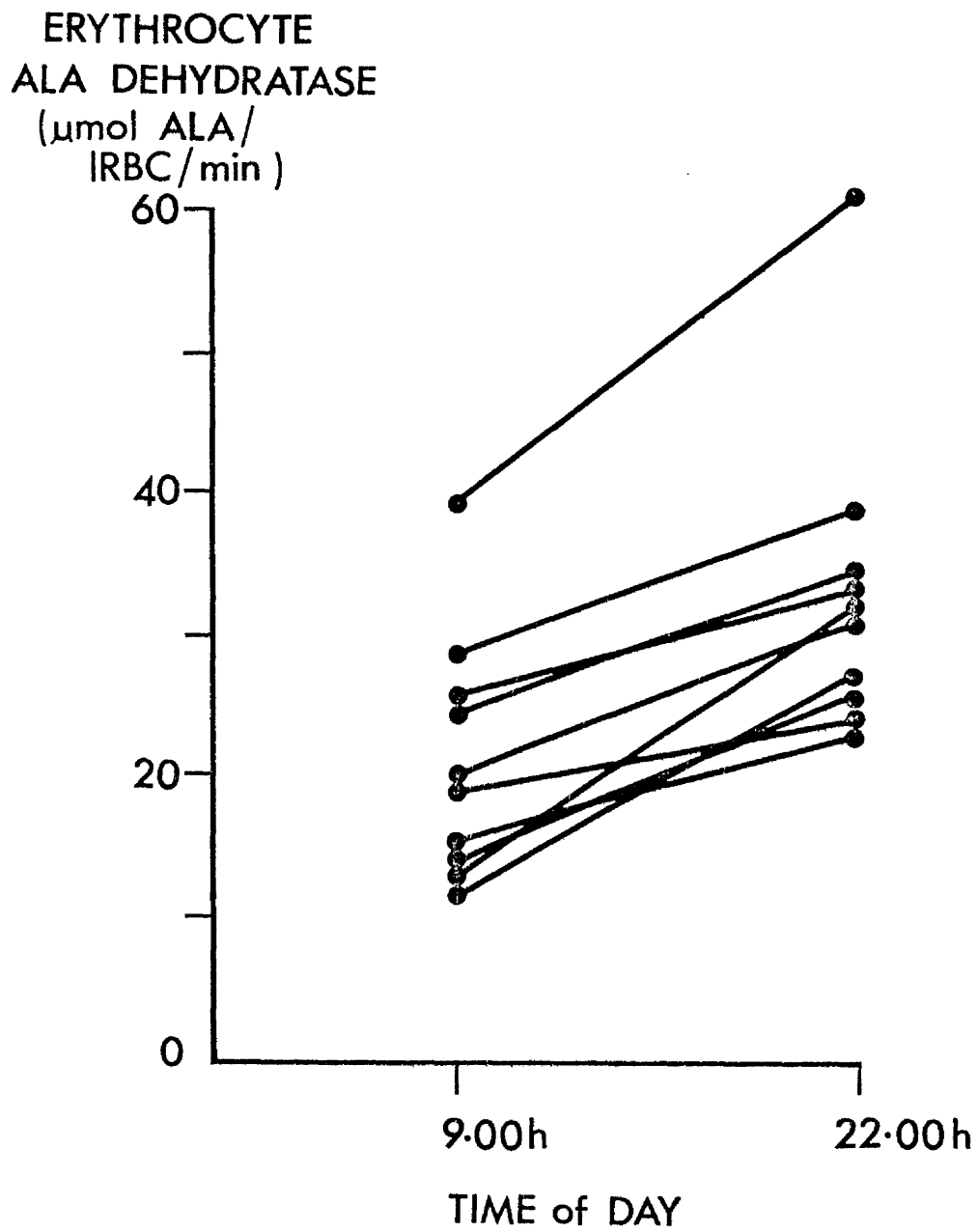


Figure 11

Erythrocyte ALA dehydratase activity was higher in the evening than in the morning in each of the 10 normal subjects studied.

	L. A L A. S	E. A L A. D.	E. U R O. S.	E. U R O. D.	L. C O P R O. O.	L. F E R R.
9.00h	240 (range 147-295)	21 (13-39)	26 (18-36)	14 (9-28)	99 (45-188)	3.4 (1.1-5.0)
22.00h	243 (range 181-300)	33 (24-61)	29 (19-39)	13 (4-26)	95 (40-154)	3.4 (1.5-8.0)
Significance	N. S.	$p < 0.01$	N. S.	N. S.	N. S.	N. S.

Table 5

Mean activities of enzymes of haem biosynthesis in peripheral blood cells of 10 normal subjects, at 9.00h and 22.00h. Statistical comparison performed using Wilcoxon's paired-samples ranking test. (for units of enzyme activities, see Table 2).

blotted dry. Aliquots were taken and weighed for ALA synthase and Cyt P450 determination. For ALA synthase, 2 g hepatic tissue was homogenized in 6 ml Homogenisation Mixture. 200 ul of the homogenate was then used for the enzyme determination as already described for leucocyte ALA synthase. Hepatic Cyt P450 content was determined on microsomal pellets using spectrophotometry, as described by Omura & Sato (1964).

3.4. PREPARATION OF HAEMATIN SOLUTION

The haematin was prepared as personally communicated by Professor C.J. Watson (Minneapolis). Hemin was first prepared by slowly adding 250 ml of centrifuged and washed human red blood cells (Hbs Ag negative) to one litre of glacial acetic acid containing 1 ml of saturated Na Cl at 105°C. The temperature was maintained and the solution stirred for 15 min. It was then allowed to cool slowly at room temperature. When the temperature reached 40°C the solution was filtered (using a 12 cm Buchner funnel and 2 black ribbon filter papers) and the hemin crystals washed successively with approximately 100 ml portions of 50% acetic acid, absolute alcohol and diethyl ether. The hemin was recrystallized by dissolving every 1 g in 5 ml pyridine, adding 8 ml of chloroform and shaking vigorously. This solution was then filtered through a medium sintered glass funnel and the undissolved material washed with a few ml of CHCl₃. What remained on the filter was discarded. The filtrate was

added slowly to 70 ml of glacial acetic acid containing 1 ml of saturated NaCl and 0.8 ml of concentrated HCl at 100-105°C. The temperature was maintained for 10 min and then allowed to cool to 40°C, as before, and filtered on a Buchner funnel (6-8 cm) using two red ribbon filter papers. The crystals were washed as described above and allowed to pull dry.

The haematin solution for intravenous administration was prepared from the recrystallized hemin with the assistance of the pharmacy departments of Stobhill Hospital and the Western Infirmary. This was done under laminar flow sterile cabinets. 300 mg of hemin was dissolved in 5 ml of 1% Na Carbonate. This solution was then filtered through a 60 ml fine sintered glass filter assisted by washing with small amounts of slightly warmed 0.25% Na Carbonate solution. The pH of the filtrate was adjusted to 8 with HCl (1.5N - 0.3N) using a pH meter. It was then filtered through a 0.2 µm Nalgene filter (catalog 120-0020) with gentle suction. The concentration of this final solution was 6-8 mg/ml and was stored in sterile ampoules at 4°C. Samples of the final solution were confirmed to be free of bacterial contamination by culturing for 2 and 7 days and to be pyrogen free by testing in rabbits.

3.5. HORMONE ESTIMATIONS

The hormone estimations were performed in collaboration with Dr. Michael Wallace of the Department of Steroid Biochemistry, Glasgow Royal Infirmary. Androstenedione, dehydroepiandrosterone (DHA), DHA sulphate, oestradiol,

progesterone and testosterone were measured in human serum by radioimmunoassay. Antisera for oestradiol, progesterone and testosterone were raised in sheep as described by Cook et al (1977). Antisera against androstenedione was obtained from Guildhay Antisera, University of Surrey (Ref. No. HP/S/673 - 1A). DHA antisera was purchased from Dr. B. Rudd, Department of Clinical Endocrinology, The Birmingham and Midland Hospital for Women. DHA sulphate was measured by a non-extraction method similar to that described by Smith et al (1975). The other steroids were measured after extraction into ether or in the case of progesterone, N-Hexane. In all radioimmunoassays incubation with labelled steroid and antiserum was for a period of at least 2 h, and antibody bound and free steroid were then separated with Dextran-coated charcoal.

3.6. ASSAYS FOR LEAD, ALCOHOL AND PHENYTOIN

Blood lead concentrations were measured by flameless atomic absorption spectrophotometry (Perkin Elmer 306 and HGA 72). The blood alcohol concentration was measured using gas liquid chromatography as described by Cooper (1971). Serum phenytoin concentration was measured by gas chromatography as described by Thoma and Bondo (1974).

CHAPTER 4

SCREENING

FOR

ACUTE HEPATIC PORPHYRIA

4. SCREENING FOR ACUTE HEPATIC PORPHYRIA

4.1. INTRODUCTION

Each of the acute hepatic porphyrias (AIP, hereditary coproporphyria and variegate porphyria) is inherited in an autosomal dominant fashion. For every case presenting in clinical attack, there is a much larger number who are asymptomatic but at risk of developing acute attacks on exposure to precipitating factors. It is important that these latent cases are identified and given appropriate counselling in order to prevent them developing attacks and to ensure early diagnosis and treatment should one occur.

There has been a number of studies of the use of urinalysis and the measurement of erythrocyte uroporphyrinogen-1-synthase (E. URO synthase) activity for screening relatives of patients with AIP (Astrup, 1978; Grellet et al, 1977; Doss & Tiepermann, 1978 and Lamon et al, 1979). Urinalysis is of limited value as increased excretion of porphyrin precursors is present in only 1 in every 3 latent cases. The measurement of the activity of E. URO synthase, which is depressed in AIP, is more sensitive but there is considerable overlap between normal and porphyric subjects, and 20-40% of relatives have values within the overlap zone. The interpretation of the E. URO synthase activity in association with the family pedigree is helpful in some cases and this combined with urinalysis permits the classification of up to 90% of relatives

(Lamon et al, 1979). There have been no reports of the use of enzyme assays to screen for latent cases of hereditary coproporphyrinemia or variegate porphyria.

We have assessed the value of measuring, in peripheral blood cells, the activities of both the enzyme which is genetically deficient and the rate-controlling enzyme, ALA synthase, when screening relatives of patients with AIP or hereditary coproporphyrinemia.

4.2. ACUTE INTERMITTENT PORPHYRIA

4.2.1. Subjects and Methods

The activities of leucocyte ALA synthase (L. ALA synthase) and E. URO synthase and the 24 h urinary excretion of ALA and PBG were measured in 19 patients with AIP and 35 blood relatives. The normal subjects described in Chapter 3 page 38 were used as controls.

The AIP patients included 7 males and 12 females and their mean age was 30 years (range 8-45 years). All had experienced one or more symptomatic attacks with associated increased urinary excretion of ALA and PBG. Nine of the patients were in clinical attack and ten in clinical remission when studied.

The 35 relatives were all first degree blood relatives of eleven of the above-mentioned cases of AIP. Their mean age was 35 years (range 4-65 years) and fifteen were males and twenty, females. None had a history of symptoms suggestive of acute porphyria.

4.2.2. Results

Patients with AIP

The mean activity of L. ALA synthase in the patients with AIP was 908 nmol ALA/g prot/h (range 273-3,011) which was significantly increased compared with the normal subjects who had a mean of 173 nmol ALA/g prot/h (range 50-372) (Figure 12). The mean activity of E. URO synthase in the AIP patients was 17 nmol URO/l RBC/h (range 8-29) and this was significantly depressed compared with the normal subjects with a mean of 33 nmol URO/l RBC/h (range 16-51) (Figure 13). There was no significant difference between the male and female porphyria patients with respect to either ALA synthase or URO synthase activities. No significant correlation was found between the activities of L. ALA synthase and E. URO synthase in the porphyria patients when examined as one group or as males and females separately.

The urinary excretion of ALA and PBG was increased in all 9 patients studied in clinical attack, but in only five of the 10 patients in clinical remission. The activities of L. ALA synthase and E. URO synthase in the patients in clinical attack were similar to those in the patients in remission (Table 6). Likewise, the activities of both enzymes in the patients with increased urinary ALA and PBG were similar to those in the patients with normal urinalysis (Table 6).

There was considerable overlap between the normal subjects and the AIP patients with respect to both L. ALA synthase and E. URO synthase activities.

Leucocyte ALA.S.

$\left(\frac{\text{nmol ALA}}{\text{g prot/h}} \right)$

● = Increased Urinary
ALA or PBG

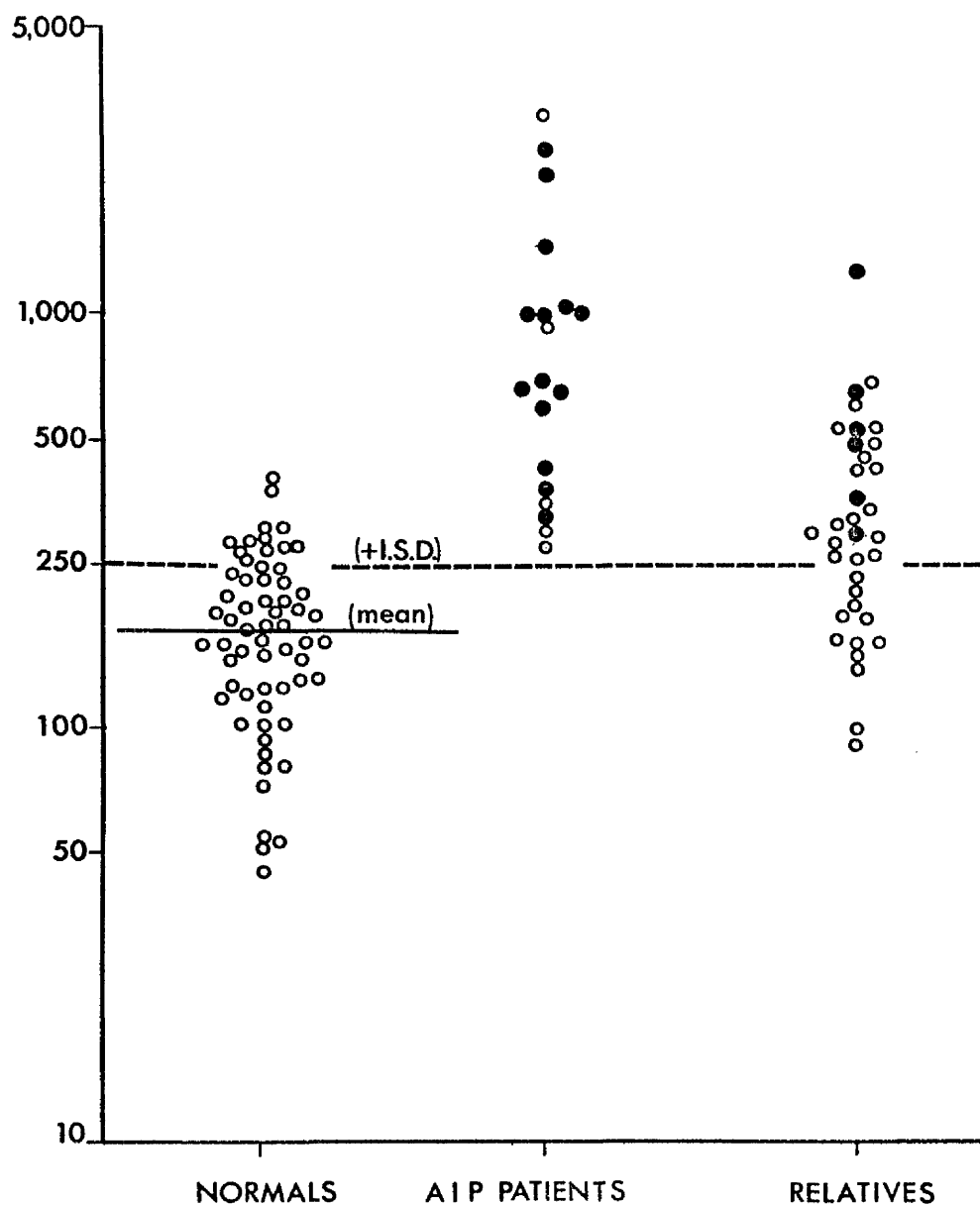


Figure 12

Activity of leucocyte ALA synthase in patients with acute intermittent porphyria and their blood relatives compared with normal subjects.

Erythrocyte URO,S.

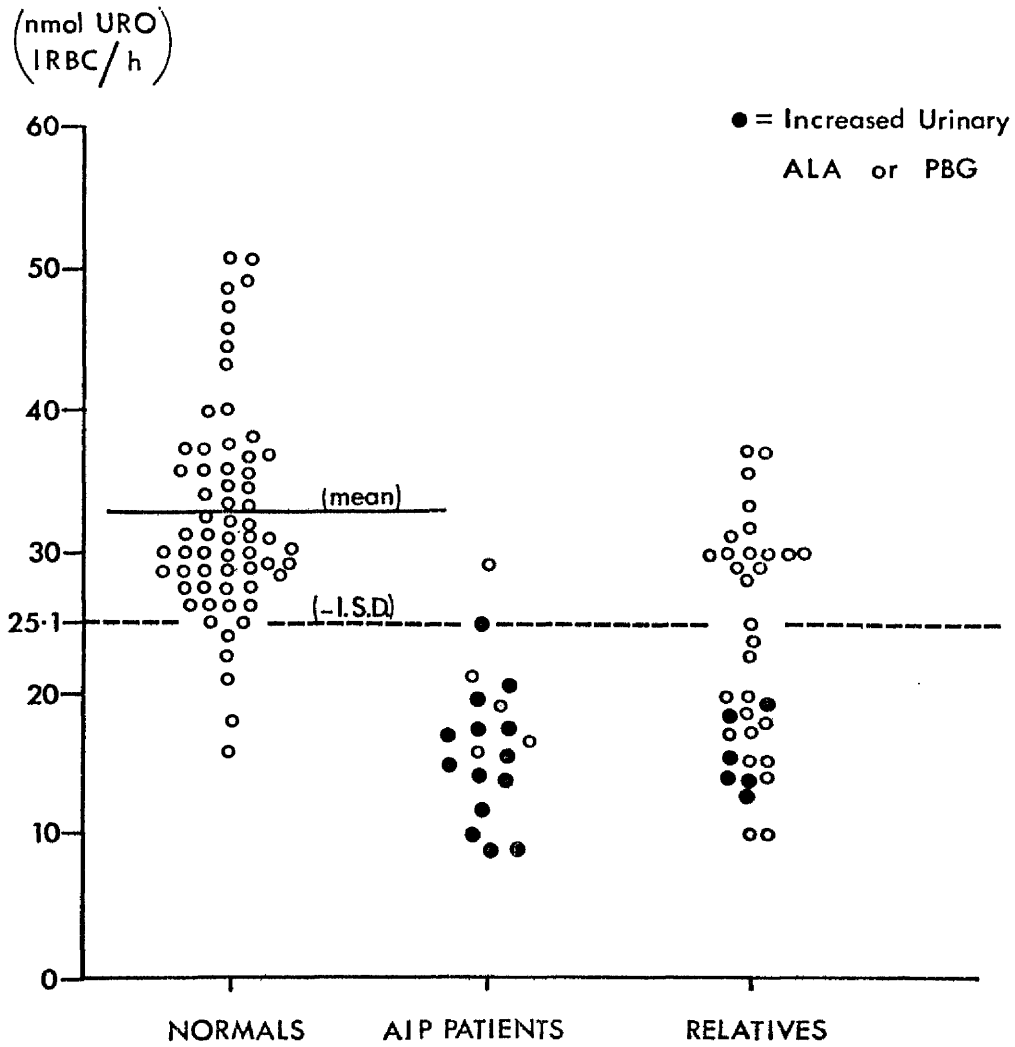


Figure 13

Activity of erythrocyte URO synthase in patients with acute intermittent porphyria and their blood relatives compared with normal subjects.

	<u>URINARY ALA and PBG</u>		<u>CLINICAL STATUS</u>	
	NORMAL (n = 5)	INCREASED (n = 14)	REMISSION (n = 10)	ATTACK (n = 9)
mean activity L. ALA. synthase (nmol ALA/g prot/h)	1,050	917	1,118	945
mean activity E. URO. synthase (nmol URO/1RBC/h)	17	16	18	15

Table 6

A activities of L. ALA. synthase and E. URO. synthase in relation to excretion of porphyrin precursors and clinical status in patients with AIP.

The best separation of normal subjects and patients with AIP with respect to L. ALA synthase was obtained by using the mean value of the normal subjects plus 1 standard deviation, i. e. 250 nmol ALA/g prot/h. All 20 AIP patients had a value of more than this and all but twelve of the 63 (19%) normal subjects had a value less than this (Table 7). Surprisingly, the most useful separation with respect to E. URO synthase was found to be equal to the mean value of the normal subjects minus 1 standard deviation, i. e. 25.1 nmol URO/l RBC/h. All but one of the 20 porphyria patients had a value of less than this and all but seven (11%) of the normal subjects had a value of more than this. Much improved separation of normals and AIP patients was obtained by using both L. ALA synthase and E. URO synthase. None of the normal subjects had both L. ALA synthase activity of more than 250 nmol ALA/g prot/h and E. URO synthase activity of less than 25.1 nmol URO/l RBC/h, whereas all but one of the AIP subjects had (Table 7).

Relatives of AIP Patients

Increased urinary excretion of ALA and/or PBG was present in only six of the 35 (17%) first degree blood relatives of AIP patients.

The activities of L. ALA synthase ranged from 96-1,301 nmol ALA/g prot/h and had a trimodal distribution, one peak corresponding to the mean of the AIP patients, one to the mean of the normal subjects and one to the overlap of the normals and AIP patients (Figure 12). The activities of E. URO synthase ranged from 10-37 nmol URO/l RBC/h with a bimodal distribution, one peak corresponding to the mean for the normal subjects and the other to the

	Normal Subjects (n = 63)	AIP Patients (n = 19)	AIP Relatives (n = 35)
L. ALA.S. >250 (nmol ALA /g prot/h)	19%	100%	66%
E. URO.S. < 25.1 (nmol URO/IRBC/h)	11%	95%	57%
BOTH	0	95%	49%

Table 7

Prevalence of increased L. ALA synthase activity and depressed E. URO synthase activity in normal subjects, patients with AIP and blood relatives of AIP patients.

mean for the AIP patient (Figure 13). Twenty three of the 35 relatives (66%) had L. ALA synthase activities of more than 250 nmol ALA/g prot/h, twenty (57%) had E. URO synthase activities of less than 25.1 nmol URO/1 RBC/h and seventeen (49%) had both increased L. ALA synthase and depressed E. URO synthase (Table 7). Each of the 6 relatives with increased urinary excretion of ALA and/or PBG had both L. ALA synthase activity of more than 250 nmol ALA/g prot/h and E. URO synthase activity of less than 25.1 nmol URO/1 RBC/h.

When relatives were separated into normals and latent AIP, the sex distribution remained very similar to that for the total group (57% females), irrespective of whether the separation was determined by increased L. ALA synthase (59% females), reduced E. URO synthase (55% females), both enzymes (56% females), or urinalysis (50% females).

The 17 relatives with increased activity of L. ALA synthase and depressed E. URO synthase activity were compared biochemically with the 10 AIP patients in clinical remission. Erythrocyte URO synthase activity was similar in the two groups but L. ALA synthase activity was significantly increased in the patients who had experienced a clinical attack and a higher percentage of the latter group had increased urinary excretion of ALA and PBG (Table 8).

4. 2. 3. Discussion

Patients with AIP

In the patients with AIP, no correlation was apparent either between the enzyme

		AIP patients in remission (n = 10)	Latent AIP subjects (n = 17)
L. ALA. S. (nmol ALA /g prot/h)	mean	988	494
	range	273-3011	317-1301
E. URO. S. (nmol URO /RBC/h)	mean	18	16
	range	9-29	10-25
Percentage with increased Urinary ALA and PBG		60%	35%

Table 8

Biochemical comparison of latent cases of AIP with patients in clinical remission. Leucocyte ALA synthase is significantly higher in the latter ($p < 0.05$, student t test).

activities in peripheral blood cells and urinary excretion of porphyrin precursors, or between the enzyme activities and clinical state of relapse or remission. The increased porphyrin precursors excreted in the urine in AIP are mainly hepatic in origin and can be expected to reflect changes in hepatic haem biosynthesis. The absence of correlation between the urinary excretion of porphyrin precursors and the enzymes of haem biosynthesis in peripheral blood cells indicates that the latter do not accurately reflect changes in the activities of their counterparts in the liver.

Relatives of AIP Patients

The genetic trait for AIP is known to be inherited in an autosomal dominant sex-independent manner. All the relatives examined were first degree blood relatives of cases of AIP and therefore approximately 50% can be expected to carry the gene. Only 17% of the relatives had increased urinary excretion of ALA and/or PBG and this is consistent with previous studies and confirms that urinalysis only detects 1 in 3 latent cases.

The measurement of E. URO synthase activity allows the detection of more latent cases but considerable overlap occurs between normal and porphyric subjects. The use of L. ALA synthase alone also suffers from the same disadvantage. The present study demonstrates that the use of both enzymes permits much improved separation of normal and porphyric subjects; none of the former and 95% of the latter having both increased L. ALA synthase and depressed E. URO synthase activities. Using both enzymes, 49% of the

relatives were classified as latent cases of AIP and there was no bias towards either sex. These findings are consistent with an autosomal dominant sex-independent mode of inheritance of AIP.

The present study demonstrates that the vast majority of patients with the genetic trait are clinically latent. None of the 17 relatives found to have the trait had experienced any symptoms. There was no clear-cut biochemical difference between the latent cases and patients in clinical remission though L. ALA synthase activity was significantly higher and the prevalence of increased urinary excretion of porphyrin precursors greater in the latter. The higher ALA synthase activity in those who have experienced a clinical attack may reflect higher circulating levels of endogenous porphyrinogenic agents and it may be worth comparing the steroid profiles of latent and manifest cases. The development of a clinical attack is, however, largely determined by exposure to exogenous precipitating factors and there is no reason for assuming that clinically latent cases are immune from subsequently developing attacks.

4.3. HEREDITARY COPROPORPHYRIA

4.3.1. Subjects and Methods

The activities of L. ALA synthase and leucocyte coproporphyrinogen (L. COPRO) oxidase and the 24 h urinary excretion of ALA, PBG and coproporphyrin and faecal coproporphyrin excretion have been studied in 6 patients with hereditary coproporphyrinuria and 9 blood relatives.

Five of the hereditary coproporphyria patients were females and one male and their mean age was 32 years (range 12-45 years). All had experienced at least one clinical attack. Two were in attack and four in remission when studied.

The 9 relatives studied consisted of 2 daughters of one of the above patients, and 3 sisters, both the parents, paternal aunt and paternal grandmother of another. Their mean age was 27 years (range 5-71 years). None had any history suggestive of cutaneous or systemic manifestations of hereditary coproporphyria.

4.3.2. Results

Patients with Hereditary Coproporphyria

In the patients with hereditary coproporphyria, the activity of L. ALA synthase was increased and the activity of L. COPRO oxidase depressed, compared with normal subjects (Figure 14). In all 6 patients the values for each enzyme lay outwith one standard deviation from the mean for the controls, i. e. L. ALA synthase more than 250 nmol ALA/g prot/h and L. COPRO oxidase less than 45 nmol Proto/g prot/h. All patients had increased urinary excretion, and all but one patient increased faecal excretion, of coproporphyrin. Increased urinary excretion of ALA and PBG was present in the two patients in attack.

Relatives of Hereditary Coproporphyria Patients

Increased faecal coproporphyrin excretion was present in 1 relative but normal urinary and faecal excretion in the other eight. Four of the relatives (44%) had

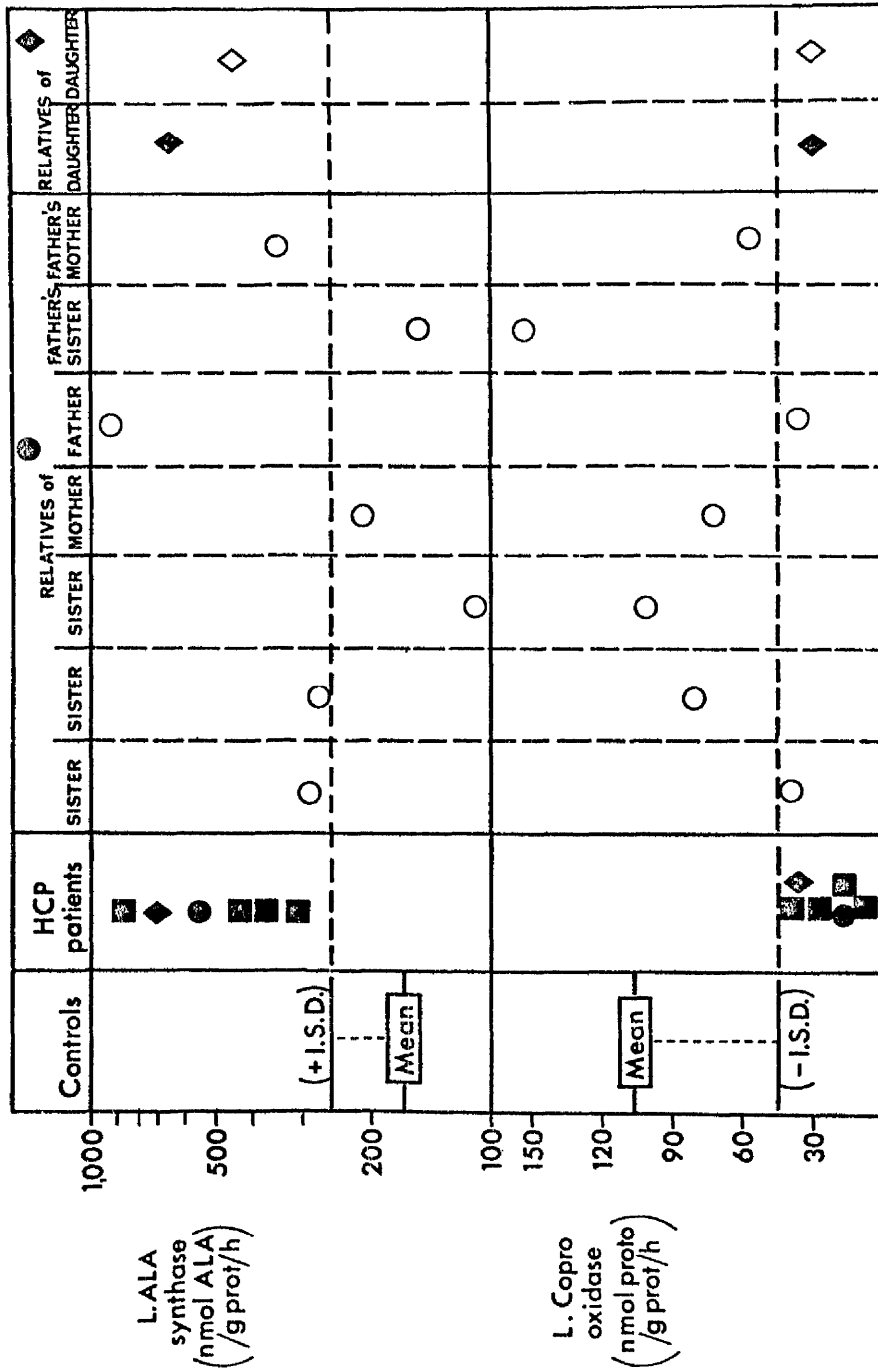


Figure 14
 Activity of ALA synthase and coproporphyrinogen oxidase in leucocytes of patients with hereditary coproporphyrinemia and in blood relatives of 2 of these patients.

L. COPRO oxidase activities of less than 45 nmol proto/g prot/h and in each of these cases the L. ALA synthase activity was more than 250 nmol ALA/g prot/h (Figure 14). These four relatives were considered to be latent cases of hereditary coproporphyrinuria. One relative had increased L. ALA synthase activity but normal L. COPRO oxidase activity and this was attributed to the inducing effect of her phenytoin anti-convulsant therapy on L. ALA synthase activity.

The 4 latent cases were comparable to the clinically manifest hereditary coproporphyrinuria patients with regards to activities of L. ALA synthase and L. COPRO oxidase though the prevalence of increased coproporphyrin excretion was higher in the patients (100%) than in the latent cases (25%).

4.3.3. Discussion

This limited study indicates that the measurement of L. ALA synthase and L. COPRO oxidase activities is a useful method for detecting latent cases of hereditary coproporphyrinuria. Urinalysis on its own is of limited value as it was abnormal in only 1 of the 4 latent cases. Our results are consistent with an autosomal dominant sex-independent mode of inheritance. As with AIP there was no clear-cut difference in enzyme activities between latent cases and patients in clinical remission, though the prevalence of increased porphyrin excretion was higher in the latter.

4.4. DISCUSSION OF SCREENING FOR ACUTE PORPHYRIA

Our studies have demonstrated that the assessment of activities of haem biosynthetic enzymes in peripheral blood cells is a reliable means of detecting latent cases of AIP and hereditary coproporphyria. The measurement of the activities of the rate controlling enzyme, L. ALA synthase, as well as the appropriate genetically deficient enzyme is considerably more discriminating than the measurement of either one on its own. It should be possible to screen for latent cases of variegate porphyria in a similar way, once a suitable peripheral blood cell assay for protoporphyrinogen oxidase is developed.

The use of these enzyme assays for screening have two minor disadvantages. Firstly, non-genetic factors may affect the activities of haem biosynthetic enzymes, resulting in misleading results. Peripheral blood reticulocytosis, elevated blood lead concentration, and the ingestion of alcohol or enzyme inducing drugs or hormones, all modify the activities of haem biosynthetic enzymes in peripheral blood cells (McColl & Goldberg, 1980). Subjects being screened should be asked about drug and alcohol ingestion and confirmed to have normal haematology. The second disadvantage is the technical complexity of the present enzyme assays as a screening test. There is a need for the development of simpler but equally reliable methods which would allow much wider screening for latent cases of acute hepatic porphyria. The present enzyme assays provide a useful standard for assessing the accuracy of any new screening test.

CHAPTER 5

DRUGS

AND HAEM BIOSYNTHESIS

5. DRUGS AND HAEM BIOSYNTHESIS

5.1. INTRODUCTION

It has been recognised for some time that the ingestion of certain drugs may precipitate attacks of acute hepatic porphyria in patients with the genetic trait. Following the introduction into medical practice of sulphonal in 1893 and barbiturates in 1903 there were numerous reports of cases of "acute toxic haematoporphyria" related to these drugs (Waldenstrom, 1937). Throughout the current century there has been a growing incidence of attacks of acute porphyria related to the increasing use of drugs many of which are porphyrinogenic (Fales, 1979). At the present time, drug ingestion is the most commonly identified precipitating factor of severe attacks, and research in this field is of great importance if more attacks are to be prevented.

Porphyrinogenic drugs all share the ability to stimulate increased activity of the initial enzyme of haem biosynthesis, ALA synthase (Smith & De Matteis, 1980). It is the overactivity of this enzyme in hepatic, and possibly also extrahepatic, tissues of patients with acute porphyria that results in the biochemical and clinical features of an acute attack. ALA synthase is under negative feedback control by "free" or "regulatory" haem and most porphyrinogenic drugs are thought to exert their effect by depleting the free haem pool (De Matteis, 1975). Drugs may do this in at least three ways.

Firstly, a high proportion of porphyrinogenic drugs induce the mixed function

oxidase enzyme system with associated increased concentration of the haemoprotein cytochrome P450 (Cyt. P450) (Rifkind et al, 1973). These drugs are thought to primarily stimulate increased synthesis of the Cyt. P450 apoprotein which combines with free haem to form the holo-cytochrome; the consequent depletion of free haem resulting in stimulation of ALA synthase (Rajamanicham et al, 1975; Bhat et al, 1977; Padmanaban, 1973) (Figure 15). Secondly, certain drugs may deplete free haem by inhibiting enzymes involved in its synthesis and examples of this include griseofulvin which inhibits ferrochelatase (Tephly et al, 1971) and sulphonamides which inhibit uroporphyrinogen-1-synthase (Peters et al, 1980). Thirdly, drugs with allyl or ethynyl substituents, such as sedormid, deplete haem by degrading it to abnormal green pigment derivatives (Ortiz de Montellano et al, 1978). It is also possible that some drugs may increase ALA synthase by mechanisms other than depleting free haem, for example by interfering with the normal feedback control mechanism.

The acute hepatic porphyrias are an example of pharmacogenetic disease; subjects with the genetic trait showing an idiosyncratic reaction to many commonly prescribed drugs. Prevention of attacks relies on accurate identification of porphyrinogenic drugs as well as detection of patients with the genetic trait. All drugs which stimulate ALA synthase activity are lipid soluble but unfortunately do not share any common chemical structure and, therefore, individual assessment of drugs is necessary. There are three main ways in which the porphyrinogenicity of a drug may be assessed (Moore, 1980). Firstly, the effect on ALA synthase activity and porphyrin

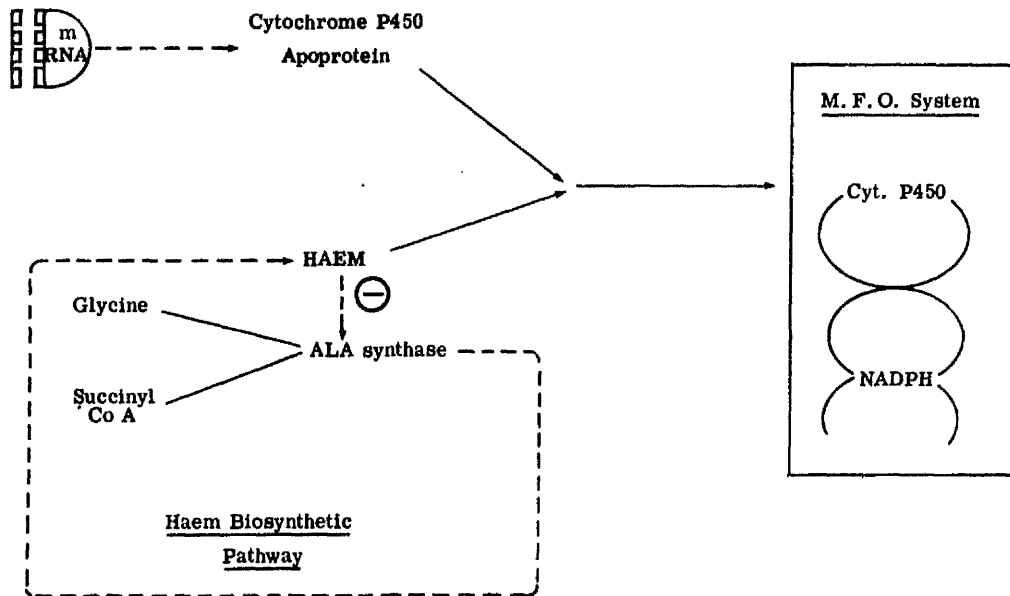


Figure 15

HAEM BIOSYNTHESIS AND THE MIXED FUNCTION OXIDASE ENZYME (M. F. O.) SYSTEM.

During induction of the M. F. O. system Cyt. P450 apoprotein combines with haem to form the holocytochrome. This depletion of haem stimulates ALA synthase activity by negative feedback control.

production in various forms of cell cultures may be examined. This is a very sensitive method and will often produce false positive results.

Secondly, it is possible to examine the effects of the drugs alone or combined with known porphyrinogenic agents on hepatic ALA synthase activity and porphyrin production in whole laboratory animals. Neither of these methods can be directly extrapolated to the human situation on account of interspecies variations in drug metabolism and enzyme induction and because of the problem of dose equivalence (Gram & Gillette, 1971). Lastly, there is anecdotal evidence of the drugs causing biochemical and clinical attacks of porphyria. This last method also produces false positive and false negative results and clearly is ethically unsuitable for routine testing of new drugs. At the present time assessment of available evidence divides the drugs which have been tested into three groups, i. e. those which are almost certainly porphyrinogenic, e. g. barbiturates and sulphonamides, those which are almost certainly safe, e. g. penicillins and phenothiazines and those with conflicting evidence, e. g. phenylbutazone and sodium valproate (Moore, 1980).

In this chapter *in vivo* studies are presented of the effects of various drugs on ALA synthase activity and Cyt. P450 content of rat hepatic tissue. In addition the *in vivo* effects of phenytoin on ALA synthase activity in peripheral blood cells of rabbits and humans are described. The relevance of these findings to the screening of drugs for porphyrinogenicity and to wider aspects of drug metabolism is discussed.

5.2. TESTING FOR PORPHYRINOGENICITY OF DRUGS IN RATS

5.2.1. Introduction

Over the past 4 years our department has received numerous enquiries regarding the safety of specific drugs in patients with acute hepatic porphyria. When no reliable information has been available from published literature, I have proceeded to test the particular drug in laboratory animals. Twenty six drugs have been tested over this period consisting of 12 drugs used in the management of arthritis, 6 drugs used in depressive illness and 8 miscellaneous drugs. All drugs were tested in rats by assessing their effect on hepatic ALA synthase activity and when possible on hepatic Cyt. P450 content.

5.2.2. Materials and Methods

Animals Fully-grown male Sprague-Dawley rats which had been in our animal house for at least 2 weeks were used. Twelve rats were used for testing each drug, 6 rats receiving the drug and 6 rats the placebo. The drugs were administered intraperitoneally or orally, once per day, for periods ranging from 2-6 days. On the final day of the study, the rats were sacrificed 1h after receiving the drug. The rats were housed separately and throughout the study had free access to water and food. The test and placebo animals were checked for comparable weights before and after the study. If any of the animals died during the study the whole study was repeated. Details of sacrifice, removal of the liver and measurement of ALA synthase and Cyt. P450 are given in Chapter 3. Statistical analysis was performed using the

non-parametric Mann Whitney U-Test.

Drugs The drugs were administered in a dosage equal to 2.5 times the recommended human dosage expressed as mg/kg body wt/day. Twenty of the drugs were administered intraperitoneally. A proprietary parenteral preparation was used when available and when not available a suitable preparation was formulated. The anti-depressant drugs were administered orally, as parenteral preparations were not possible due to problems of drug solubility. The placebo consisted of a similar volume of the solvent or carrier of the drug being tested. Phenobarbitone was used to test the model. The first 10 drugs tested were assessed for porphyrinogenic effects after both 2 days and 6 days of drug administration requiring 24 animals for each drug tested. Thereafter, all drugs were assessed only after 3 days.

5.2.3. Results and Discussion

Phenobarbitone resulted in a marked and significant increase in hepatic ALA synthase activity and Cyt. P450 content when assessed at both 2 days and 6 days after commencing intraperitoneal administration (Table 9). This confirmed the efficacy of the rat model. Eleven of the 26 drugs tested resulted in a significant increase in activity of ALA synthase. This consisted of 3 of the 12 anti-arthritic drugs (namely, azopropazone, sodium aurothiomalate and chloroquine) (Table 9), 5 of the 6 anti-depressants (namely, amitriptyline, imipramine, mianserin, phenelzine and viloxazine (Table 10) and 3 of the 8

DRUG	DOSE mg/kg/day	Administered for 2 days		Administered for 6 days	
		ALA.S. (percentage change from controls)	CYT. P450 (percentage change from controls)	ALA.S. (percentage change from controls)	CYT. P450 (percentage change from controls)
Phenobarbitone	10	+214 (p < 0.01)	+111 (p < 0.05)	+180 (p < 0.01)	+150 (p < 0.01)
Aspirin	80	+31	-10	-20	+10
Azopropazone	50	+ 55 (p < 0.05)	-11	+18	+ 20 (p < 0.05)
Alcofenac	100	- 4	-30	+16	-10
Ibuprofen	40	+ 4	+22	+43	-20
Ketoprofen	10	+10	0	- 5	-20
Flurbiprofen	15	-23	+33	+ 4	+10
Phenylbutazone	20	-14	+33	- 5	+10
Naproxen	30	- 6	-22	+11	-20
Prednisolone	4	0	+ 4	+ 6	- 4
Penicillamine	20	+ 8	+30	+10	-20
Administered for 3 days					
		ALA.S.		CYT. P450	
Gold	0.5	+ 56 (p < 0.05)		+ 17	
Chloroquine	25	+ 71 (p < 0.05)		+ 47 (p < 0.02)	

Table 9

Effect of anti-arthritic drugs (and phenobarbitone) on hepatic ALA synthase activity and Cytochrome P450 content in rats. All drugs administered intraperitoneally.

DRUG	DOSE (mg/kg/day)	ALA. S. (Percentage change from controls)	CYT. P450
Amitriptyline	10	+110 (p<0.01)	not tested
Imipramine	10	+205 (p<0.01)	not tested
Mianserin	12	+ 42 (p<0.05)	+ 25
Phenelzine	6	+500 (p<0.01)	not tested
Tryptophan	400	-3	+ 10
Viloxazine	4	+ 73 (p<0.01)	+ 19

Table 10

Effect of anti-depressant drugs on hepatic ALA synthase activity and Cytochrome P450 content. Animals sacrificed 3 days after commencing oral dosing.

DRUG	DOSE (mg/kg/day)	ALA. S. (Percentage change from controls)	CYT. P450 (Percentage change from controls)
Chloramphenicol	300	+ 10	+ 11
Disopyramide	25	+ 4	+ 1
Ergotamine	0.1	+ 2	+ 23
Hydrallazine	20	+ 4	- 26
Isoniazid	50	+102 (p<0.02)	+ 51 (p<0.05)
Metronidazole	100	+ 67 (p<0.02)	+ 57 (p<0.05)
Streptomycin	100	+ 56 (p<0.05)	+114 (p<0.05)
Cimetidine	50	+ 10	- 24

Table 11

Effect of miscellaneous drugs on hepatic ALA synthase activity and cytochrome P450 content. Drugs were administered intraperitoneally and rats sacrificed 3 days after commencing dosing.

miscellaneous drugs (namely, isoniazid, metronidazole, and streptomycin) (Table 11). The increase in ALA synthase produced by these 11 drugs varied from 42% to 500%. The phenobarbitone increased ALA synthase activity by 200%. None of the drugs significantly depressed ALA synthase activity or Cyt. P450 content. The high proportion (5 out of 6) of the positive anti-depressant drugs may be related to their high lipid solubility.

There was a positive correlation of increased Cyt. P450 and increased ALA synthase activity. Of the 24 drugs in which both were monitored, 6 significantly increased both ALA synthase and Cyt. P450, 3 increased ALA synthase alone and none increased Cyt. P450 alone. Following phenobarbitone and azopropazone, both of which increased ALA synthase and Cyt. P450, it was noted that the increase in the former was more marked at 2 days and the latter at 6 days. The mechanism of the relationship of the increase of ALA synthase activity and Cyt. P450 content is discussed more fully in the following section.

5.3. SEQUENTIAL STUDIES OF HEPATIC ALA SYNTHASE ACTIVITY AND CYT. P450 CONTENT AFTER COMMENCING PHENYTOIN ADMINISTRATION TO RATS

5.3.1. Introduction

Phenobarbitone is an example of a drug which results in a marked increase in activity of hepatic ALA synthase and which is also a potent inducer of the mixed function oxidase enzyme system. Sequential studies have shown that following the commencement of continued phenobarbitone administration to rats, the increase in ALA synthase is most marked initially and thereafter

returns towards pre-treatment values, whereas the rise in Cyt. P450 content is sustained (Meyer, 1978). We have monitored in rats changes in hepatic ALA synthase activity and Cyt. P450 content following the commencement of another porphyrinogenic anticonvulsant drug, phenytoin.

5.3.2. Materials and Methods

Thirty fully-grown adult male Sprague-Dawley rats ranging in weight from 200 g to 252 g were studied. They received daily intraperitoneal injections of phenytoin in a dosage of 15 mg/kg body weight. Six of the rats were sacrificed on day 0 after receiving only an injection of sterile water and a further six on each of days 1, 3, 6 and 12 of phenytoin injections. A further thirty similar rats were studied in an identical manner except for the substitution of phenytoin by sterile water. The animals had free access to food and water. Hepatic ALA synthase activity and Cyt. P450 content were measured as previously described (Chapter 3).

5.3.3. Results and Discussion

Hepatic ALA synthase activity was significantly increased after 1, 3 and 6 days but not after 12 days of phenytoin administration when compared with pre-treatment values (Figure 16). Hepatic Cyt. P450 content showed little change on days 1 and 3 but was significantly elevated on days 6 and 12 (Figure 16). There was no significant change in hepatic ALA synthase activity or Cyt. P450 content following placebo injections (Figure 17).

Phenytoin is a further example of a drug which both stimulates ALA synthase activity and induces the mixed function oxidase system. This sequential study

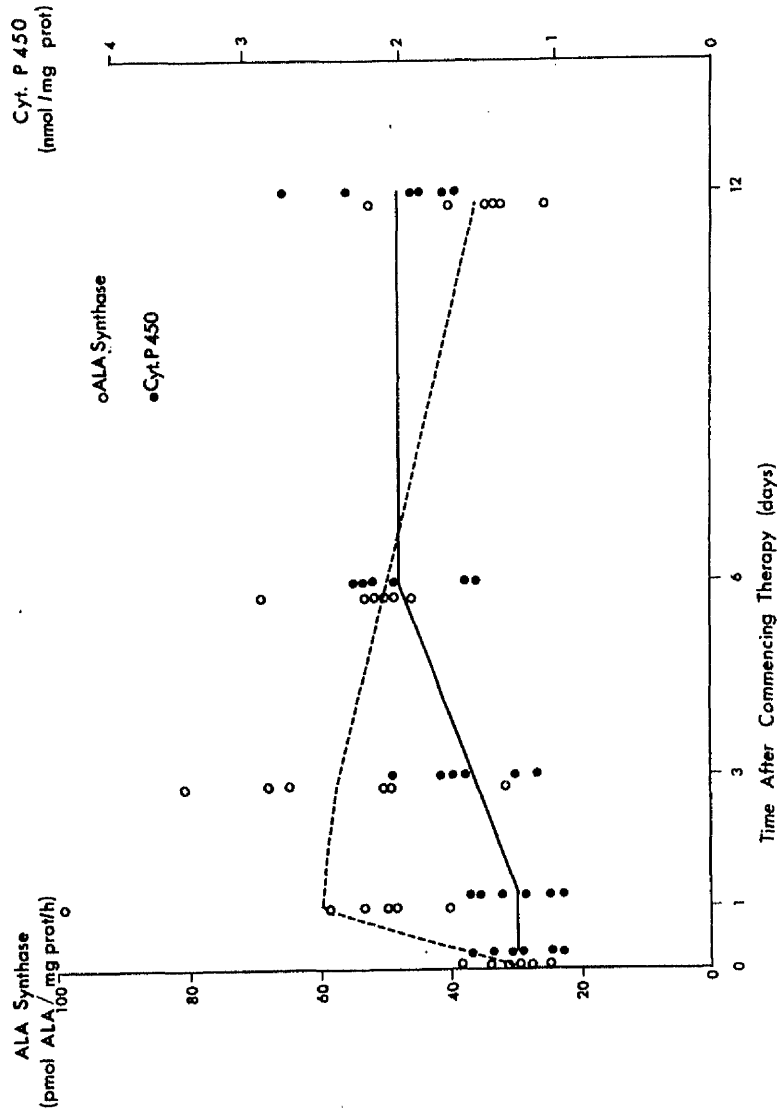


Figure 16

Hepatic ALA synthase activity and cytochrome P450 content following commencement of phenytoin in rats. ALA synthase is significantly increased on days 1, 3 and 6 ($p < 0.02$ for each) and cytochrome P450 on days 6 and 12 ($p < 0.01$ for each) compared to pre-treatment values (day 0). Statistical analysis by Mann Whitney U-Test.

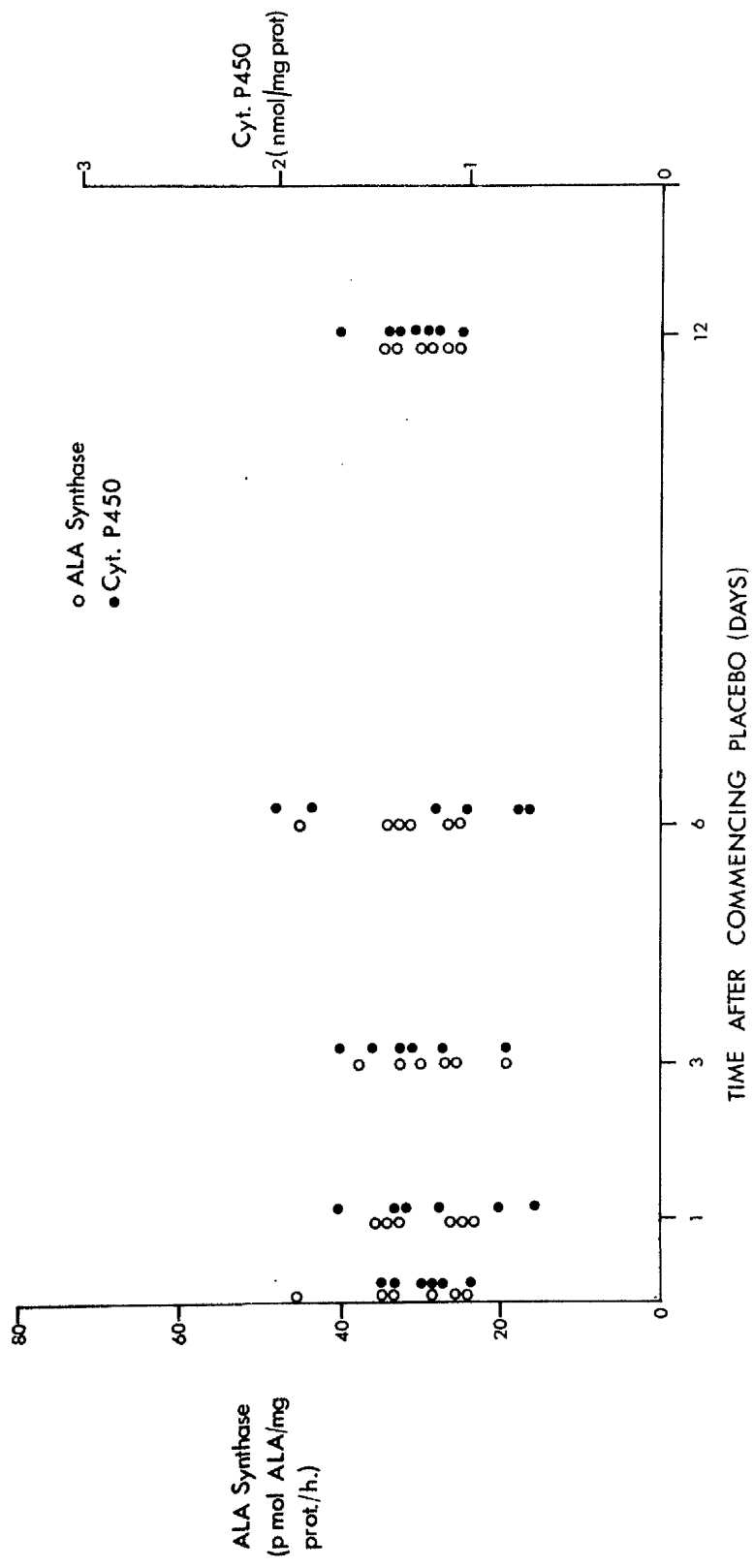


Figure 17
 Hepatic ALA synthase activity and cytochrome P450 content following commencement of placebo injections in rats.

indicates that the increase in ALA synthase activity is most pronounced during the induction phase of the mixed function oxidase system, when the Cyt. P450 level is rising. When the latter reaches its plateau, ALA synthase activity returns towards normal. This most probably indicates, as one would expect, that increased haem requirement for Cyt. P450 synthesis is most marked during the initial induction phase of the mixed function oxidase system.

5.4. PHENOBARBITONE ADMINISTRATION AND ALA SYNTHASE ACTIVITY IN PERIPHERAL LEUCOCYTES OF RABBIT

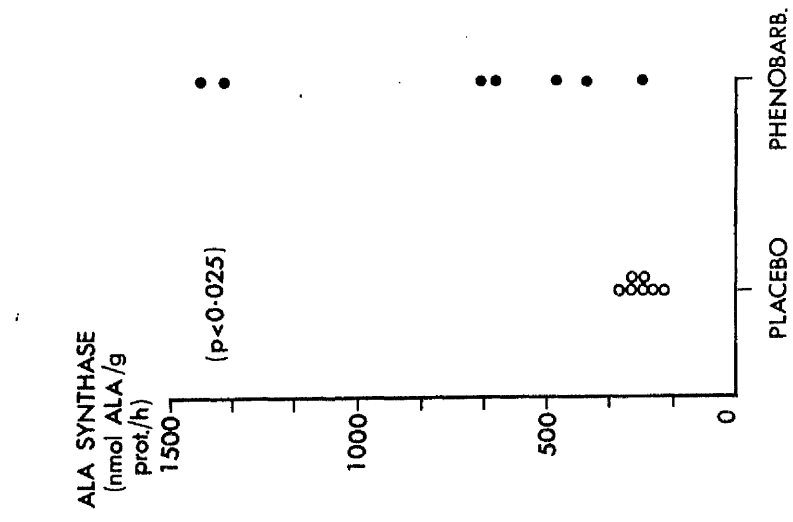
5.4.1. Introduction

There is little known about the effects of therapeutic drugs on ALA synthase activity in extra-hepatic tissues. Percy & Shanley (1979) found no increase in rat brain ALA synthase activity following phenobarbitone administration. We have studied ALA synthase activity in peripheral leucocytes and liver of rabbits receiving phenobarbitone.

5.4.2. Materials and Methods

Fourteen fully-grown adult male New Zealand rabbits were studied. Seven received daily intraperitoneal injections of phenobarbitone in a dosage of 50 mg/kg body weight and seven received placebo injections of sterile water. The animals were each housed separately and consumed similar quantities of food. They were sacrificed 1 h after the third injection by a traumatic blow to the cranium. The animals were immediately exsanguinated by cardiac

LEUCOCYTES



LIVER

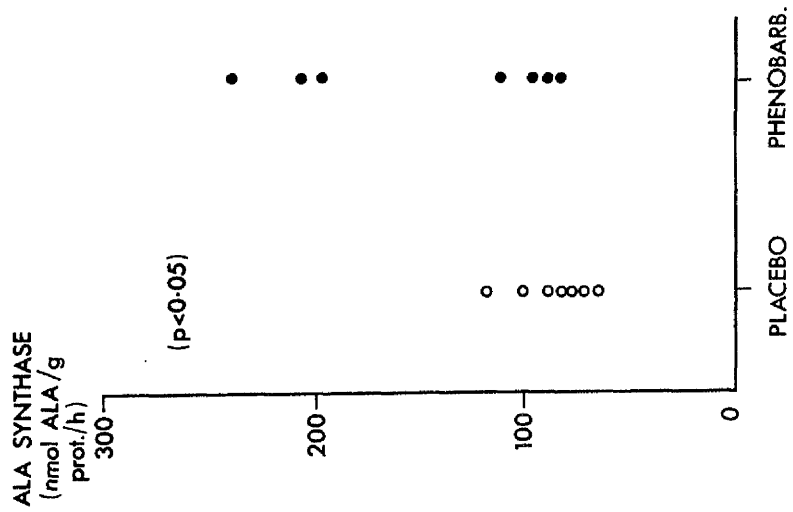


Figure 18
Effect of phenobarbitone on ALA synthase activity in leucocytes and liver of rabbits. Statistical analysis by Mann Whitney U-Test.

aspiration and their livers excised. ALA synthase activity was measured in liver and peripheral leucocytes as described in Chapter 3.

5.4.3. Results and Discussion

ALA synthase activities in hepatic tissue and leucocytes of the rabbits receiving placebo injections were similar to those in corresponding human tissues. The activity of ALA synthase was significantly increased in leucocytes as well as in hepatic tissue of the phenobarbitone-treated rabbits (Figure 18). The percentage increase in activity was greater in the leucocytes than in the liver. In order to accurately compare the increase in enzyme activity in these two tissues, however, it would be necessary to sacrifice the animals at varying time intervals after commencing the drug as the timing of the peak rise in activity may differ in the liver and leucocytes.

5.5. ALA SYNTHASE ACTIVITY IN PERIPHERAL LEUCOCYTES OF NON-PORPHYRIC SUBJECTS ON PHENYTOIN ANTICONVULSANT THERAPY

5.5.1. Patients and Methods

Single venous blood samples for measurement of leucocyte ALA synthase activity were obtained from 16 out-patients who had been taking phenytoin anti-convulsant therapy for varying periods of time.

Sequential blood samples were obtained from a further 6 out-patients newly commenced on the drug in a dosage of 100 mg three times daily. The first sample was obtained before commencement of therapy and 5-7 further samples were obtained from each patient over the subsequent 2 weeks. These 6 patients had been referred to the clinic for investigation of convulsions.

None of the patients was taking any other medication and all had normal haematological indices and blood lead concentrations. All samples were obtained at the same time of day.

5.5.2. Results and Discussion

The 16 patients in whom single estimations were performed had widely varying activities of leucocyte ALA synthase. Eight showed marked elevation and eight similar values compared to control subjects. There was no correlation with age, sex, haematological indices, drug dosage or drug concentration. There was a relationship between the enzyme activity and duration of therapy. The enzyme activity was normal in those who had been on therapy for less than 3 days, markedly increased in patients who had been taking the drug for 3-11 days and normal or slightly increased with longer therapy (Table 12).

The 6 subjects in whom leucocyte ALA synthase activity was measured sequentially following commencement of therapy all showed a similar pattern. There was no change in activity during the first 2 days of therapy. All showed a marked increase in activity between the third and eleventh day of therapy, returning to normal by the fourteenth day (Figure 19). The mean peak rise in enzyme activity as compared to pre-treatment values was eight times (range six to twenty times). In the 6 subjects studied, there was no correlation between the degree of rise in enzyme activity and age, sex or drug concentration.

Age	Sex	Daily Dose (mg)	Serum Phenytoin (μ mol/litre)	Duration of therapy	L.ALA.S. (nmol/ALA/g protein/hour)
18	F	100 T.I.D.	7	2 days	166
50	M	100 T.I.D.	15	2 "	124
60	M	100 T.I.D.	14	3 "	335
45	M	100 T.I.D.	20	3 "	722
31	M	100 T.I.D.	30	6 "	1137
50	F	100 T.I.D.	34	7 "	1800
35	M	100 T.I.D.	28	7 "	2273
36	F	100 Q.I.D.	50	11 "	850
18	M	100 Q.I.D.	60	16 "	240
25	M	100 Q.I.D.	120	6 months	140
28	F	100 T.I.D.	70	5 years	286
40	F	100 T.I.D.	40	6 "	322
22	F	100 T.I.D.	50	7 "	46
40	M	100 B.D.	68	10 "	270
71	M	100 T.I.D.	160	15 "	234
45	F	100 T.I.D.	24	17 "	250

Normal Range = 57 - 292

Table 12

Activity of leucocyte ALA synthase in 16 non-porphyric subjects on phenytoin anti-convulsant therapy.

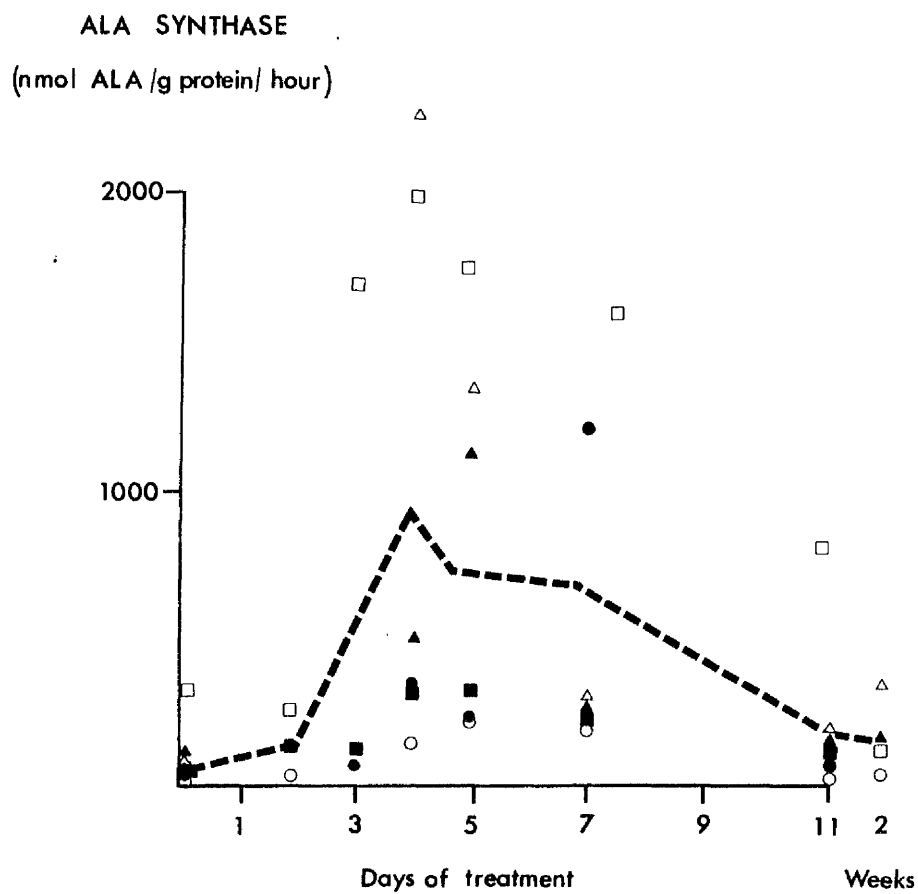


Figure 19

Sequential study of leucocyte ALA synthase activity in 6 non-porphyric subjects following commencement of phenytoin anti-convulsant therapy. Each patient is identified by different symbol and mean values indicated by broken line.

These results indicate that in non-porphyric human subjects therapeutic doses of phenytoin stimulate ALA synthase activity in circulating leucocytes. This is consistent with our observations in the rabbit. The transient nature of the increase of the leucocyte ALA synthase activity is similar to that noted in the hepatic studies. As already discussed, the rise in hepatic ALA synthase activity following commencement of phenytoin administration is most probably the result of utilization of free haem for the increased synthesis of Cyt. P450. The stimulation of leucocyte ALA synthase by phenytoin may indicate that this drug is inducing a haem-requiring enzyme system in leucocytes, as well as in the liver. Several drugs have been noted to induce enzymes in extra-hepatic tissues of laboratory animals and tissue culture preparations (Bacq, 1971). There is, however, very little information regarding in-vivo drug-related enzyme induction in human extra-hepatic tissues.

An alternative explanation for the stimulation of leucocyte ALA synthase by phenytoin is that induction of hepatic Cyt. P450 results in increased hepatic utilization not only of hepatic free haem but of total body free haem. This would result in a depletion of haem in other tissues and a compensatory increase in ALA synthase activity. Studies of the effects of phenytoin on ALA synthase in isolated leucocyte culture preparation might resolve this issue.

5. 6. DISCUSSION OF DRUGS AND HAEM BIOSYNTHESIS

The most interesting observation in this chapter is that therapeutic doses of phenytoin stimulate ALA synthase activity in leucocytes as well as in the liver. This most probably indicates increased utilization of leucocyte free

haem for the induction of a haem-requiring enzyme system within the leucocyte or within the liver. Whatever the explanation, phenytoin is clearly modifying haem metabolism within the leucocyte. The major use of haem synthesized within the leucocyte is for the formation of haem-containing peroxidases which are important in the oxidation of phagocytosed circulating debris. It would be interesting to assess the effect of phenytoin or similar drug on this aspect of leucocyte function.

Human studies of drug-related enzyme induction have to date relied on indirect methods or single estimations of enzyme activity in liver biopsy tissue. Our findings that enzyme induction or changes related to enzyme induction are occurring within human leucocytes may considerably facilitate in vivo human studies of enzyme induction as leucocytes are considerably more accessible than hepatic tissue and sequential samples can be obtained. The extent to which the changes in the leucocytes reflect those occurring in hepatic tissue remains to be discovered.

As discussed at the beginning of the chapter, there is no ideal way of testing the safety of drugs for patients with acute porphyria. It is worth considering whether studies of the effects of drugs on leucocyte ALA synthase activity might be useful in this respect. This could be performed in-vivo examining drug-related changes in leucocyte ALA synthase in non-porphyrin subjects or in-vitro using cultures of leucocytes from normal subjects or patients with acute porphyria. Though these models would overcome the problem of variation in enzyme induction between species (Bacq, 1971) they would be

subject to the disadvantage that the changes in the leucocytes may not accurately reflect changes in the liver.

The observation that drug-related stimulation of ALA synthase activity is often transient should be considered when interpreting results of drug porphyrinogenicity studies. If ALA synthase activity is measured too early or too late after commencing the drug, false negative results may occur.

It has often been debated whether drugs which induce attacks of acute porphyria may be deleterious to patients with the non-acute erythropoietic porphyrias, i. e. congenital porphyria and erythropoietic protoporphyria. The observation that ALA synthase is induced in blood cells as well as in the liver is strong evidence that porphyrinogenic drugs will increase the overproduction of porphyrins in these erythropoietic porphyrias and thus aggravate the cutaneous manifestations.

CHAPTER 6

ALCOHOL

AND HAEM BIOSYNTHESIS

6. ALCOHOL AND HAEM BIOSYNTHESIS

6.1. INTRODUCTION

Acute alcohol ingestion is an important factor for precipitating attacks of acute hepatic porphyria. Chronic alcohol ingestion, on the other hand, is important in the aetiology of cutaneous hepatic porphyria (Goldberg et al, 1978). Haem biosynthesis is modified by alcohol in normal as well as in porphyric subjects. In 1935, Franke and Fickentschir noted in normal subjects, that drinking 1 litre of beer or 90ml of cognac generally doubled urinary coproporphyrin excretion (Goldberg & Rimington, 1962). Orten et al (1963) noted that chronic alcoholics had increased urinary excretion of coproporphyrin, mainly isomer III, but normal excretion of uroporphyrin, ALA and PBG. Acute alcohol dosing increases the activity of ALA synthase and depresses the activities of ALA dehydratase and ferrochelatase, in rat hepatic tissue (Shanley et al, 1968, Moore et al, 1974). Increased hepatic ALA synthase activity also occurs with chronic alcohol dosing of rats (Rubin et al, 1970). In 1977, Bonkowsky and Pomeroy reported increased activity of hepatic ALA synthase in human chronic alcoholics with liver disease. Alcohol also modifies haem biosynthesis in extra-hepatic tissue, causing a marked depression of ALA dehydratase activity in circulating erythrocytes of both rats and humans (Moore, 1971). In patients with alcohol-related sideroblastic anaemia, there is increased activity of bone marrow ALA synthase (Fraser & Schacter, 1977) and accumulation of protoporphyrin and

coproporphyrin in erythrocytes (Ali & Sweeney, 1974).

In this chapter the effects of acute and chronic alcohol consumption on haem biosynthesis in non-porphyrin human subjects are studied.

6.2. ACUTE ALCOHOL INGESTION AND HAEM BIOSYNTHESIS

6.2.1. Subjects and Methods

Studies were performed on 8 healthy male volunteers. They ranged in age from 19 to 31 years and each was confirmed to have normal haemoglobin concentration, total white cell count and blood lead concentration. None was taking any form of drug therapy and all abstained from alcohol for 7 days prior to the commencement of the study. Throughout the study each subject consumed his normal diet.

On the first day, at 09.00 hours, each subject drank 200 ml of 69.5% proof Vodka (1.316 mol ethanol) over 15 min. Whole blood (35 ml) was withdrawn immediately prior to his consuming the alcohol and again 2, 6, 24 and 30 h after completion of the drink. Of this, 30 ml was heparinized and used for the enzyme assays. The activities of the mitochondrial enzymes ALA synthase, coproporphyrinogen oxidase and ferrochelatase were measured in leucocytes and of the cytosolic enzymes ALA dehydratase, uroporphyrinogen-I-synthase and uroporphyrinogen decarboxylase in erythrocytes. On each occasion the remaining 5 ml of blood was used for measuring the haemoglobin concentration, total white cell count, reticulocyte count and ethanol concentration. All urine

passed in the 24 h prior to and 48 h following the alcohol consumption was collected and the 24h urinary excretion of ALA, PBG, uroporphyrin and coproporphyrin measured. In four of the subjects the enzymes were monitored over a similar 30 h period without alcohol consumption.

6.2.2. Results

The mean whole blood alcohol concentration of the 8 subjects was 22 mmol/l (range 18-24) at 2 h falling to 3 mmol/l (range 1-9) at 6 h and undetectable at 24 h and 30 h following the alcohol ingestion. There were no consistent alterations in the haemoglobin concentration, total white cell count or reticulocyte count throughout the period of the study. There was significant alteration in the activity of each of the enzymes studied as described below. Alterations in the mitochondrial enzymes are shown in Figure 20 and in the cytosolic enzymes in Figure 21 .

Mitochondrial enzymes:

Leucocyte ALA synthase. There was a rise in the mean activity of this enzyme, being most marked at 6 h and returning to the pre-alcohol level at 30 h.

Leucocyte coproporphyrinogen oxidase. This was depressed at 6 h and similar to pre-alcohol values at 2, 24 and 30 h.

Leucocyte ferrochelatase. This showed the most marked alterations. Its activity was significantly depressed at 2, 6, 24 and 48 h, this being most pronounced at 6 h.

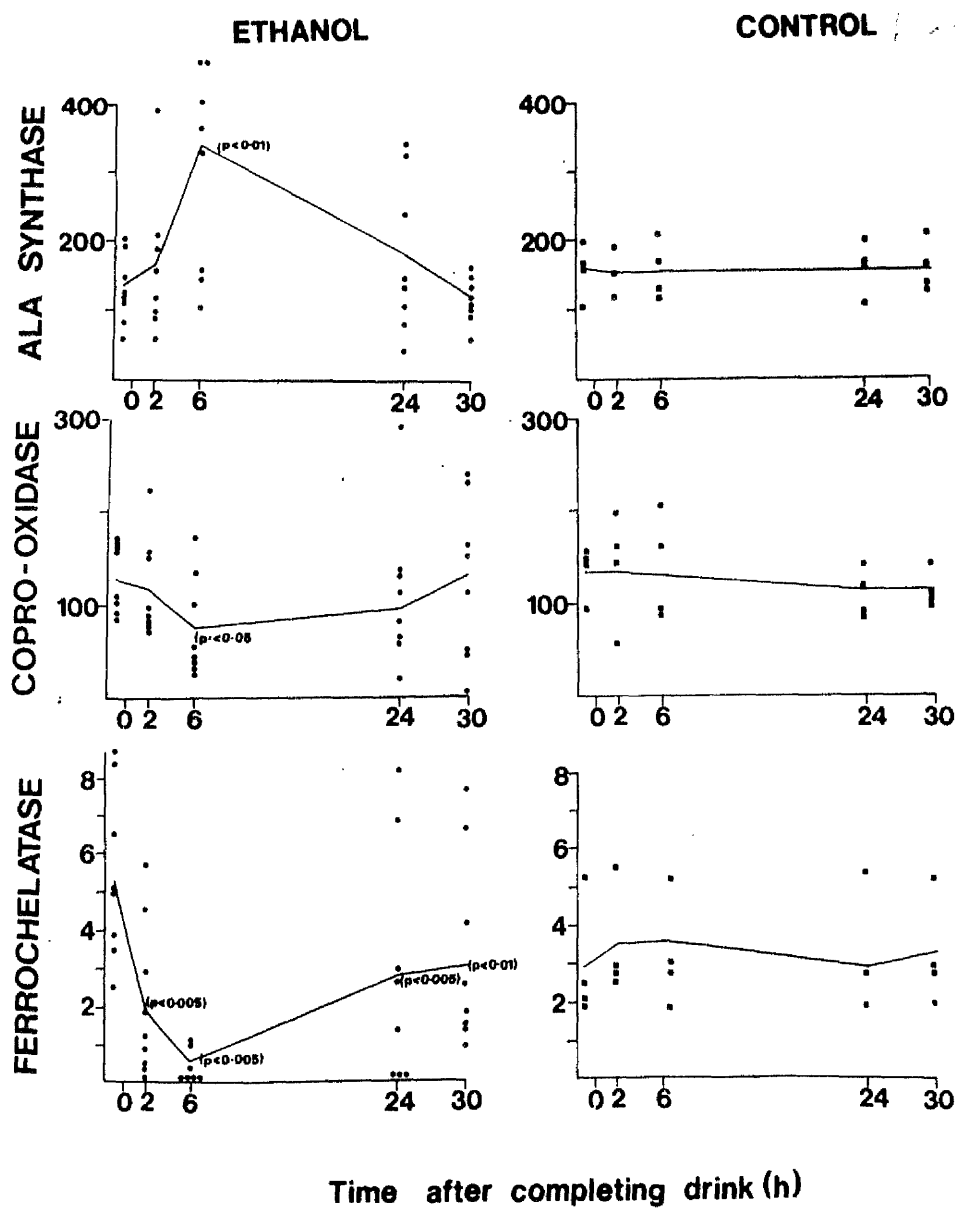


Figure 20

Effect of consuming 200 ml Vodka on the activities of ALA synthase, coproporphyrinogen oxidase and ferrochelatase in peripheral leucocytes of 8 healthy subjects. The values at each time studied have been compared with the pre-ethanol values (time 0) using the Wilcoxon paired samples ranking test. Activities of these enzymes in four of the subjects over a similar control period is also shown. For units of enzyme activities, see Table 2, Chapter 3.

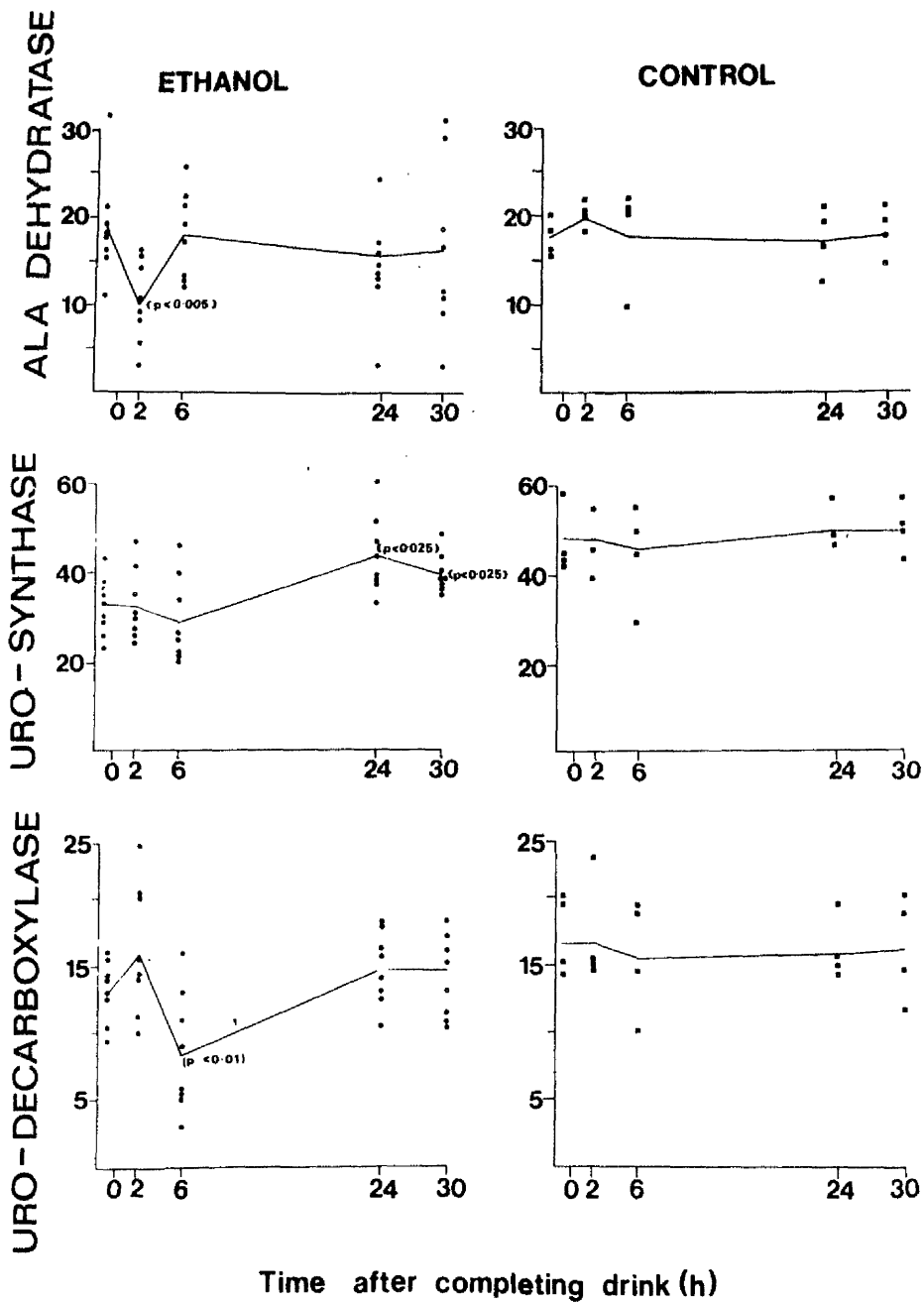


Figure 21

Effect of consuming 200 ml Vodka on the activities of ALA dehydratase, uroporphyrinogen-1-synthase and uroporphyrinogen decarboxylase in peripheral erythrocytes of healthy subjects.

Cytosolic enzymes:

Erythrocyte ALA dehydratase. The mean activity was markedly depressed at 2 h and similar to the pre-alcohol level at 6, 24 and 30 h.

Erythrocyte uroporphyrinogen-I-synthase. There was little alteration at 2 and 6 h but the activity was significantly increased at 24 and 30 h.

Erythrocyte uroporphyrinogen decarboxylase. This was depressed at 6 h and similar to pre-alcohol values at 2, 24 and 30 h.

There was a significant negative correlation of alteration of activities of leucocyte ALA synthase and leucocyte ferrochelatase by linear regression analysis:

$$\text{Ferrochelatase} = -75.7 - 0.084 \times \text{ALA synthase}$$

$$(r = -0.712, P < 0.05)$$

There was also a significant positive correlation of depression of erythrocyte uroporphyrinogen decarboxylase and leucocyte ferrochelatase activities.

$$\text{Ferrochelatase} = -74.5 + 0.423 \times \text{Uroporphyrinogen decarboxylase}$$

$$(r = 0.882, P < 0.005)$$

The above two correlations were also significant using the non-parametric Spearman rank correlation test ($P < 0.05$ and $P < 0.02$ respectively). No significant correlation was found for ethanol concentration at 1 h post drink and alteration in enzyme activity.

No consistent alteration was found in the activity of the enzymes repeated in four of the subjects over a similar time without alcohol ingestion (Figures 20 and 21).

The 24 h urine volume was increased in each subject on the day of the test compared with the previous and subsequent 24 h period (Table 13).

The mean 24 h urinary excretion of ALA and PBG was also higher on the day of the test. Uroporphyrin could not be detected in any of the samples. The most marked and only significant increase was in the coproporphyrin excretion which, on the day of the test, exceeded our upper limit of the normal range (432 nmol/24 h) in three of the 8 subjects. The mean urinary coproporphyrin excretion was also increased on the day following the alcohol consumption but this was not statistically significant.

6.2.3. Discussion

The alcohol consumption in this acute study resulted in increased activities of leucocyte ALA synthase and erythrocyte uroporphyrinogen-I-synthase and depressed activities of the other four enzymes studied. The mechanism of these alcohol-induced changes is not clear. ALA synthase is under negative feedback control by haem and therefore decreased free haem concentration due to reduced formation of haem, increased utilization of haem or destruction of haem could explain the rise in activity. The alcohol-related depression of four of the enzymes of the pathway could result in decreased haem synthesis. The negative correlation of alteration of ALA synthase activity and ferrochelatase activity would support this hypothesis. Alcohol may also result in increased utilization of haem as it induces the formation of the haemoprotein cytochrome

	Pre-ethanol	Post-ethanol	
	24 hours	0-24 hours	24-48 hours
ALA (umol/24 h)	18 (8-20)	22 (11-26)	19 (10-22)
PBG (umol/24 h)	3.5 (0-11)	9 (0-15)	6 (0-13)
URO (nmol/24h)	0	0	0
COPRO (nmol/24h)	109 (5-149)	796* (100-1735)	815 (80-1200)
Urine Volume (ml)	1701 (1550-2000)	2781* (2100-3502)	1667 (1200-2100)

Table 13

Effect of ethanol on 24 h urinary volume and excretion of porphyrins and precursors in 8 subjects. Values are mean (range shown in parentheses). *The urine volume and coproporphyrin excretion is significantly increased ($p < 0.025$ for each) during the 24 hours following ethanol ingestion compared with the pre-ethanol values. Statistical analysis performed using Wilcoxon paired samples ranking test.

P450 (Rubin et al, 1968). Increased hepatic concentration of cytochrome P450 has been noted within 2 h of enteral alcohol dosing of rats (Pennington et al, 1978). A third mechanism may be important. The intramitochondrial NADH/NAD⁺ ratio is of importance in the regulation of ALA synthase (Labbe et al, 1970). Alcohol alters the intramitochondrial redox state and may thereby increase ALA synthase activity (Shanley et al, 1968). The only other enzyme noted to increase in activity was uroporphyrinogen-1-synthase and this occurred later, at 14 and 30 h post alcohol ingestion, at a time when no detectable alcohol remained in the blood. This delayed response may indicate that the rise in activity of uroporphyrinogen-1-synthase is a secondary compensatory phenomenon. Uroporphyrinogen-1-synthase has recently been suggested to play a secondary rate-controlling role in the pathway when ALA synthase is derepressed (Brodie et al, 1977). It is therefore interesting to note that the two enzymes which increased in activity are the two enzymes which are of importance in controlling the rate of the pathway. The mechanism by which alcohol or one of its metabolites depresses the activities of ALA dehydratase, uroporphyrinogen decarboxylase, coproporphyrinogen oxidase and ferrochelatase remains unclear.

The increased urinary excretion of coproporphyrin observed during the 24 h following the alcohol consumption may be due to increased synthesis of this porphyrin and/or increased renal clearance. There is little information available on the mechanism of renal handling of coproporphyrin. If tubular reabsorption is important the alcohol induced diuresis might temporarily

increase renal clearance. The relative magnitude of the increase in coproporphyrin excretion compared with the increased urine volume is against a purely renal explanation.

These enzyme changes noted in peripheral blood cells may be relevant to some of the haematological effects of alcohol consumption. Alcohol is known to depress erythropoiesis (Myrhed et al, 1977; Houribane & Weir, 1970) and this is thought to be the result of primary depression of haem synthesis with consequent depression of globin synthesis (Ibrahim et al, 1979; Freedman et al, 1975). The very marked depression of ferrochelatase activity noted may be of particular relevance to the aetiology of alcohol-related sideroblastic anaemia. Ferrochelatase is the final enzyme in the pathway and responsible for the insertion of iron into protoporphyrin to form haem. Sideroblastic anaemia is characterised by accumulation of non-haem iron in the mitochondria of blood cell precursors. Patients with alcohol-related sideroblastic anaemia have also been found to have increased activity of ALA synthase in bone marrow (Fraser & Schacter, 1977) and accumulation of protoporphyrin and coproporphyrin in erythrocytes (Ali & Sweeney, 1974). All these changes may be explained by depression of ferrochelatase.

It is probable that the alcohol ingestion is causing similar alterations in the enzymes of haem biosynthesis in hepatic cells to those noted in peripheral blood cells. In rats, acute alcohol ingestion produces similar changes in ALA synthase, ALA dehydratase, uroporphyrinogen decarboxylase and ferrochelatase to those which we have observed in human peripheral blood

cells (Moore, 1974; Doss, 1980). The increased urinary coproporphyrin excretion may indicate depression of hepatic coproporphyrinogen oxidase activity. If similar changes are occurring in the human liver following alcohol ingestion, these may be relevant to our understanding of the mechanisms of certain alcohol-related hepatic disorders. Alcohol is an important aetiological agent in the development of cutaneous hepatic porphyria. Patients with this condition are thought to have a genetic deficiency of hepatic uroporphyrinogen decarboxylase (Kushner et al, 1976; Elder et al, 1978). A further acquired factor, e.g. alcohol excess, certain drugs and chemicals or liver disease, is necessary before the patients excrete excess urinary uroporphyrin and develop symptoms of the disease (Tiepermann & Doss 1978). Our finding of alcohol-related depression of uroporphyrinogen decarboxylase and increase of ALA synthase, may explain the mechanism by which alcohol can precipitate this condition in patients possessing the genetic trait. Hepatic siderosis is also a feature of this disease and alcohol has been incriminated as a factor in the development of this. Alcoholism and alcoholic liver disease are also associated with increased hepatic iron deposition (Jakobovits et al, 1978) and alcohol is an important aetiological factor in the development of the so-called, idiopathic haemochromatosis (McDonald, 1964). The alcohol-related marked depression of ferrochelatase, which inserts iron into protoporphyrin to form haem, may play a role in the accumulation of non-haem iron in hepatic tissue.

Most of the alcohol-related haematological and hepatic disorders occur in the context of chronic rather than acute alcohol ingestion. It is, therefore, necessary to examine the effect of chronic alcohol ingestion on haem biosynthesis.

6. 3. CHRONIC ALCOHOL INGESTION AND HAEM BIOSYNTHESIS

6. 3. 1. Subjects and Methods

Haem biosynthesis has been studied in 11 male chronic alcoholics who were admitted to hospital for alcohol withdrawal. Their mean age was 50 years (range 36-65 years). None had taken any form of medication in the month prior to admission and the consultant psychiatrists in charge agreed to withhold their routine therapy of chlordiazepoxide and multivitamin preparations, and instead manage the patients with psychological support and adequate nutrition and hydration. Informed consent was obtained from the patients. Details of the alcohol history were obtained from the patients and their relatives and are shown in Table 14. The estimated mean daily alcohol consumption was 230 g (range 100-400 g) and mean duration of alcohol abuse 11 years (range 2-25 years). All patients reported drinking heavily up until the day prior to their arranged admission. Screening on admission showed the blood ethanol concentration to be 13 mmol/litre in one patient but none was detected in the remainder. The patients were closely supervised during their admission in order to prevent and detect any occult alcohol consumption. Severe withdrawal symptoms developed in 2 patients and they received appropriate therapy and were withdrawn from the sequential part of the study.

Patient	Ethanol intake (grammes/day)	Type	Duration (years)	Admission blood ethanol (mmol/l)
1	260	Lager + Whisky	25	undetectable
2	240	Whisky + Vodka	10	"
3	260	Whisky	10	"
4	130	Whisky	10	"
5	260	Whisky	10	"
6	240	Whisky + Vodka	5	13
7	400	Whisky	10	undetectable
8	200	Cider	10	"
9	300	Fortified Wine	10	"
10	100	Fortified Wine	2	"
11	130	Whisky	15	"

Table 14

Ethanol History in Chronic Alcoholics

All patients had a full general medical assessment on admission (Table 15). One patient was found to have hepatomegaly, peripheral neuropathy and Korsakoff's psychosis. A further patient had peripheral neuropathy, amblyopia and Korsakoff's psychosis and a further two patients had hepatomegaly. None of the patients had clinical stigmata of hepatocellular dysfunction. Routine liver function tests performed on the day of admission showed elevated activity of gamma glutamyltranspeptidase in all 11 patients, elevated aspartate transaminase in eight and elevated alanine transaminase in seven (Table 16). Vitamin studies showed that nine of the patients had subnormal leucocyte ascorbic acid, three thiamine deficiency and one pyridoxine deficiency (Table 17). Haematological screening showed a haemoglobin concentration less than 13.5 g/dl in 3 patients, elevated mean corpuscular volume in seven and low platelet counts in 2 patients (Table 18). All patients had normal reticulocyte count and total white cell count. Haematinic screening showed the iron saturation elevated in 4 patients and reduced in one; the whole blood folate was low in 1 patient and none had Vit B12 deficiency (Table 17).

Venous blood was obtained from each patient within 18 h of admission for the measurement of the activities of the enzymes of haem biosynthesis and the erythrocyte porphyrin and lead concentrations. In 8 patients it was possible to obtain further samples on the second, fifth and between the tenth and twentieth days of admission. The blood samples for the enzyme studies were always obtained at the same time of day. A 24 h urine collection was obtained from

Patient	Age (years)	Mental State	Neuropathy	Liver Disease
1	51	normal	-	-
2	60	delirium tremens	-	-
3	42	normal	-	4cm hepatomegaly
4	58	normal	-	-
5	60	Korsakoffs Psychosis	Peripheral Neuropathy	6cm hepatomegaly
6	36	normal	-	-
7	65	delirium tremens	-	-
8	43	normal	-	4cm hepatomegaly
9	65	Korsakoffs Psychosis	Peripheral Neuropathy + amblyopia	-
10	41	normal	-	-
11	36	normal	-	-

Table 15

Clinical Status of Chronic Alcoholics

Patient	Albumin (g/l)	Globulin (g/l)	Bilirubin (μ mol/l)	Alk. Phos. (iu/l)	Cholesterol (mmol/l)	AST (iu/l)	ALT (iu/l)	LDH (iu/l)	γ GT (iu/l)
1.	37	27	9	83	4.4	30	20	609	56
2.	37	27	13	69	5.9	137	130	416	78
3.	43	31	11	89	5.9	245	225	507	474
4.	37	31	18	144	5.6	125	79	377	101
5.	33	41	108	380	7.1	299	84	669	1008
6.	41	29	8	77	6	43	33	234	44
7.	39	25	30	49	6.6	120	65	445	95
8.	42	26	11	96	5.2	134	168	341	482
9.	37	29	19	74	6.2	28	36	307	145
10.	36	29	5	166	3.9	32	22	515	62
11.	37	33	15	66	6.1	62	64	375	100
normal range	36-52	26-36	5-20	40-115	3.0-8.5	13-42	11-55	250- 525	0-40

Table 16 Liver function tests in Chronic Alcoholics

Patient	Fe ($\mu\text{mol/l}$)	TIBC ($\mu\text{mol/l}$)	% Sat.	Folate ($\mu\text{g/l}$)	Vit C ($\text{nmol}/10^8 \text{ WBC}$)	Vit B ₁ (RBC transketolase % activation by thiamine)	Vit B ₆ (RBC AST % activation by pyridoxine)	Vit B ₁₂ (ng/l)
1.	25	57	44	7.4	51	41	16	1,050
2.	24	60	40	3.4	119	7	2	720
3.	28	54	52	4.5	169	17	3	840
4.	5	30	17	3.4	66	2	7	1,000
5.	30	36	83	2.8	17	31	5	2,000
6.	13	66	20	3.8	70	5	10	300
7.	33	45	73	8.1	43	58	16	580
8.	29	51	57	3.9	86	9	17	820
9.	23	57	40	1.5	59	8	13	1,300
10.	9	45	20	2.2	74	4	44	620
11.	23	57	40	8.0	77	4	14	510
normal range	14-29	45-72	25-45	> 1.9	> 102	< 25	< 20	> 200

Table 17 Vitamin and Haematinic Status of Chronic Alcoholics

Patient	Hb (g/dl)	MCV (fl)	Retics (%)	WCC ($\times 10^9/l$)	Platelets ($\times 10^3/l$)
1	13.9	98	1	7.3	140
2	13.8	90	1	9.1	250
3	14.0	99	1	5.6	102
4	11.8	87	1	4.9	104
5	9.0	103	1	5.8	72
6	14.5	103	1	5.7	190
7	12.6	103	1	4.5	132
8	16.0	95	1	7.2	240
9	17.0	103	1	7.2	260
10	14.4	92	1	8.6	400
11	15.6	99	2	7.8	220

Table 18

Haematological status of Chronic Alcoholics

each patient on the first day of admission for the measurement of porphyrin and precursor excretion.

Eleven healthy male subjects (mean age = 40 years, range 21-69 years) admitted to hospital for elective arthroscopy of the knee were used as control subjects. Blood was obtained from them within 18 h of hospital admission for haem enzyme studies. Statistical analysis of comparison of test and control subjects was performed using the non-parametric Mann Whitney U-Test.

6.3.2. Results

Studies on Day of Admission

The activity of leucocyte ALA synthase was significantly increased and the activities of erythrocyte ALA dehydratase and uroporphyrinogen decarboxylase significantly decreased on the day of admission compared with the control subjects (Table 19). Erythrocyte uroporphyrinogen-I-synthase activity was elevated compared with the controls but this difference did not reach statistical significance. There was no statistical difference between the chronic alcoholics and controls with regards to leucocyte coproporphyrinogen oxidase or ferrochelatase activities, though in two of the chronic alcoholics (numbers 5 and 7) ferrochelatase activity was markedly depressed and in one of these (number 7) coproporphyrinogen oxidase activity was also depressed.

The erythrocyte porphyrin studies showed elevated protoporphyrin and coproporphyrin concentrations in one patient (number 7) elevated protoporphyrin

Patient	Blood Lead (pmol/l)	L. ALA. S.	E. ALA. D	E. URO. S.	E. URO. D.	L. COPRO. O.	L. FERR.
1.	1.1	140	10	40	8	86	1.5
2.	0.5	1297	11	41	11	51	4.6
3.	2.3	381	10	52	18	100	1.7
4.	1.6	656	8	30	12	90	2.4
5.	1.6	642	2	57	3	37	0.6
6.	0.7	134	20	76	23	48	1.7
7.	1.9	430	1	39	5	7	0.8
8.	2.5	264	3	46	7	54	2.1
9.	1.5	400	7	25	7	84	3.4
10.	1.5	93	11	28	6	68	2.8
11.	2.0	259	9	42	7	62	3.2
Mean	1.6	427	8.3	43	9	62	2.3
Control Mean (range)	1.3 (0.6-2.0)	176 (80-372)	22 (12-40)	35 (23-49)	16 (9-42)	78 (20-191)	2.5 (1.0-5.1)
Significance	N. S.	p < 0.05	p < 0.01	N. S.	p < 0.05	N. S.	N. S.

Table 19

Enzymes of haem biosynthesis in peripheral blood cells of chronic alcoholics. (for units of enzyme activities, see Figure 2, Chapter 3). Statistical analysis performed by Mann Whitney U-Test.

in two further patients (numbers 3 and 4) and elevated coproporphyrin in a fourth patient (number 5) (Table 20). The 24 h urinary excretion of ALA, PBG, uroporphyrin and coproporphyrin was in all cases within our range for normal subjects. The blood lead concentration in the chronic alcoholics was slightly increased (mean 1.6 $\mu\text{mol/l}$, range 0.5-2.5 $\mu\text{mol/l}$) compared with the controls (mean 1.3 $\mu\text{mol/l}$, range 0.6-2.0 $\mu\text{mol/l}$).

Sequential Enzymes Studies

Leucocyte ALA synthase activity, which was elevated on the day of admission, remained elevated on the first and fourth days post admission but was similar to control subjects when measured between the tenth and twentieth days post admission (Figure 22). Erythrocyte ALA dehydratase activity, which was depressed on the day of admission, remained depressed on the first and fourth days post admission but again had returned to normal when measured between the tenth and twentieth days (Figure 23). Erythrocyte uroporphyrinogen decarboxylase activity, which was depressed on the day of admission, was still depressed the following day but similar to controls when measured on the fourth day and between the tenth and twentieth days (Figure 24). Sequential studies of activities of erythrocyte uroporphyrinogen-I-synthase and leucocyte coproporphyrinogen oxidase and ferrochelatase in the chronic alcoholics as a group showed no significant alterations from control subjects or from day of admission. The marked depression of leucocyte ferrochelatase activity in patients numbers 5 and 7 and of coproporphyrinogen oxidase activity in patient number 7, noted on the day of admission, corrected within 5 days of alcohol withdrawal.

Patient	Erythrocyte		Coproporphyrin (nmol/24h)	Uroporphyrin (nmol/24h)	Urinary		
	Coproporphyrin (nmol/1 RBC)	Protoporphyrin (nmol/1 RBC)			ALA (umol/24h)	PBG (umol/24h)	
1.	13	191	54	Undetectable	12	Undetectable	Undetectable
2.	16	273	27	"	9	"	"
3.	24	999	200	"	12	"	"
4.	37	1206	107	"	22	9.4	9.4
5.	154	625	79	"	1.9	2.2	2.2
6.	24	483	98	"	11	Undetectable	Undetectable
7.	126	1871	304	"	31.7	"	"
8.	18	627	279	"	6	"	"
9.	28	766	244	"	10	"	"
10.	30	500	145	"	11	3	3
11.	19	335	95	"	24	8	8
normal range	64	800	432	49	40		16

Table 20

Erythrocyte porphyrin concentrations and urinary excretion of porphyrins and precursors in eleven chronic alcoholic subjects.

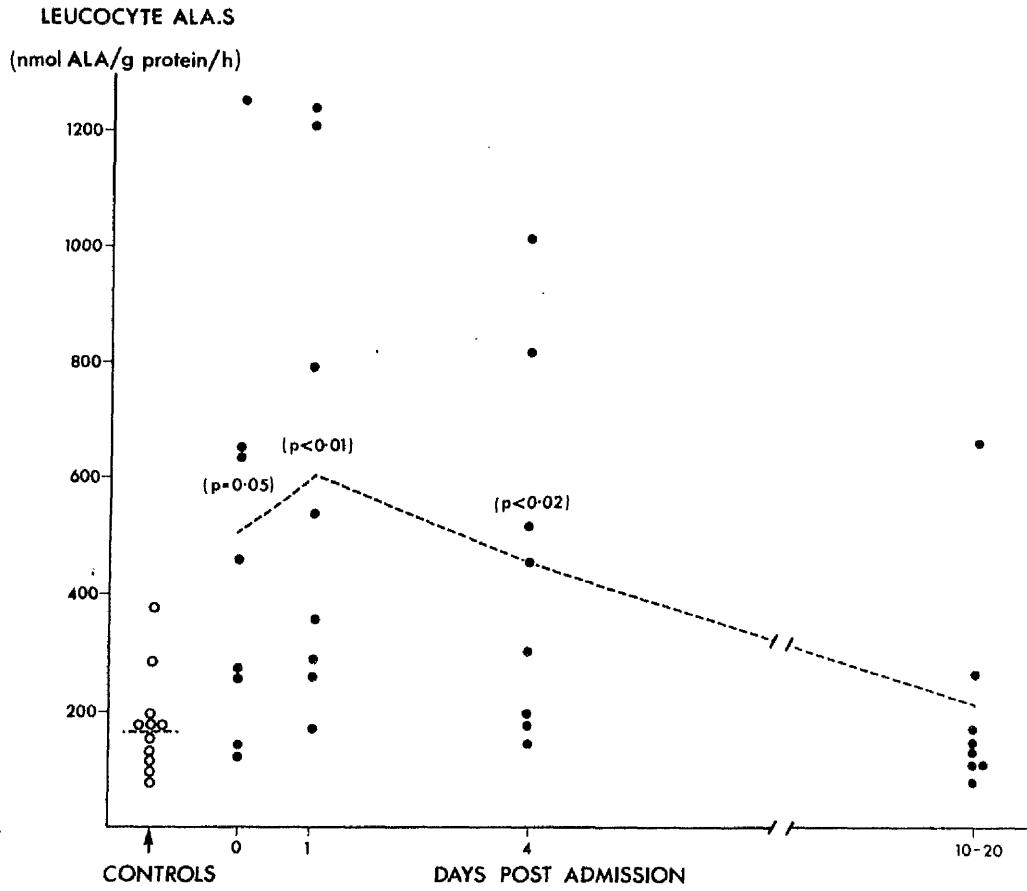


Figure 22

Leucocyte ALA synthase activity in Chronic Alcoholics following cessation of drinking. Statistical comparison of alcoholics versus controls by Mann Whitney U-Test.

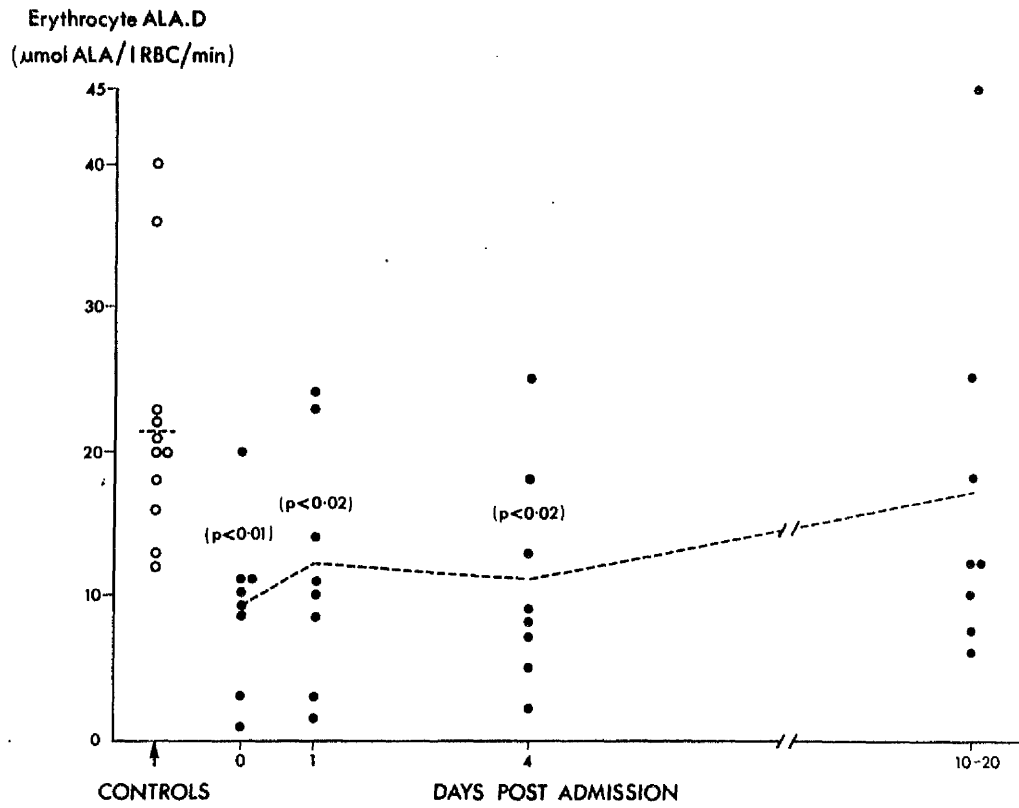


Figure 23

Erythrocyte ALA dehydratase activity in chronic alcoholics following cessation of drinking.

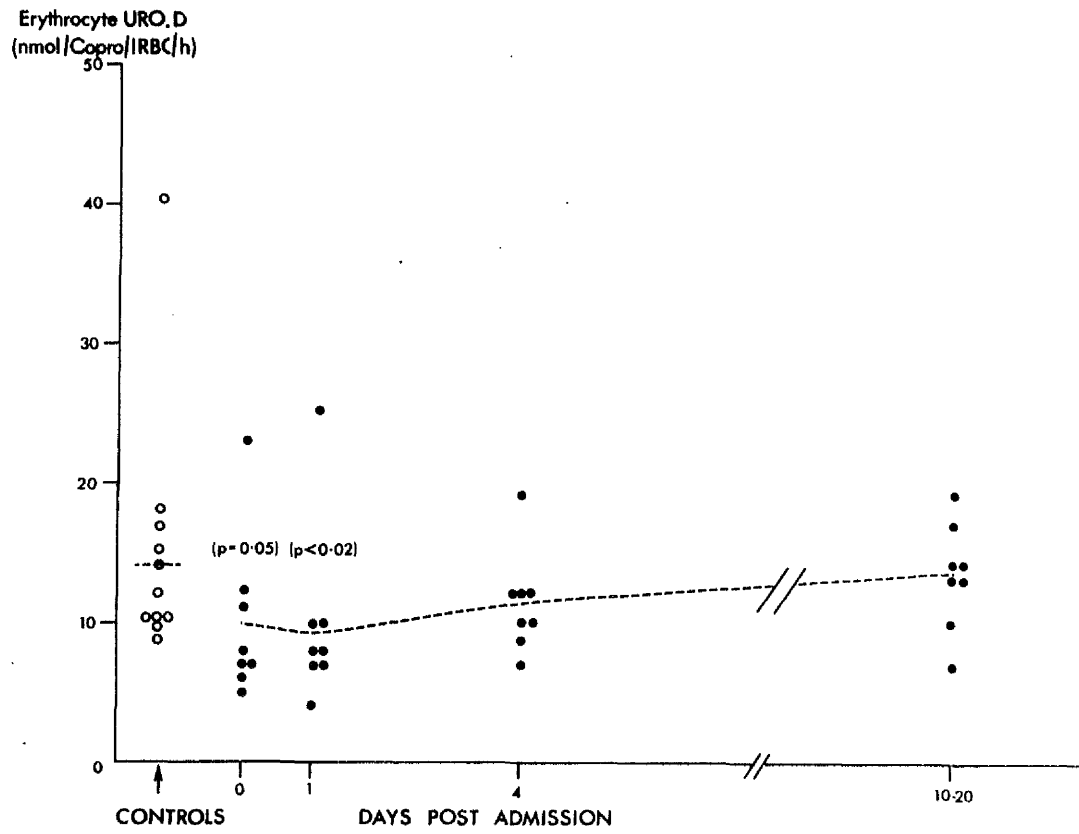


Figure 24

Erythrocyte uroporphyrinogen decarboxylase activity in chronic alcoholics following cessation of drinking.

6.3.3. Discussion

This study demonstrates that chronic alcohol ingestion is associated with abnormal haem biosynthesis in peripheral blood cells which reverts to normal with withdrawal of alcohol. The observations in the chronic alcoholics are similar, but not identical, to those noted in the acute alcohol study in healthy subjects. Increased activity of ALA synthase and depressed activities of ALA dehydratase and uroporphyrinogen decarboxylase were features of both studies. Increased activity of erythrocyte uroporphyrinogen-I-synthase was also noted in both, though it did not reach statistical significance in the chronic study. Depressed activities of leucocyte coproporphyrinogen oxidase and ferrochelatase noted in the acute study were not seen in the chronic alcoholics. The fact that the enzymatic changes noted in the present study were also seen following acute alcohol ingestion in healthy subjects is strong evidence that these changes are due to the alcohol rather than any of the nutritional deficiencies present in the chronic alcoholics.

The urinary excretion of porphyrins and precursors was normal in the chronic alcoholics, in contrast to the acute alcohol study where increased urinary excretion of coproporphyrin was noted. The increased coproporphyrin in the acute alcohol situation may have been the result of the depression of coproporphyrinogen oxidase which was not present in the chronic study. The absence of increased urinary coproporphyrin excretion in the chronic situation is in agreement with the observation in rats that the coproporphyrinuria occurring on commencing alcohol is transient and does not persist with

continued dosing (Doss et al, 1980). Increased urinary coproporphyrin excretion, however, has been noted in chronic alcoholics (Elder, 1976) and its absence in this study may be in part explained by the absence of liver disease and the effects of acute alcohol withdrawal.

Four of the chronic alcoholics had increased erythrocyte porphyrin concentrations. The increased erythrocyte protoporphyrin in patient number 4 may be explained by the iron deficiency anaemia and in patient number 3 by the elevated blood lead. The increased erythrocyte coproporphyrin in patient number 5 and increased erythrocyte protoporphyrin and coproporphyrin in patient number 7 are more interesting as neither patient had evidence of iron deficiency or elevated blood lead. Excluding patient number 4 with iron deficiency anaemia, patients numbers 5 and 7 were the only anaemic chronic alcoholics. In both cases the anaemia was macrocytic in type with normal folate and Vit B12 levels. The iron saturation was markedly increased in both cases suggesting a failure of iron utilization. The enzymes of haem biosynthesis were particularly interesting in these two patients, both having markedly depressed activities of leucocyte ferrochelatase and in addition, patient number 7 having depressed coproporphyrinogen oxidase activity. The activities of these two enzymes were similar to the controls in all the other non-anaemic alcoholics. It is reasonable to conclude that in these 2 patients the chronic alcohol ingestion was depressing the activities of these enzymes resulting in accumulation of their substrates (coproporphyrin, protoporphyrin and iron) and producing

anaemia due to deficient haem synthesis. Both these enzymes had been noted to be depressed by acute alcohol ingestion and it is likely that in certain patients this persists with chronic alcohol ingestion resulting in alcohol-induced anaemia. Patients with alcohol-related sideroblastic anaemia have increased bone marrow ALA synthase (Fraser & Schacter, 1977), accumulation of protoporphyrin and coproporphyrin in erythrocytes (Ali & Sweeney, 1974) and accumulation of non-haem iron (Figure 25). All these features are explained by depressed activity of coproporphyrinogen oxidase and ferrochelatase.

If the enzymatic changes noted in the peripheral blood cells of the chronic alcoholics are also occurring in the liver they may be relevant to the role of alcohol in cutaneous hepatic porphyria. Most patients with this condition have a hereditary partial deficiency of hepatic uroporphyrinogen decarboxylase (Kushner et al, 1976; Elder et al, 1978). The alcohol-related accumulation of uroporphyrin in these patients may be explained by the alcohol further depressing uroporphyrinogen decarboxylase activity as well as increasing the activity of the initial and rate-controlling enzyme of the pathway, ALA synthase.

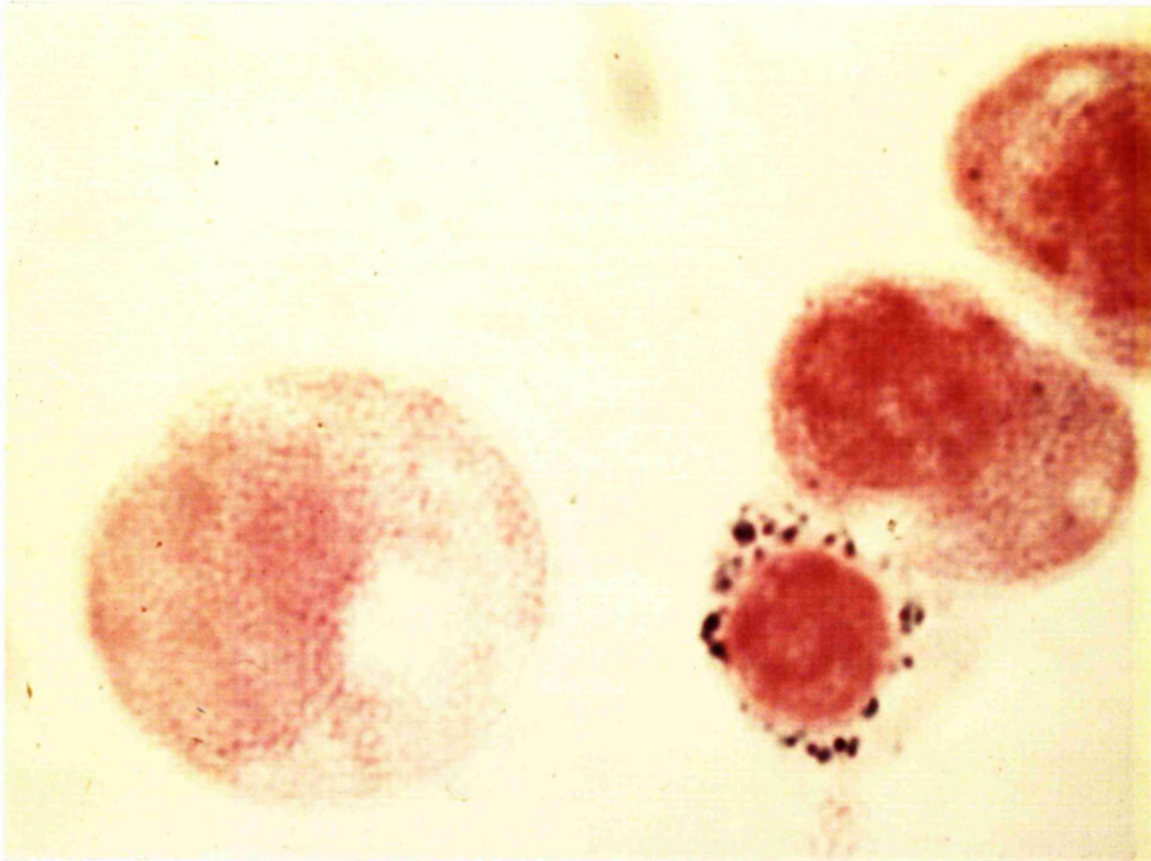


Figure 25

Perinuclear accumulation of iron in alcohol-induced sideroblastic anaemia. Stained with Prussian Blue.

CHAPTER 7

HORMONES

AND HAEM BIOSYNTHESIS

7. HORMONES AND HAEM BIOSYNTHESIS

7.1. INTRODUCTION

Hormonal factors affect haem biosynthesis and play an important role in the aetiology of attacks of acute hepatic porphyria. Attacks are more common in females than males and rarely occur before puberty, being most common in the third decade (Tschudy, 1974). Pregnancy may precipitate acute attacks and some women experience regular attacks occurring in the week prior to menstruation (Lamon et al, 1978). The contraceptive pill and other forms of exogenous oestrogens may also precipitate attacks of acute porphyria and are also important in the aetiology of porphyria cutanea tarda (Goldberg et al, 1978). There is evidence that hormonal influences affect haem biosynthesis in normal subjects; increased urinary excretion of porphyrins and precursors being reported during pregnancy (Lyberatos et al, 1972; De Klerk et al, 1975; Brodie et al, 1977), and increased urinary excretion of ALA in women on the contraceptive pill (Koskelo et al, 1966).

Animal studies have shown that the activity of ALA synthase in rat hepatic tissue varies throughout the female sexual cycle, being highest in oestrus and lowest in dioestrus (Held & Przerwa, 1976) and a similar pattern is observed in the harderian gland of the female hamster (Moore et al, 1978). Certain steroid hormones have been noted to increase the activity of ALA synthase in rat hepatic tissue. Among the most potent tested to date are

androstenedione, androstenediol, dehydroepiandrosterone (DHA) 17-hydroxypregnenolone and aetiocholanolone (Paxton et al, 1974). The mechanism by which hormones increase ALA synthase activity is not clearly understood. ALA synthase is under negative feedback control by haem and any lowering of the intramitochondrial free haem concentration results in its induction. Certain synthetic acetylenic substituted steroids, including norethindrone and ethynyl oestradiol, have been shown to destroy microsomal haem and cytochrome P450 in rats (White, 1979). The haem degrades to abnormal green coloured derivatives similar though not identical to the green pigments formed by allylisopropylacetamide - a chemical used to produce experimental porphyria in animals. These animal studies, however, may be of little relevance to the human situation as the hormones studied were synthetic rather than endogenous and the concentrations many times greater than occur physiologically. Animal studies have shown that the steroid hormones which induce ALA synthase all have a similar structure (Granick & Kappas, 1967). It is therefore possible that these hormones are able to competitively inhibit the feedback of haem by occupying its receptor site. The observation that both haem (Waxman et al, 1966) and oestradiol (Tschudy et al, 1967) cause similar oscillatory changes in hepatic ALA synthase would be in favour of them both acting in a similar way such as occupying a common receptor.

In patients with AIP there is an increased ratio of 5 beta/5 alpha steroids with increased urinary excretion of DHA and aetiocholanolone and increased plasma

concentrations of DHA (Moore et al, 1973). This is the result of a deficiency of 5 alpha reductase activity (Bradlow et al, 1973). The relationship between this altered steroid metabolism and the abnormality of haem biosynthesis is now known. The overproduction of these porphyrinogenic steroids, however, may be of importance in the aetiology of the acute attack.

In the following section we have studied the interrelationship of steroid hormones and haem biosynthesis in both porphyric and normal subjects.

7.2. ACUTE PORPHYRIC ATTACKS AND THE FEMALE MENSTRUAL CYCLE

7.2.1. Subjects and Methods

The timing of hospital admission in attack of AIP was examined in relation to the phase of the menstrual cycle in 3 patients (J. Mc. - 23 years old, J. C. - 24 years old and P. W. - 30 years old), who suffered frequent attacks. They had experienced between them a total of 26 attacks requiring hospital admission and without apparent exogenous precipitating factors over the three year period studied. All had regular menstrual cycles. As a result of our self-referral hospital admission system their admissions were usually within 1 or 2 days of the onset of symptoms of an attack.

7.2.2. Results

In the 3 patients studied, attacks were most common pre-menstrually, seventy per cent of the admissions occurring within the 7 days prior to the onset of menstruation (Figure 26).

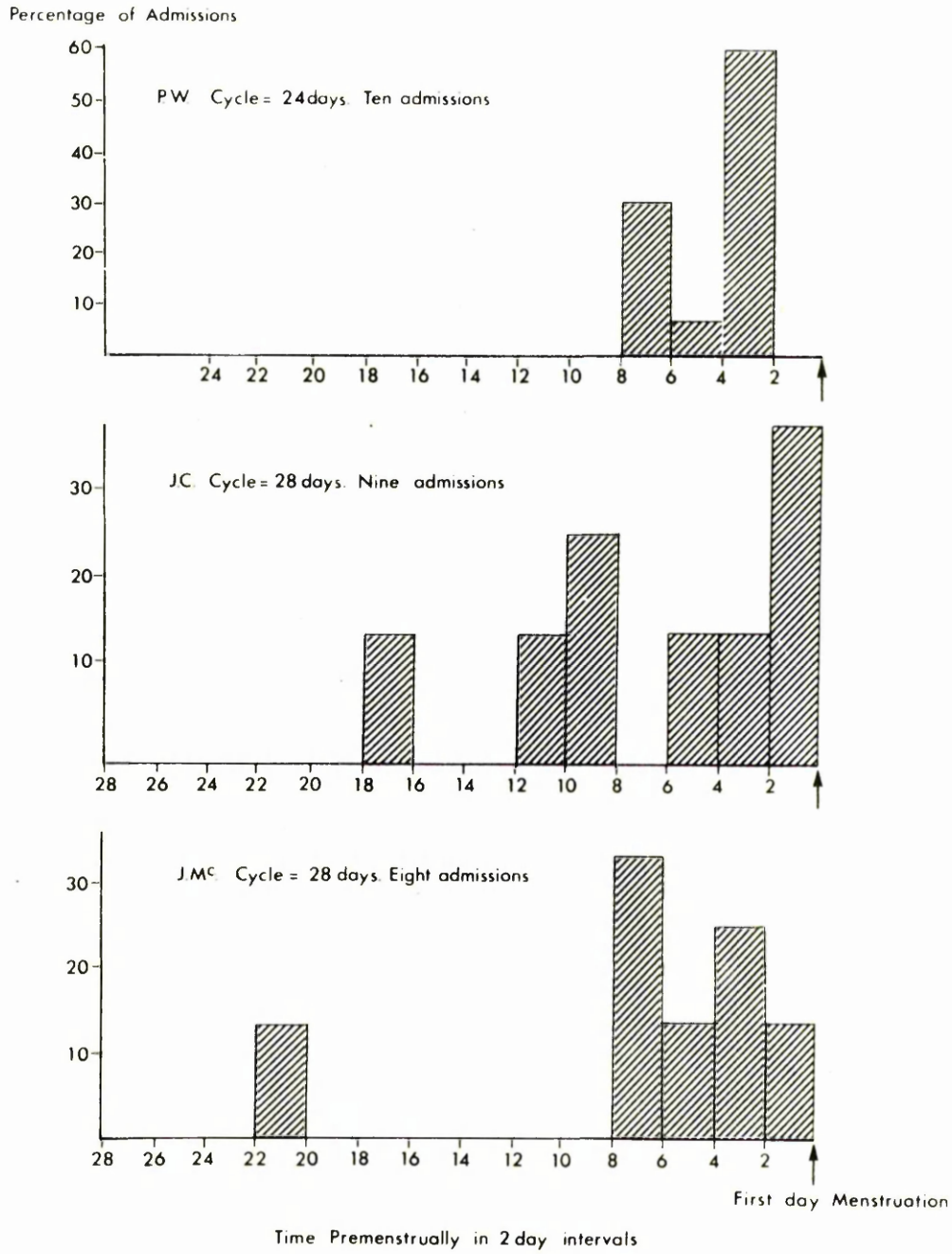


Figure 26

Timing of admission in acute attack in relation to menstrual cycle in 3 females with acute intermittent porphyria.

7.3. HAEM BIOSYNTHESIS THROUGHOUT MENSTRUAL CYCLE IN NORMAL FEMALES AND IN PATIENT WITH LATENT AIP

7.3.1. Subjects and Methods

Studies of haem biosynthesis were made throughout the complete menstrual cycle in 6 healthy female volunteers (mean age - 30 years, range 26-45 years) and over a similar time period in 6 healthy male volunteers (mean age - 30 years, range 24-44 years). All subjects had normal blood lead and haemoglobin concentrations and none was taking any form of drug therapy. All abstained from alcohol throughout the study. Blood was withdrawn for assessment of leucocyte ALA synthase activity each Monday, Wednesday and Friday between 9a.m. and 10a.m. In four of the males and four of the females the activity of URO synthase in peripheral erythrocytes was also monitored. The urinary excretion of ALA and PBG was monitored in four males and three females. Fifty ml spot urine samples were used and results expressed in relation to creatinine excretion. In two of the females the plasma concentrations of androstenedione, dehydroepiandrosterone (DHA) and DHA sulphate were also monitored and in one female the plasma concentrations of oestradiol, progesterone and testosterone.

Similar studies were performed throughout one complete menstrual cycle in a 26 year old female (H.B.) with latent AIP. The diagnosis was based on depression of the activity of erythrocyte URO synthase and increased activity of leucocyte ALA synthase in the presence of normal urinary excretion of porphyrins and precursors. The condition had been inherited from the patient's

mother who showed the same biochemical disorder. The patient had never experienced a symptomatic attack of porphyria but has subsequently developed "idiopathic" hypertension. Throughout the menstrual cycle the activities of leucocyte ALA synthase, erythrocyte URO synthase and the urinary excretion of ALA and PBG were monitored as described for the normal females. Plasma concentrations of androstenedione, DHA and DHA sulphate were also monitored.

7.3.2. Results in Normal Subjects

Leucocyte ALA synthase

The mean ALA synthase activity was slightly higher in the females (150 nmol ALA/g prot/h) than in the males (122 nmol ALA/g prot/h) but this difference was not statistically significant. The female subjects showed more fluctuation of activity of leucocyte ALA synthase than the males (Figure 27, Table 21). In the 6 females studied, it was not possible to discern any association between the fluctuation in activity and phase of menstruation (Figure 27). There was no association of degree of fluctuation and subject age or body weight.

Erythrocyte URO synthase

The mean activity of erythrocyte URO synthase was 48.5 nmol URO/1 RBC/h in the males and 41 nmol URO/1 RBC/h in the females. The degree of fluctuation of activity was similar in the different sexes (Figure 28). No

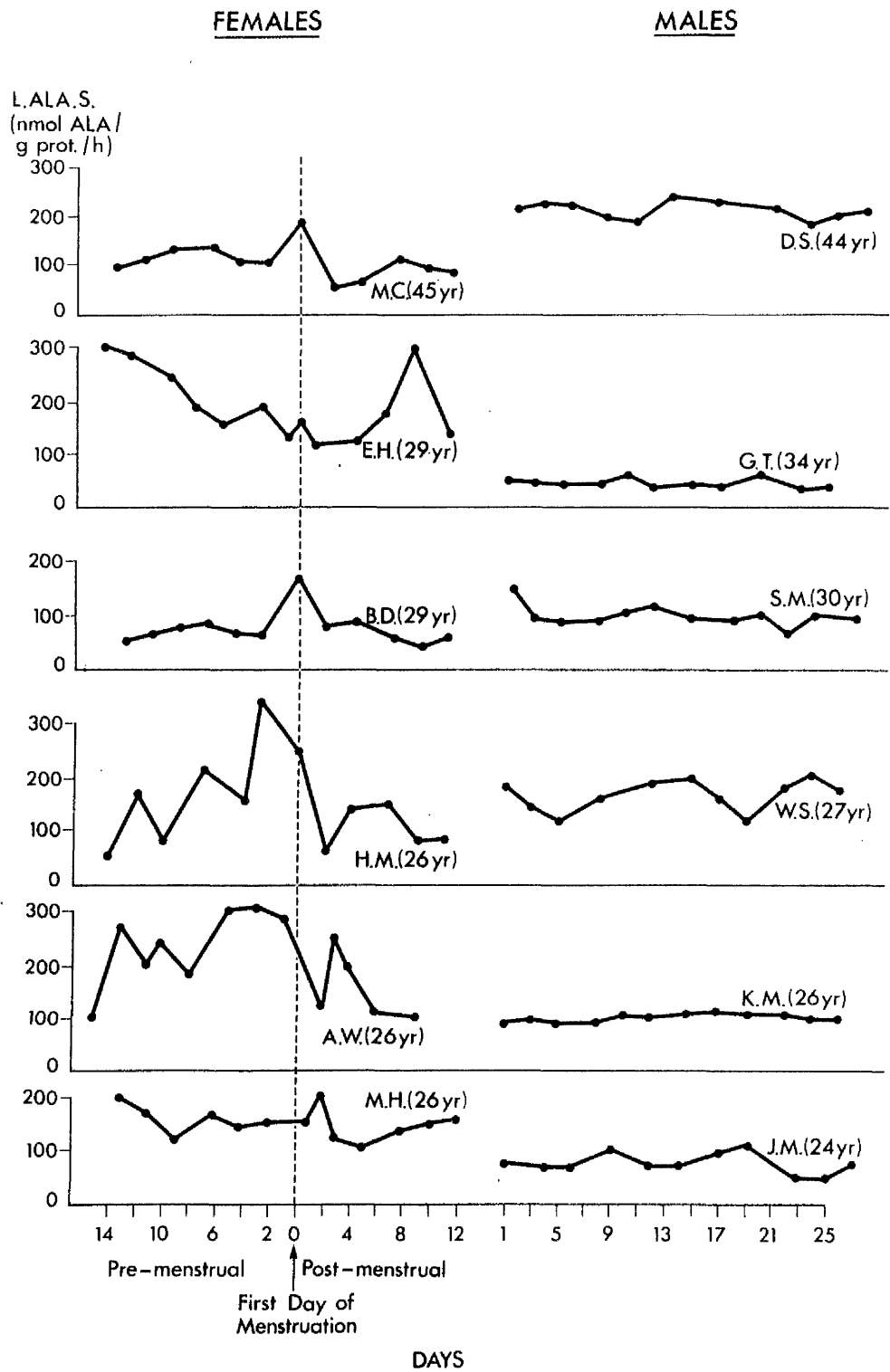


Figure 27

Activity of leucocyte ALA synthase throughout complete menstrual cycle in 6 normal females and throughout similar time period in 6 normal males.

Subjects	Mean Activity (n mol ALA /g protein/h)	Average fluctuation from mean	
		(nmol ALA /g protein/h)	(as % of mean)
<u>MALES</u>			
G. T.	68	4	6
K. M.	98	7	7
D. S.	215	21	10
S. M.	103	16	15
J. M.	75	15	20
W. S.	175	26	15
Mean for all males	122	15	12
<u>FEMALES</u>			
M. C.	115	19	17
B. D.	77	16	21
F. H.	192	46	24
A. W.	218	61	28
M. H.	149	20	13
H. M.	152	74	49
Mean for all females	150	26	25
<u>LATENT AIP FEMALE</u>	315	100	28

Table 21

Leucocyte ALA synthase activity throughout month in normal males and females and in patient with latent AIP.

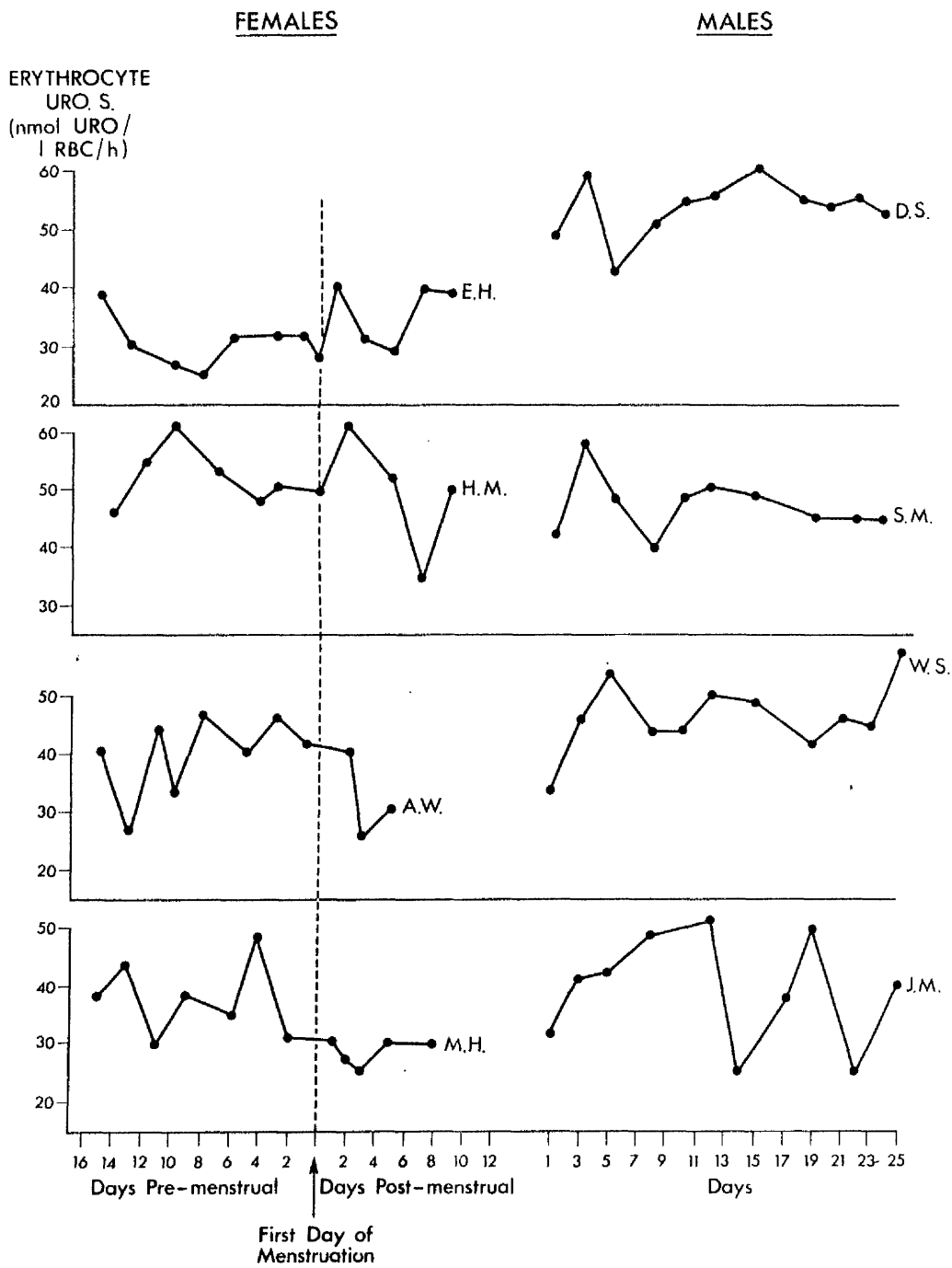


Figure 28

Activity of erythrocyte URO synthase throughout complete menstrual cycle in 4 normal females and over similar time period in 4 normal males.

association was apparent between the fluctuations in ALA synthase and URO synthase activities.

Urinary ALA and PBG excretion

The mean urinary excretion and degree of fluctuation of excretion of ALA (Figure 29) and PBG (Figure 30) were similar in the two sexes. There was no discernable association between the urinary excretion of porphyrin precursors and the enzyme activities, nor was there any association between the porphyrin precursor excretion and phase of the menstrual cycle.

Hormonal Studies

In subject E.H., in whom the activity of leucocyte ALA synthase and plasma concentrations of oestradiol, testosterone and progesterone were monitored simultaneously throughout the menstrual cycle, there was no clear association between the enzyme activity and concentration of any of these hormones (Figure 31). In subjects A.W. and M.H., there was no association between leucocyte ALA synthase activities and the plasma concentrations of the porphyrinogenic steroids DHA, DHA sulphate and androstenedione (Figures 32, 33).

7.3.3. Results in Patient with Latent AIP

The mean activity of leucocyte ALA synthase in the patient with latent AIP (315 nmol ALA/g prot/h) was considerably higher than in any of the normal subjects studied. The degree of fluctuation in leucocyte ALA synthase activity was more than occurred in the normal females when expressed as

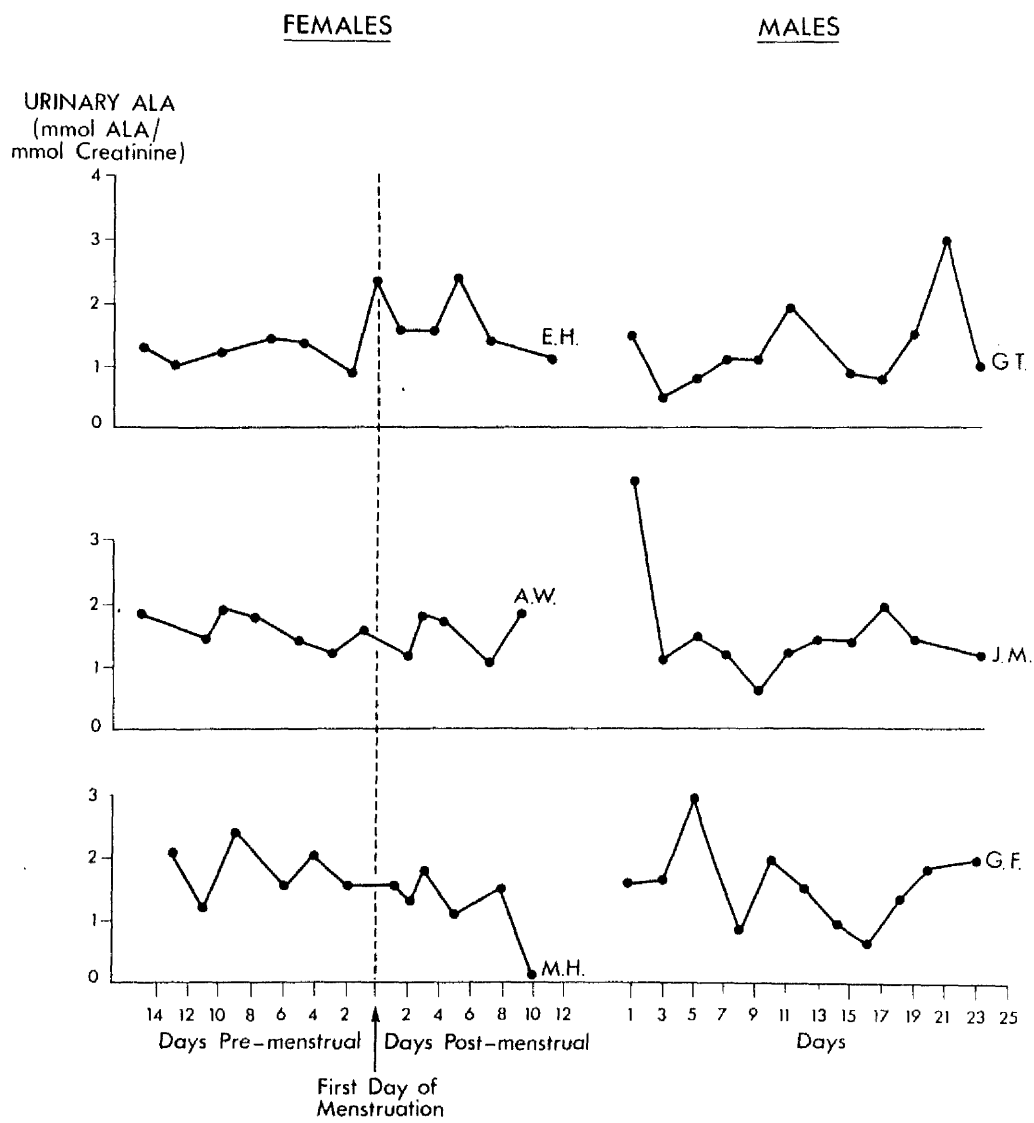


Figure 29

Urinary excretion of ALA throughout menstrual cycle in 3 normal females and over similar time period in 3 normal males.

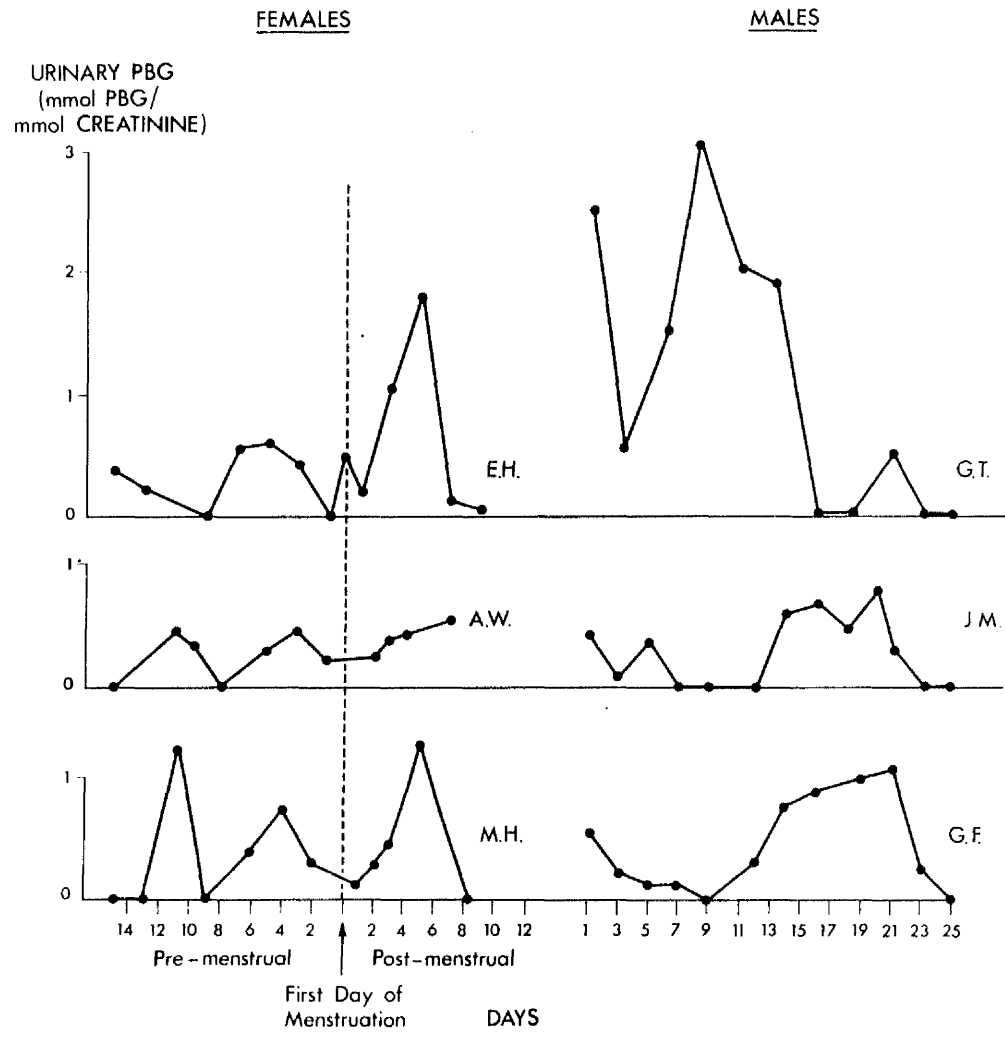


Figure 30

Urinary excretion of PBG throughout menstrual cycle in 3 normal females and over similar time period in 3 normal males.

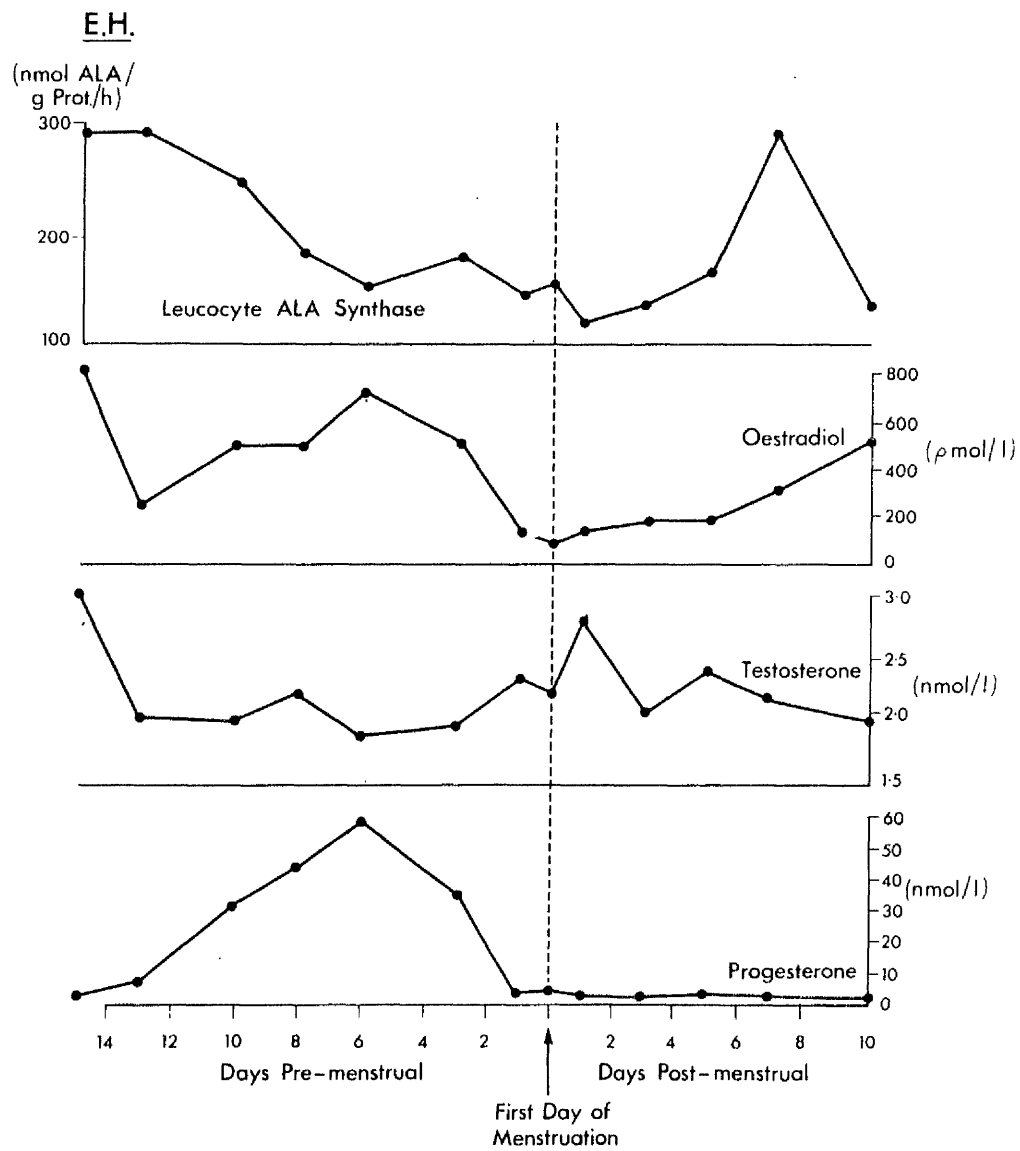


Figure 31

Activity of leucocyte ALA synthase and plasma concentrations of oestradiol, testosterone and progesterone throughout menstrual cycle of normal female (E.H.).

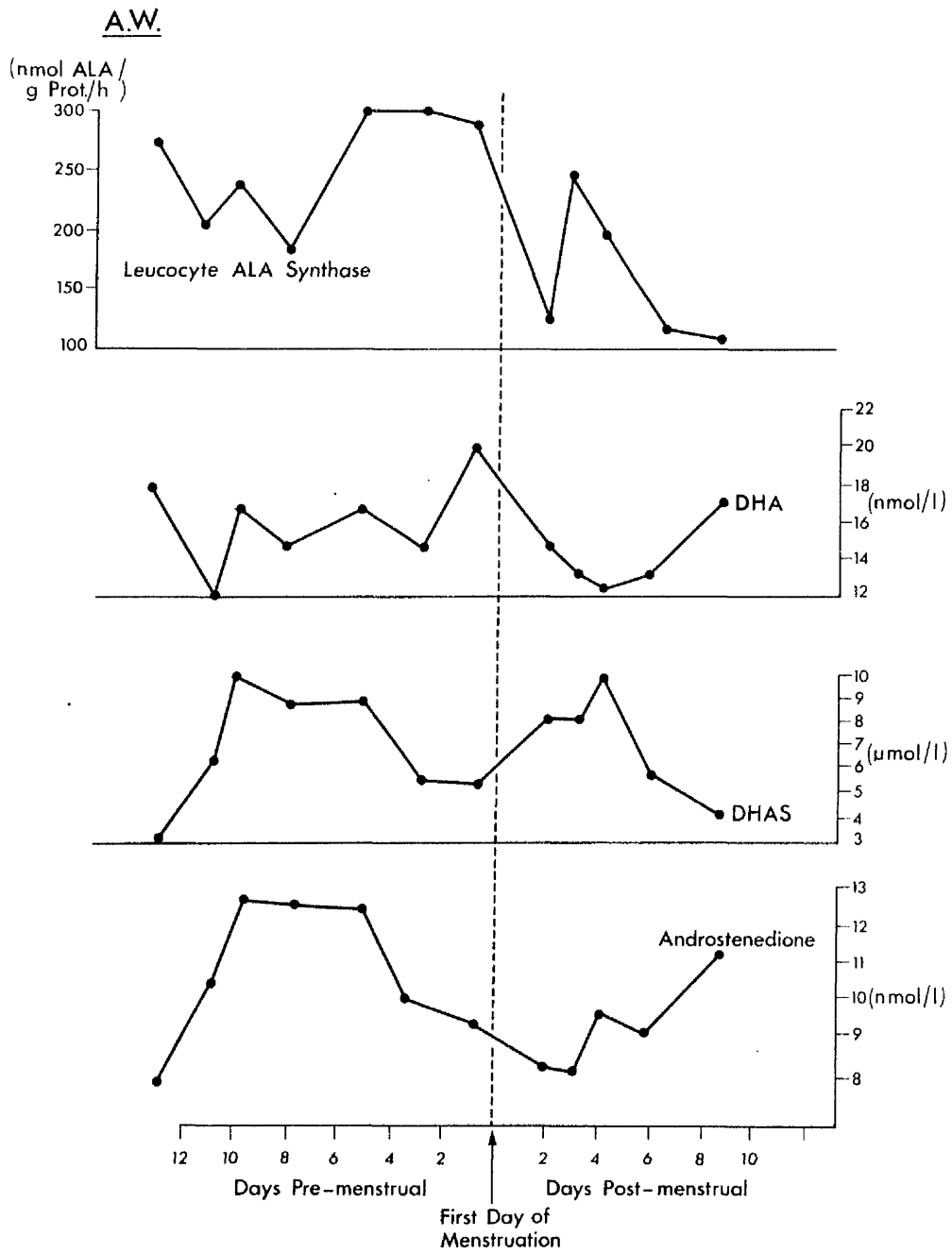


Figure 32

Activity of leucocyte ALA synthase and plasma concentrations of dehydroepiandrosterone (DHA), dehydroepiandrosterone sulphate (DHAS) and androstenedione throughout menstrual cycle in normal female (A.W.).

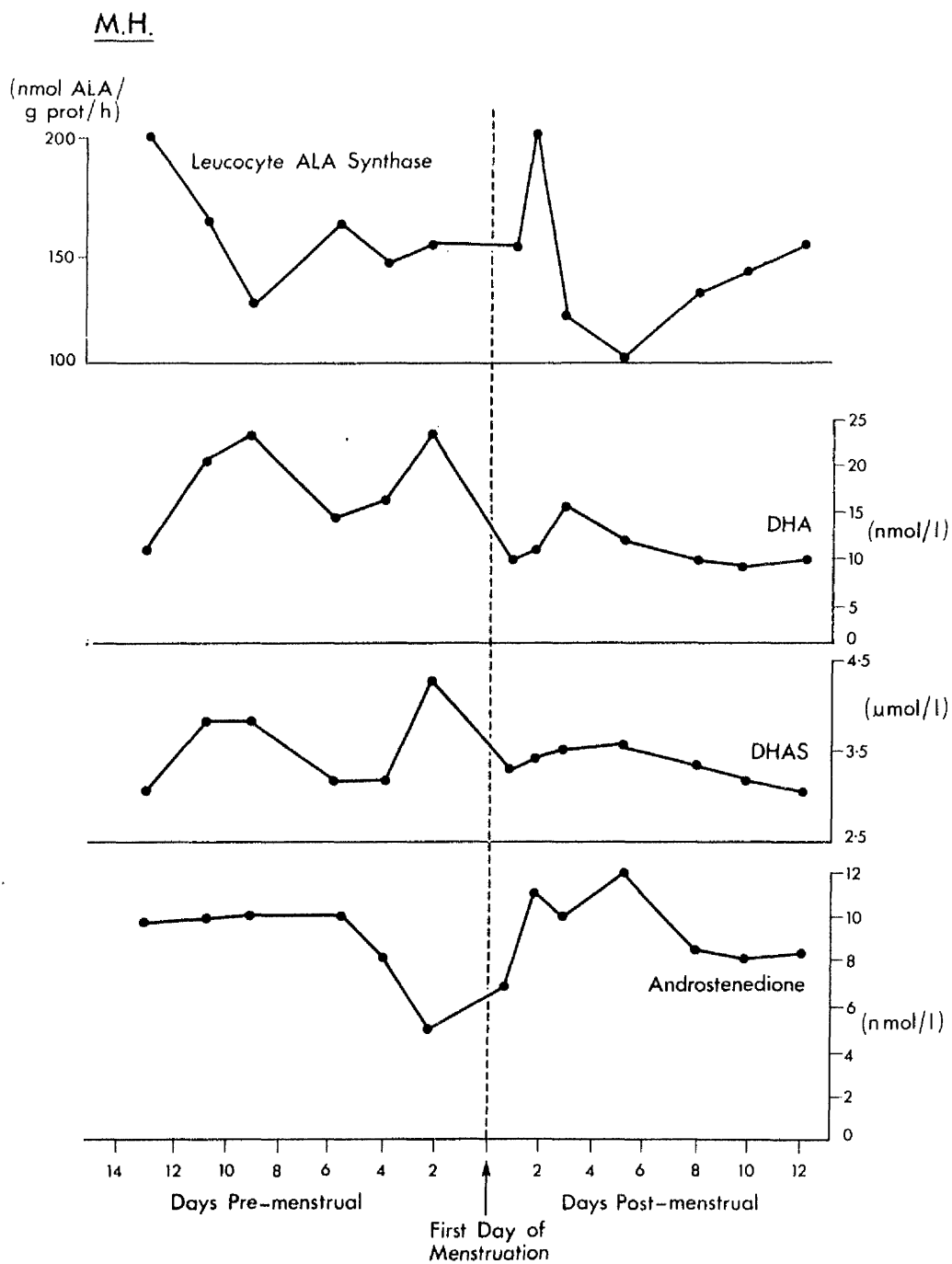


Figure 33

Activity of leucocyte ALA synthase and plasma concentrations of dehydroepiandrosterone (DHA), dehydroepiandrosterone sulphate (DHAS) and androstenedione throughout menstrual cycle of normal female (M.H.).

absolute values (Table 21). The mean erythrocyte URO synthase activity (21 nmol URO/l RBC/h) was lower than that of any of the normal subjects and below our lower limit of normal which is 25.1 nmol URO/l RBC/h. The degree of fluctuation of erythrocyte URO synthase activity was similar to that noted in the normal females and males. Leucocyte ALA synthase activity was highest and erythrocyte URO synthase lowest at the time of menstruation (Figure 34). Urinary excretion of ALA and PBG was similar to the normal males and females and showed no association with phase of menstruation or activity of the enzymes studied (Figure 34). No association was found between leucocyte ALA synthase activity and plasma concentrations of androstenedione, DHA or DHA sulphate (Figure 35). The plasma DHA concentration in this patient was above the normal female range (0.5- 11 nmol/l) on 5 of the 14 occasions measured.

7.4. DISCUSSION

The studies in the 3 patients with recurrent attacks of AIP, confirm that attacks are most common premenstrually and this suggests that the hormonal fluctuations associated with the female menstrual cycle are modifying hepatic haem biosynthesis in these patients. The fluctuation of leucocyte ALA synthase activity in the normal females and female with latent AIP, indicates that the female menstrual cycle is affecting haem biosynthesis in normal females as well as in patients with latent and manifest AIP. Further substantiative evidence that the fluctuations in enzyme activity were the result of the hormonal changes associated with the menstrual cycle might have been obtained by examining pre-pubertal or post-menopausal females but it was impossible

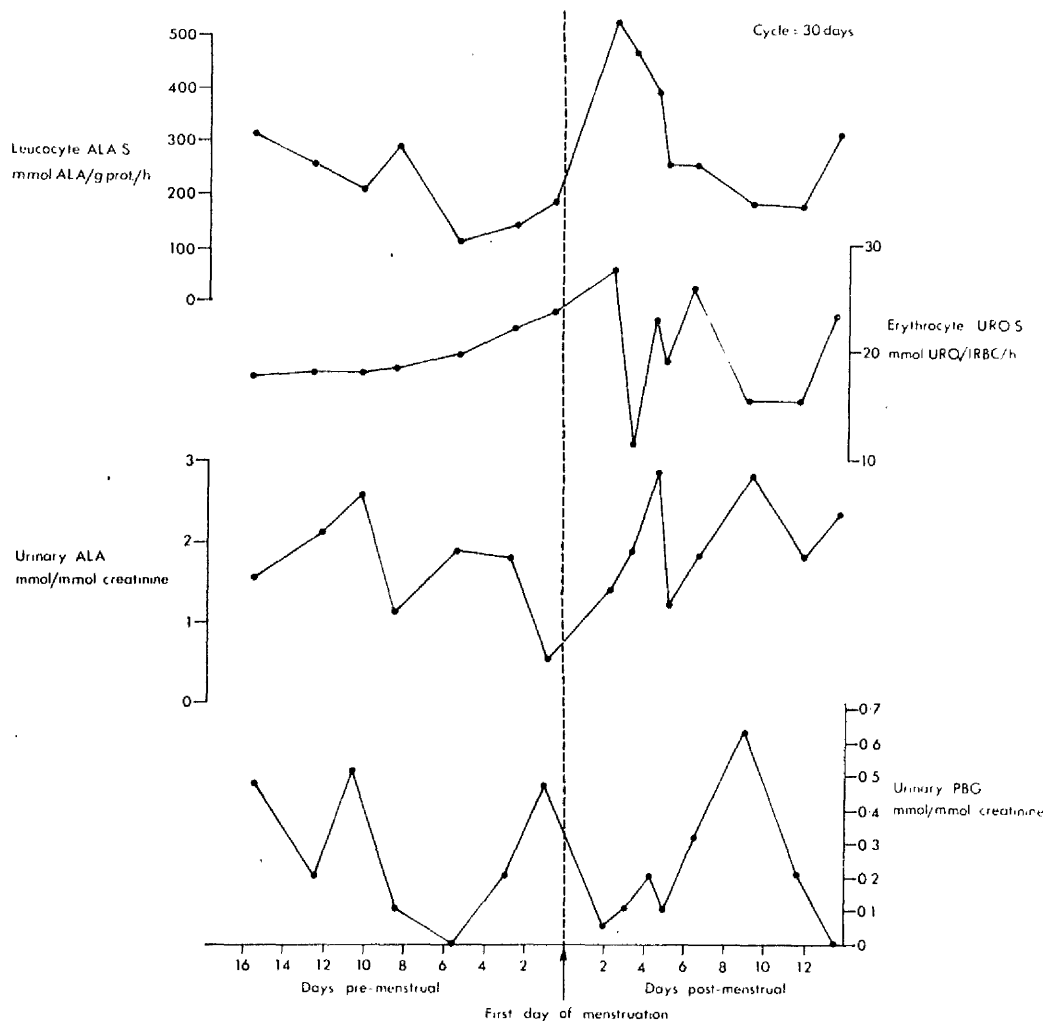


Figure 34

Activities of leucocyte ALA synthase and erythrocyte URO synthase and urinary excretion of ALA and PBG throughout menstrual cycle of 26 year old woman (H. B.) with latent acute intermittent porphyria.

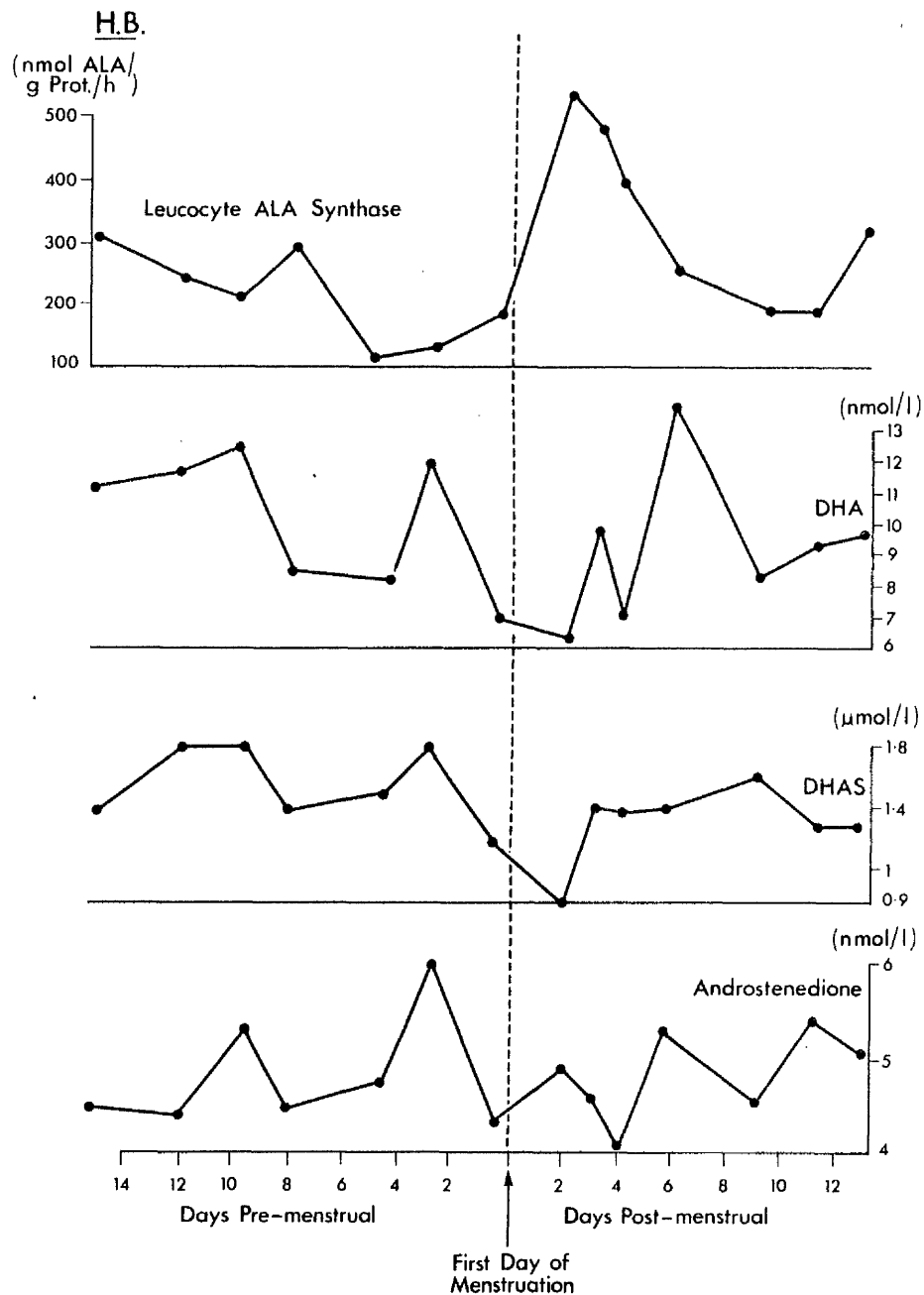


Figure 35

Activity of leucocyte ALA synthase and plasma concentrations of dehydroepiandrosterone (DHA), dehydroepiandrosterone sulphate (DHAS) and androstenedione throughout menstrual cycle of 26 year old woman (H. B.) with latent acute intermittent porphyria.

to obtain suitable volunteers. The fact that the fluctuations in ALA synthase activity were noted in leucocytes indicates that the hormonal fluctuations are modifying haem biosynthesis in haematopoietic as well as hepatic tissue.

No correlation was apparent between the fluctuations in the activity of leucocyte ALA synthase and the phase of the menstrual cycle or the plasma levels of certain hormones. A number of explanations are possible. Firstly, the hormonal fluctuations associated with the menstrual cycle are complex and ALA synthase is probably influenced by several of these hormones and also by their metabolites. Clear association between the alteration in one hormone and ALA synthase activity may be obscured by the influence of other hormones on the enzyme. Secondly, a delay in the response of the enzyme to altering concentrations of an inducing steroid would be expected which might further complicate the association. Animal studies have shown that a single injection of oestradiol results in oscillations in the activity of ALA synthase lasting for up to 90 hours (Tschudy et al, 1967).

In the patient with latent AIP, the magnitude of the fluctuation of the activity of leucocyte ALA synthase ~~through the cycle~~ was more than that seen in the normal females. This is consistent with the partial block in the haem pathway at the level of URO synthase which exists in haematological as well as hepatic tissue of such patients. Animal studies have demonstrated that induction of ALA synthase by porphyrinogenic agents is considerably enhanced by the

presence of a partial enzymatic block in the pathway (Maxwell & Meyer, 1975; Sinclair & Granick, 1978).

The observation that the human female menstrual cycle is modifying haem biosynthesis in haematological as well as hepatic tissue may be of relevance to the understanding of sex hormone-related alterations in haematopoiesis. Healthy women are known to have a reduced haemoglobin, red cell count and packed cell volume compared to their male counterparts (Wintrobe, 1967). Castration of the male rat results in a fall in the red cell count which is reversed with testosterone administration whereas castration of female rats causes a rise in the red cell count which is reversed by oestrogens (Gordon & Charipper, 1956). The stimulatory effect of androgens on haematopoiesis has been utilized with varying degrees of success in the treatment of certain forms of aplastic anaemia in man (Kennedy, 1962). Our studies indicate that sex hormones may modify haematopoiesis as a result of their effect on the rate-controlling enzyme of haem biosynthesis, ALA synthase. Haem synthesis is known to control globin synthesis (Freedman et al, 1974). Granick (1965) showed that steroids which induce ALA synthase in embryonic liver cells also accelerate haem and haemoglobin synthesis in chick blastoderm cultures. Like these steroids the hormone, erythropoietin which plays a major role in stimulating erythropoiesis, increased ALA synthase activity in rabbit bone marrow cultures (Bottomley & Smithee, 1969). Thus ALA synthase may be an important control point for the hormonal regulation of haematopoiesis.

The marked fluctuation noted in the activity of leucocyte ALA synthase in normal females and more particularly in females with latent AIP should be borne in mind when using this enzyme as a screening test for latent cases of acute porphyria.

CHAPTER 8

NUTRITION AND HAEM

BIOSYNTHESIS

8. NUTRITION AND HAEM BIOSYNTHESIS

8.1. INTRODUCTION

It has been recognised for some time that dieting or fasting can precipitate attacks of acute porphyria (Knudsen et al, 1967). In patients with AIP a reduction in carbohydrate or total caloric intake results in increased urinary excretion of porphyrin precursors and sometimes clinical attack, whereas carbohydrate and caloric supplementation results in reduced excretion of porphyrin precursors (Felsher & Redeker, 1967; Verspohl et al, 1979). There is also evidence that the administration of carbohydrate, either orally or as intravenous laevulose, results in biochemical and clinical improvement in patients in a acute attack of porphyria (Brodie et al, 1977).

Studies in rats have demonstrated that carbohydrate or protein restriction increases hepatic ALA synthase activity whereas carbohydrate or protein supplementation represses hepatic ALA synthase activity (Tschudy et al, 1964). Allylisopropylacetamide (A.I.A.) or drug-mediated induction of ALA synthase is similarly affected by carbohydrate restriction and supplementation (Rose et al, 1961). The mechanism of this "glucose effect", which also affects other enzymes, is unknown (Goldberg, 1974). Alteration of caloric intake results in hormonal changes which may affect ALA synthase activity (Kappas & Granick, 1968). In chick embryo liver, A.I.A. - induced porphyrin biosynthesis is maximal at low insulin to glucagon molar ratios (Fischer et al, 1978). The "glucose effect" on ALA synthase may also be due to changes in concentrations of free haem as starvation augments and glucose-loading diminishes haem catabolism as measured by carbon monoxide evolution

(Lundh & Ugander, 1976). Several other mechanisms have been postulated as discussed by Tschudy (1978). At present, however, both the mechanism and the physiological function of the "glucose effect" remain unclear.

In addition to the effect of the total caloric intake, certain minor dietary constituents may affect haem biosynthesis. Pyridoxal phosphate is the cofactor for ALA synthase and the only vitamin derivative known to serve as a cofactor for any of the enzymes of the pathway. Both normal rabbits and rabbits with chemically-induced porphyria excrete less ALA and PBG when rendered pyridoxine deficient with deoxypyridoxine (Druyan & Haegar-Aronsen, 1965). In the rat, however, dietary pyridoxine deficiency does not impair hepatic ALA synthase induction (Chabner et al, 1970). Oral iron in the form of ferric citrate has a pronounced synergistic effect on the induction of hepatic ALA synthase in experimental animals (Stein et al, 1969). This may be due to iron depleting the "free haem pool" by increasing haem degradation (De Matteis & Sparks, 1973), or by decreasing haem synthesis by inhibiting uroporphyrinogen decarboxylase activity (Kushner et al, 1975). Though iron excess plays an important role in the aetiology of cutaneous hepatic porphyria, there is no evidence that either iron excess or deficiency have any effect on the acute hepatic porphyrias. Folic acid and other pteridine derivatives exert a protective and enhancing effect on the activity of URO synthase (Piper et al, 1979; Piper et al, 1977). There is some evidence that oral folic acid administration results in clinical and biochemical improvement in patients in attack of AIP (Wider de Xifra et al, 1979).

We have assessed the value of the intravenous administration of laevulose to patients in attack of AIP. In addition we have studied in non-porphyric obese subjects, the effect of fasting on the urinary excretion of ALA and PBG and on the activity of ALA synthase in peripheral leucocytes.

8.2. LAEVULOSE THERAPY IN ACUTE PORPHYRIA

8.2.1. Patients and Methods

We have assessed the effect of intravenous laevulose infusions on the duration and severity of attacks in 3 female patients (J. Mc, J. C. and P. W.) with AIP. The study was retrospective and covered a 4 year period. During the first 2 years, each patient received intravenously 20% laevulose (2 litres/day) during the first 3 days of each admission in attack. After 2 years, this practice was discontinued because of its damaging effects on the patients veins. In the second 2 year period, we attempted to maintain the patients' caloric intake using the glucose polymer Caloreen (Roussel) which the patients either drank or received via a fine bore nasogastric tube. Inevitably, the daily carbohydrate and total caloric intake was considerably less over the second 2 year period.

8.2.2. Results

The frequency of admissions was similar over the "laevulose" and the "no-laevulose" periods (Figure 36). In each patient the mean duration of admission in attack was considerably longer during the "no-laevulose" period (Figure 36). Peripheral neuropathy complicated 3 attacks in 2 patients during the "no-

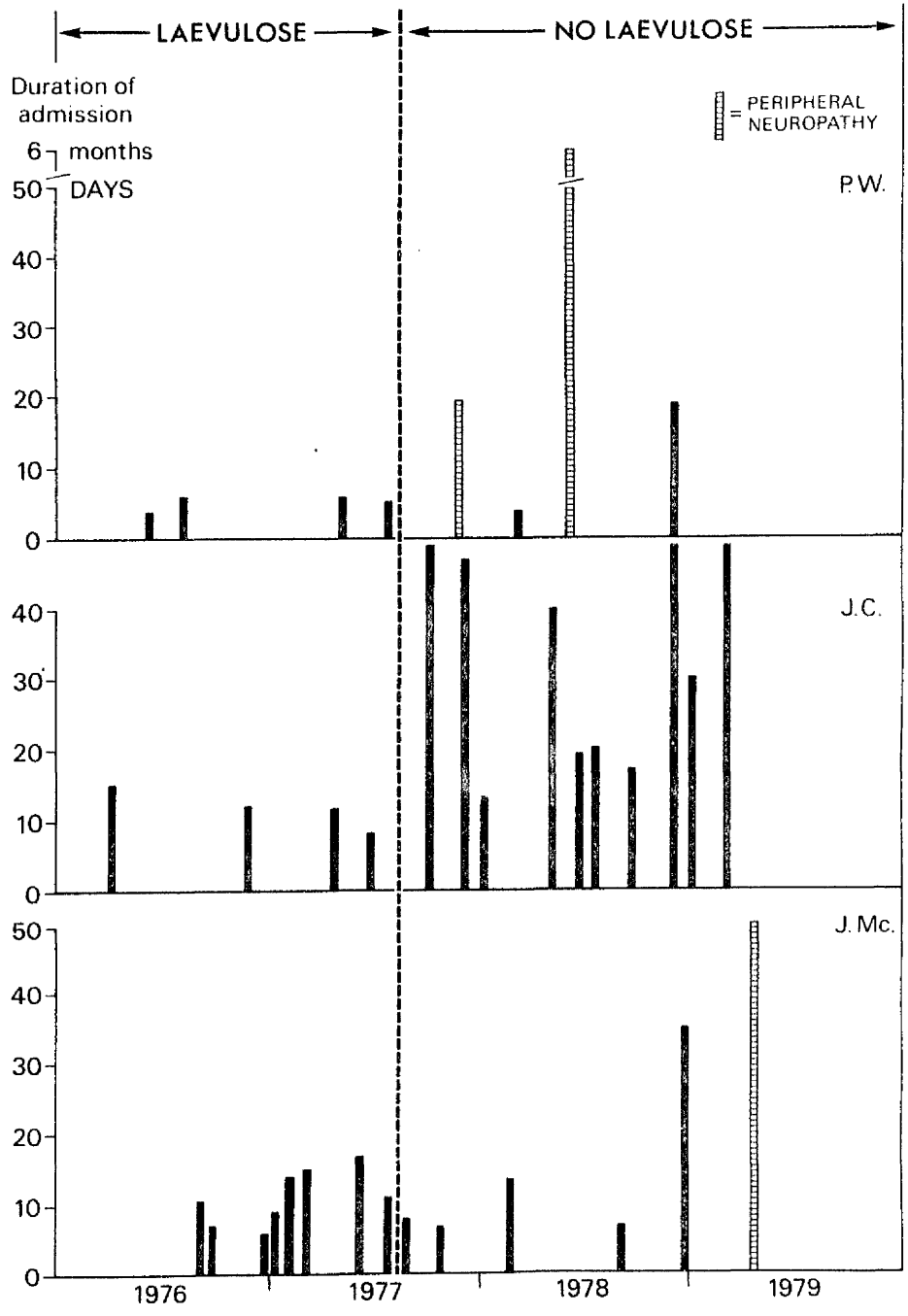


Figure 36

Effect of laevulose infusions on the duration and severity of attacks in 3 female patients with acute intermittent porphyria. Each bar represents one admission in attack.

laevulose" 2 year period whereas neurological complications did not occur in any of the attacks treated with laevulose.

8.2.3. Discussion

Though this study is retrospective and subject to many varying factors, it supports previous reports that the intravenous infusion of laevulose is beneficial in the management of patients in attack of AIP. The reduced effectiveness of enterally administered Caloreen can be explained by the smaller doses received. The nausea and vomiting, which is an almost invariable feature of an attack of AIP, considerably compromises any attempts at enteral feeding. This study also highlights the fact that repeated intravenous infusions of 20% laevulose, whether via peripheral or central line, result in considerable venous damage and so complicates the management of future attacks. In patients with frequent attacks the early construction of an arterio-venous fistula should be considered in order to facilitate venous access.

8.3. STARVATION AND HAEM BIOSYNTHESIS

8.3.1. Patients and Methods

Five female and one male patient admitted to hospital for weight reduction were studied. Their mean age was 35 years (range 19-54 years) and their mean body weight on admission was 106kg (range 89-122kg). All were suffering from simple obesity but were otherwise healthy. None was

taking any form of drug therapy.

During the first 2 days of admission they were maintained on the diet which they had been taking at home, which in each case was 800 kcalories. On the morning of the third day of admission all food was withdrawn and the patients received only water and physiological doses of vitamins B₁, B₅, B₆, C and D. The fasting was continued for a mean of 21 days (range 15-35 days). Within 48 h of commencing the "water diet", all patients developed ketonuria which gave a strongly-positive reaction with the ketostix test (Ames). The ketonuria persisted throughout the fasting period in each patient confirming adherence to the diet. The mean weight loss during fasting was 11.8kg (range 20.4 - 6.8 kg). Routine biochemical monitoring showed the well recognised metabolic changes associated with fasting, namely increased plasma concentrations of cholesterol, creatinine and urate and reduced plasma concentration of urea. The plasma glucose concentration and routine haematology remained normal throughout the period of study.

Blood was withdrawn at 9.00a.m. for measurement of leucocyte ALA synthase activity on the day prior to commencement of fasting and on each of the first 5 days of fasting and again on the tenth and fifteenth days of fasting. On each of these days a 24 h urine collection was obtained for measurement of ALA and PBG excretion.

8.3.2. Results

Prior to the commencement of fasting the activity of leucocyte ALA synthase and the urinary excretion of ALA and PBG were within our normal range in all subjects. There was no significant alteration in either the enzyme activity or the porphyrin precursor excretion with fasting (Figures 37, 38).

8.3.3. Discussion

Our study indicates that in non-porphyric subjects, fasting does not alter the activity of ALA synthase in leucocytes. This is in contrast to the increase in ALA synthase activity which occurs in hepatic tissue of healthy laboratory animals. The differing enzymatic responses to fasting in leucocytes and liver may be a reflection of the different roles these two tissues play in the regulation of carbohydrate metabolism.

This study also demonstrates that fasting does not result in overproduction of ALA or PBG in normal subjects. Hepatic overproduction of these porphyrin precursors requires both increased activity of the initial enzyme ALA synthase and a partial block later in the pathway. Induction of hepatic ALA synthase activity by fasting, will only result in overproduction of porphyrin precursors in the presence of an enzymatic block later in the pathway such as exists in patients with any of the acute hepatic porphyrias.

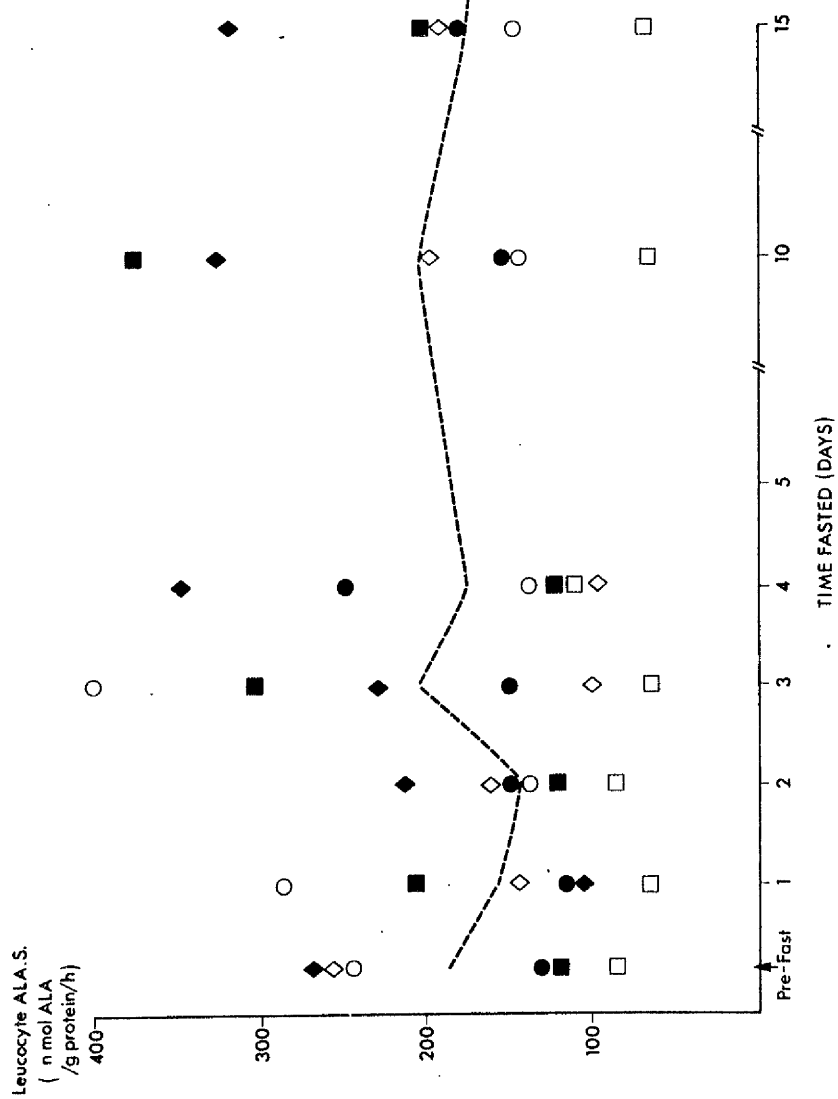
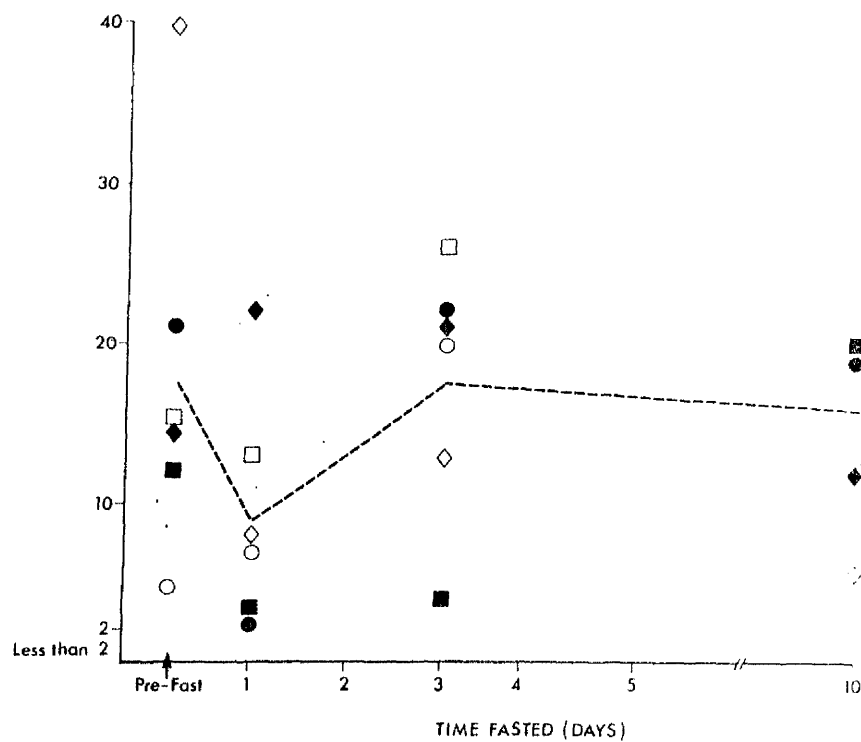


Figure 37

Effect of fasting on leucocyte ALA synthase activity in non-porphyrinic obese subjects. Each subject is represented by a different symbol. The broken line joins the mean values.

Urinary
ALA
($\mu\text{mol}/24\text{h}$)



Urinary
PBG
($\mu\text{mol}/24\text{h}$)

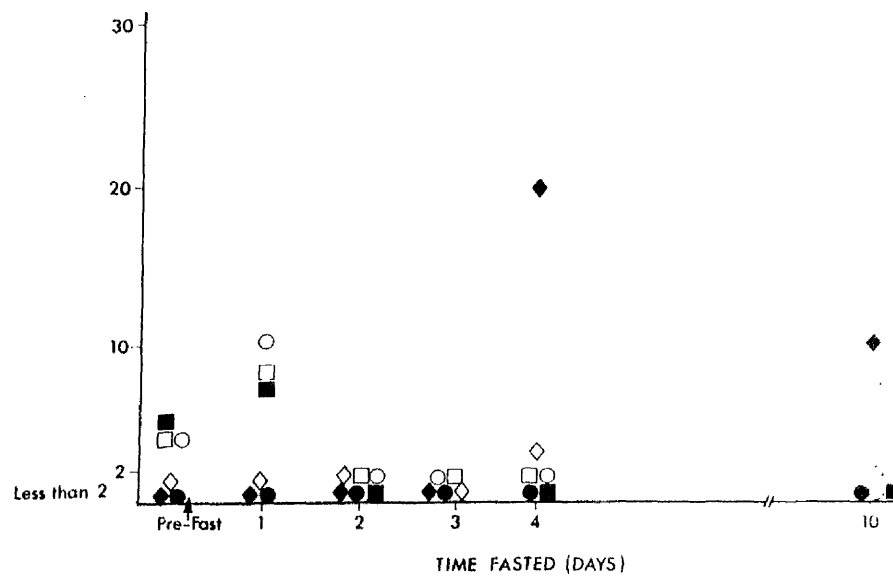


Figure 38

Effect of fasting on urinary excretion of ALA and PBG in 6 obese non-porphyric subjects. Each subject represented by different symbol.

CHAPTER 9

HAEMATIN THERAPY FOR ACUTE
HEPATIC PORPHYRIA

9. HAEMATIN THERAPY FOR ACUTE HEPATIC PORPHYRIA

9.1. INTRODUCTION

The administration of haematin or haemin will suppress the activity of chemically-induced ALA synthase in laboratory animals and cell cultures (Granick, 1966). This is presumed to be the result of supplementation of the intra-mitochondrial free haem pool. The exogenous haematin is incorporated into functionally active hepatic cytochrome P450 and tryptophan pyrrolase (Correia et al, 1979) and increased mono-oxygenase activity has been noted following haematin administration to hepatic and extrahepatic (including brain) tissue preparations (Jonen et al, 1978; Omiecinski et al, 1978; Omiecinski et al, 1980). Exogenous haematin induces the activity of haem oxygenase and is itself catabolized by this enzyme in the same way as endogenously synthesized haem (Tenhunen et al, 1970; Landaw et al, 1970). The induction of haem oxygenase may explain the increased rate of destruction and lowered hepatic concentrations of cytochrome P450 and catalase which have been noted following administration of large doses of haem (Marver et al, 1968; Hamato & Higashi, 1979; Sassa & Granick, 1970; Bock et al, 1973).

The demonstration that haematin was able to suppress the induction of ALA synthase raised the possibility that it might be of value in treating attacks of acute hepatic porphyria. Information regarding its safety in animal studies is required prior to embarking on clinical trials.

9.2. HAEMATIN TOXICITY IN ANIMALS

As far back as 1911, W.H. Brown, in his studies of malaria, noted that the intravenous injection of large doses of haematin to dogs produced a dose related pyrexial reaction (Brown, 1911, 1912, 1913). This was probably related to impure preparations and it has been found more recently that pyrogenic reactions can be eliminated by crystallisation and re-crystallisation of the haemin (Dhar et al, 1975). Brown (1913) also noted that the administration of haem in a dosage of 20mg/kg was consistently followed by albuminuria and the appearance of granular and cellular casts in the urine. He found similar, though less severe, changes using a dose of 10mg/kg. Anderson (1942) noted that the intravenous administration of haematin to dogs sufficient to cause an initial plasma concentration between 10 and 72mg/100ml resulted in a generalised vascular reaction with vasodilatation, haemorrhage and thrombosis. Haemorrhage in the subarachnoid space and central nervous system caused convulsions and renal, endocardial and myocardial haemorrhages also occurred. Very rapid intravenous injection frequently resulted in a marked toxic reaction and death. Corcoran and Page (1945) noted a transitory renal hyperaemia in dogs given intravenous haematin in a dosage of 14.5mg/kg at a rate of 0.28mg/kg/min. A larger dose of 23.7mg/kg at a rate of 0.49mg/kg/min caused intense efferent arteriolar vasoconstriction resulting in uraemia and proteinuria. At a higher level still of 32.6mg/kg administered at a rate of 0.835mg/kg/min there were generalised petechial haemorrhages with ischaemia of glomeruli and tubular degeneration. Lips (1978) examined the toxicity of intravenous haematin in rats. The L.D. 50 was 0.43mg/kg with 95% confidence limits of 0.54 and

0.35 mg/kg. At toxic levels the rats showed hypotension, widespread haemorrhaging and renal failure. Increasing the intravenous injection time from 1 min to 2 min in one rat appeared to reduce the toxic effects.

9.3. FATE OF HAEMATIN ADMINISTERED INTRAVENOUSLY TO HEALTHY ANIMALS AND HUMANS

Intravenously administered haematin is handled in the same way as haem released by intravascular haemolysis. It is rapidly bound to the haem-binding B-globulin, haemopexin (Hanstein & Muller-Eberhard, 1968; Heide et al, 1964). When this becomes saturated, excess haematin is bound to albumin forming methaemalbumin (Dhar et al, 1978). In healthy human subjects the circulating haemopexin concentration averages 77 mg/100 ml which is sufficient to bind 19 mg haematin. Above a plasma haematin concentration of 0.6 mg/100ml haemopexin will be saturated and the excess haematin binds to albumin in a 2:1 molar ratio (Rosenfeld & Surgenor, 1950). An average healthy person has 125 g of circulating albumin which should be sufficient to bind 2.6 g haematin. Theoretically, therefore, a haematin dose of up to 2.6 g (serum concentration of 100 mg/100 ml) would be protein bound. The rate of administration, however, may be important as rapid injection without adequate time for equilibration could result in circulating free haematin.

The haem-haemopexin complex is rapidly taken-up by the liver in both experimental animals (Snyder & Schmid, 1965; Sears & Huser, 1966; Muller-Eberhard et al, 1969; Muller-Eberhard et al, 1970; Muller-Eberhard & Liem, 1974), and man (Sears, 1969) and the haemopexin then

released back into the circulation (Smith & Morgan, 1978). Albumin serves only as a temporary storage site from which haematin is transferred to haemopexin as the latter becomes available. Injected haematin is taken-up almost exclusively by the liver (Muller-Eberhard & Liem, 1974). The I.V. administration of ^{59}Fe -haematin in man results in rapid accumulation of radioactivity over the liver with only slight increases occurring over the spleen and sacrum (Sears, 1970; Liem et al, 1975).

9.4. REVIEW OF PREVIOUSLY PUBLISHED EXPERIENCE OF HAEMATIN IN ACUTE HEPATIC PORPHYRIA

9.4.1. Patients and Methods

A review of the world literature has allowed us to collate clinical and biochemical details of haematin therapy of 45 attacks of acute hepatic porphyria in 32 patients over the past 10 years (Bonkowsky et al, 1971; Watson et al, 1973; Dhar et al, 1975; Peterson et al, 1976; Watson et al, 1978; Brezis et al, 1979; Lamon et al, 1979). Twenty six of the patients had AIP and six, porphyria variegata. The mean age was 35 years (range 18-52 years) and twenty four were females and eight, males. Nearly all the patients had received a trial of high dose glucose orally or intravenously prior to the haematin. The haematin was administered as intravenous infusions over 13-30 min and the infusions were given every 12 or 24 h. The total daily dose varied from 1.8 - 8.0 mg/kg body weight and the courses lasted from 1-13 days. High glucose intake was maintained during most

courses of haematin therapy.

9.4.2. Biochemical Response

In all patients studied the haematin reduced the plasma concentrations of ALA and PBG by 65-100% of their pre-haematin values. The reduction in ALA has been slightly more pronounced than PBG. In patients in whom the ALA and PBG levels were closely monitored, they have fallen within 24 h of the first infusion, reached a trough usually on the final day of the infusions and then risen again on discontinuing the haematin to plateau, several days later, at a level of 50-100% of the pre-haematin level. The haematin has resulted in a similar reduction in the urinary excretion of uroporphyrin.

Using the data presented by Lamon et al (1979), it is possible to compare the significance of the reduction in urinary excretion of ALA and PBG with haematin courses of more than, and less than, 25mg/kg body weight. With the nine high dose courses, the reduction in ALA was significant at $p < 0.002$ in eight and reduction in PBG at $p < 0.002$ in seven. With the ten lower dose courses, the reduction in ALA was significant at $p < 0.002$ in only four and the reduction in PBG significant at $p < 0.002$ in only five.

In the series by Watson et al (1978) the mean reduction in serum ALA and PBG concentrations in courses of more than 1,000mg haematin was 90% compared to 77% with smaller courses. The percentage reduction in ALA and PBG following haematin appears to be independent of the pre-haematin levels of these porphyrin precursors. In the series by Watson et al (1978) the haematin lowered the plasma ALA and PBG concentrations by a similar

percentage (83%), irrespective of whether the pre-haematin concentrations were more than or less than 165ug/100ml.

The clearance of exogenous haematin administered to patients in attack of acute porphyria has been studied by Lamon et al (1979) and Petryka et al (1976). Both noted that the curve of plasma haematin disappearance could be resolved into two exponential components. The half-life of the first component was between 4.5 h and 15.5 h and for the second component between 20 h and 53.5 h. The first component is thought to represent the rate of hepatic uptake of haem-haemopexin complexes. Once all available haemopexin is taken up by the liver, the remaining haem bound to albumin must await the release of haemopexin from the liver in order to bind it and allow further hepatic uptake. The second component, therefore, reflects the rate of release of haemopexin from the liver. The administration of haematin results in a precipitous fall in the serum haemopexin concentration which returns to normal within 7-10 days of completion of haematin administration.

9.4.3. Clinical Response

Critical analysis of the published reports shows that the clinical response has not been as consistent as the biochemical response. Twenty four (54 per cent) courses have been associated with sustained clinical improvement, ten (22 per cent) with temporary improvement then relapsing within 2-14 days (3 fatal) and in eleven (24 per cent) there was no apparent benefit with three of these patients dying in attack. The clinical response to the haematin therapy

in these patients must be viewed in association with their clinical severity. Twenty six of these attacks in 17 patients can be classified as Class A (Watson et al, 1978) as there was a combination of gastrointestinal symptoms, mental disturbance, hypertension and tachycardia but no significant peripheral neuropathy. In these attacks, the haematin resulted in sustained improvement in fifteen (58 per cent), temporary improvement in seven (27 per cent) and no change in four (15 per cent) (Table 22). Ten attacks in 8 patients were of Class B severity, as there was, in addition, severe peripheral neuropathy. The haematin therapy was associated with improvement of the neuropathy in six (60 per cent) but no change in four (40 per cent) (Table 23). Nine attacks in 7 patients were of Class C severity, there being severe generalised neuropathy resulting in respiratory embarrassment. In three (33 per cent) of these attacks the haematin therapy was associated with maintained improvement of the neuropathy, in three temporary improvement initially occurred but the patients later died and in three there was no improvement and this was followed by death (Table 24). In most cases where improvement of neuropathy occurred it commenced about the final day of the haematin infusion and continued slowly for several months, however, in a few cases dramatically rapid improvement has been reported.

No serious side-effects have been observed following haematin administration to patients in acute attack of porphyria. In a small number of cases the haematin has produced phlebitis around the injection site with associated mild pyrexia. One 40 year old male with AIP in remission developed transitory acute renal failure following the rapid bolus intravenous injection

<u>Age</u>	<u>Sex</u>	<u>Type of porphyria</u>	<u>Clinical response</u>	<u>Reference</u>
{31	M	AIP	Improved	Watson et al, 1973
32	"	"	Improved	Dhar et al, 1975
{35	"	"	Improved	Watson et al, 1978
52	F	AIP	Improved	Dhar et al, 1975
50	F	AIP	No change	" "
42	M	AIP	Temporary improvement	Watson et al, 1978
35	F	AIP	Improved	" "
{34	F	PV	Temporary improvement	" "
{34	"	"	Improved	" "
31	F	PV	Improved	" "
{27	F	AIP	Improved	" "
{27	"	"	Improved	" "
30	M	AIP	Temporary improvement	" "
33	M	PV	No change	Lamon et al, 1979
49	F	AIP	No change	" "
25	F	AIP	Temporary improvement	" "
31	F	AIP	Temporary improvement	" "
{21	F	AIP	Temporary improvement	" "
{21	"	"	Improved	" "
43	M	AIP	No change	" "
33	F	AIP	Temporary improvement	" "
{31	F	AIP	Improved	" "
31	"	"	Improved	" "
31	"	"	Improved	" "
31	"	"	Improved	" "
{31	"	"	Improved	" "

Brackets on left side of table group attacks in individual patients

Table 22

Clinical response to haematin therapy of 26 Class A attacks in 17 patients.

<u>Age</u>	<u>Sex</u>	<u>Type of porphyria</u>	<u>Abdominal pain</u>	<u>Neuropathy</u>	<u>Reference</u>
36	F	PV	no data	improved	Watson et al, 1978
36	"	"	no data	improved	" "
45	F	AIP	no data	improved	" "
22	F	AIP	no data	no change	" "
19	F	AIP	improved	improved	" "
31	F	AIP	improved	no change	Lamon et al, 1979
21	F	AIP	improved	improved	" "
26	F	AIP	temporary improvement	no change	" "
24	F	AIP	improved	improved	" "
24	"	"	no change	no change	" "

Table 23

Clinical response to haematin therapy of 10 Class B attacks in 8 patients.

<u>Age</u>	<u>Sex</u>	<u>Type of porphyria</u>	<u>Neuropathy</u>	<u>Reference</u>
33	F	AIP	no improvement - died	Bonkowsky et al, 1971
56	F	PV	Improved	Dhar et al, 1975
21	F	AIP	Improved	Peterson et al, 1976
19	M	AIP	early improvement-died	Watson et al, 1978
42	F	PV	early improvement-died	" "
18	M	AIP	Improved	Brezis et al, 1979
49	M	AIP	early improvement	Lamon et al, 1979
49	"	"	no improvement died	" "
49	"	"	no improvement	" "

Table 24

Clinical response to haematin therapy of 9 Class C attacks in 7 patients.

of 1,000mg haematin (Dhar et al, 1978). Following the injection the patient developed oliguria passing only 200ml over the subsequent 24 h. The urine was initially almost black in colour and haematin was readily demonstrable spectroscopically. The urine also contained protein (13.8g/l), red blood cells (25/high power field), white blood cells (5/high power field) and granular casts. The patient was treated with intravenous diuretics and alkalinization of the urine and the urinary output and blood urea and creatinine concentrations returned to normal within 4 days. The transitory renal failure was thought to be the result of circulating free haematin due to the sudden injection of such a large quantity.

9.5. PERSONAL EXPERIENCE OF HAEMATIN THERAPY IN ACUTE HEPATIC PORPHYRIA

9.5.1. Patients and Methods

Over the past 3 years we have administered 14 courses of haematin to 8 patients with acute hepatic porphyria in relapse. Six of the patients were females and 2 males. The mean age was 27 years (range 19-56 years). All had AIP except one female who had porphyria variegata (case 5). They all had both clinical and biochemical evidence of attack at the time of haematin administration and had failed to respond adequately to carbohydrate administration. All but two of the patients were treated in our own wards. Cases numbers 5 and 6 were both unfit for transfer and I personally delivered the haematin and administered it in their local hospitals in Birmingham and Liverpool.

The haematin solution was prepared as described in Chapter 3 and administered within 7 days of preparation. It was stored at 4°C and removed from the

refridgerator 30 min prior to administration. The appropriate volume was drawn up into a plastic syringe through a 5 μ m filter aspiration needle (Monoject 303, Sherwood Medical Industries Inc., St. Louis, Missouri, U.S.A.). The haematin was then injected via a 0.22 μ m filter (Millipore, Millex) into the rubber bung of a slowly running intravenous infusion of physiological saline. The dose of each injection was 4mg/kg body weight and patients received injections at either 12-hourly or 24-hourly intervals. The duration of the courses of haematin varied from 3-5 days.

The response to the haematin therapy was assessed from both a biochemical and clinical point of view. The 24 h urinary excretion of ALA, PBG, uroporphyrin and coproporphyrin was monitored throughout each course, except for the sixth course administered to case number one. The activity of leucocyte ALA synthase was monitored daily throughout 9 courses to 6 patients. The activity of erythrocyte URO synthase was monitored throughout 5 courses and the activities of erythrocyte ALA dehydratase, erythrocyte uroporphyrinogen decarboxylase, leucocyte coproporphyrinogen oxidase and leucocyte ferrochelatase throughout 3 courses. Routine haematology and biochemistry was monitored daily in all patients. During 4 courses to 4 patients the plasma concentrations of dehydroepiandrosterone were also monitored. In an attempt to obtain some objective evidence of clinical response the analgesic requirement was monitored in each conscious patient throughout the period of study. The patients received their analgesia "as required for pain" and, therefore, the daily analgesic consumption could be used as a means of quantitating their pain and clinical condition. This has previously been found to be the most effective means of monitoring

clinical condition in patients with porphyria.

9.5.2. Haematin Therapy and Urinary Porphyrin and Precursor Excretion

The urinary ALA was increased in all cases immediately prior to haematin therapy, the mean being 220 $\mu\text{mol}/24\text{h}$ (range 51-374). In all but one of the courses there was an associated reduction in the urinary ALA excretion. The mean reduction, comparing the 3 days pre-haematin with days of therapy, was 43 per cent (Table 25). The urinary PBG excretion was increased in all but one patient prior to haematin administration, the mean being 274 $\mu\text{mol}/24\text{h}$ (range 8-471). All but 2 of the courses were associated with a reduction in urinary PBG excretion, with a mean reduction of 35 per cent. Urinary uroporphyrin was increased in all subjects prior to the haematin (mean 2,700 $\text{nmol}/24\text{h}$) and all but two courses were associated with reduced excretion (mean reduction = 36 per cent). The urinary coproporphyrin excretion was slightly increased prior to five of the courses and this showed little change during haematin therapy. After completion of the haematin therapy the urinary excretion of ALA, PBG and uroporphyrin rose over the following three days plateauing at a level of 50-100 per cent of the pre-haematin values (Table 26). No patient demonstrated any "rebound" phenomenon. There was no significant difference in the depression of urinary porphyrin or precursor excretion in relation to the two dose schedules employed (Table 27).

9.5.3. Haematin Therapy and activity of enzymes of haem biosynthesis in peripheral blood cells

Prior to haematin therapy leucocyte ALA synthase activity was increased in each case studied with a mean activity of 631 $\text{nmol}/\text{ALA}/\text{g protein}/\text{h}$

	Haematin Dose (mg/kg/24h)	Duration of therapy (days)	Percentage change in urinary excretion of			
			ALA	PBG	URO	COPRO
Case 1						
course i	4	3	-24	-53	-23	+24
course ii	8	3	+13	+ 5	-20	+50
course iii	4	3	-51	-44	-19	+39
course iv	4	5	-50	-45	-68	+19
course v	4	3	-33	-22	+20	+116
Case 2	4	4	-12	- 7	-50	+37
Case 3						
course i	8	3	-68	-70	- 9	-32
course ii	4	3	-54	-20	-56	+19
Case 4	4	3	-76	-70	-81	+71
Case 5	8	3	- 4	+12	-36	-40
Case 6	8	3	-84	-33	-46	-42
Case 7	8	3	-46	-66	0	-31
Case 8	4	3	-64	-41	-75	-22
MEAN			-43	-35	-36	+16

Table 25

Percentage change in urinary excretion of ALA, PBG, uroporphyrin (URO) and coproporphyrin (COPRO) following commencement of haematin therapy in patients in attack of acute porphyria. Values during haematin therapy have been compared with values obtained over 3 days immediately prior to therapy.

	Mean Urinary excretion of			
	ALA (umol/24h)	PBG (umol/24h)	URO (nmol/24h)	COPRO (nmol/24h)
3 days pre-haematin	220	274	2,700	640
during haematin	125	178	1,727	600
3 days post-haematin	145	214	2,010	450

Table 26

Mean urinary excretion of porphyrins and precursors before, during and following haematin therapy.

	No. of courses	Mean percentage change in urinary excretion during haematin treatment			
		ALA	PBG	URO	COPRO
Haematin 4mg/kg/24h	9	-45	-38	-46	+51
Haematin 8mg/kg/24h	5	-38	-30	-20	-19

Table 27

Effect of Haematin dosage on reduction of urinary porphyrin and precursor excretion.

(range 310-1,500). Seven of the courses repressed the activity of ALA synthase, in four of these to within the normal range (Fig. 39). The enzyme assays were performed 20-24 h after each haematin infusion and in each of these 7 courses the enzyme activity was reduced within this period of time. On one occasion leucocyte ALA synthase activity was assessed 1 h after the haematin infusion and was found to be only 45 nmol ALA/g protein/h, which is below our lower limit for normal subjects. The activity of ALA synthase rose again within 48 h of completion of each of these 7 courses to levels similar or slightly lower than the pre-treatment values. There was no evidence of rebound phenomenon. Two courses of haematin were complicated by severe phlebitis and in these leucocyte ALA synthase activity was not repressed (Fig. 40) though the urinary excretion of ALA, PBG and uroporphyrin was reduced.

There was no significant change of the activities in peripheral blood cells of ALA dehydratase, URO synthase, uroporphyrinogen decarboxylase, coproporphyrinogen oxidase or ferrochelataase following haematin administration (Table 28).

9.5.4. Haematin Therapy and plasma Dehydroepiandrosterone levels.

The plasma concentrations of DHA prior to haematin administration were above our upper range of normal (i.e. 11 nmol/l) in three of the 4 patients studied (Fig. 41). In each patient haematin administration resulted in a fall in the DHA level. On completion of haematin therapy the plasma DHA concentration rose again in 2 patients within 48 h but remained depressed

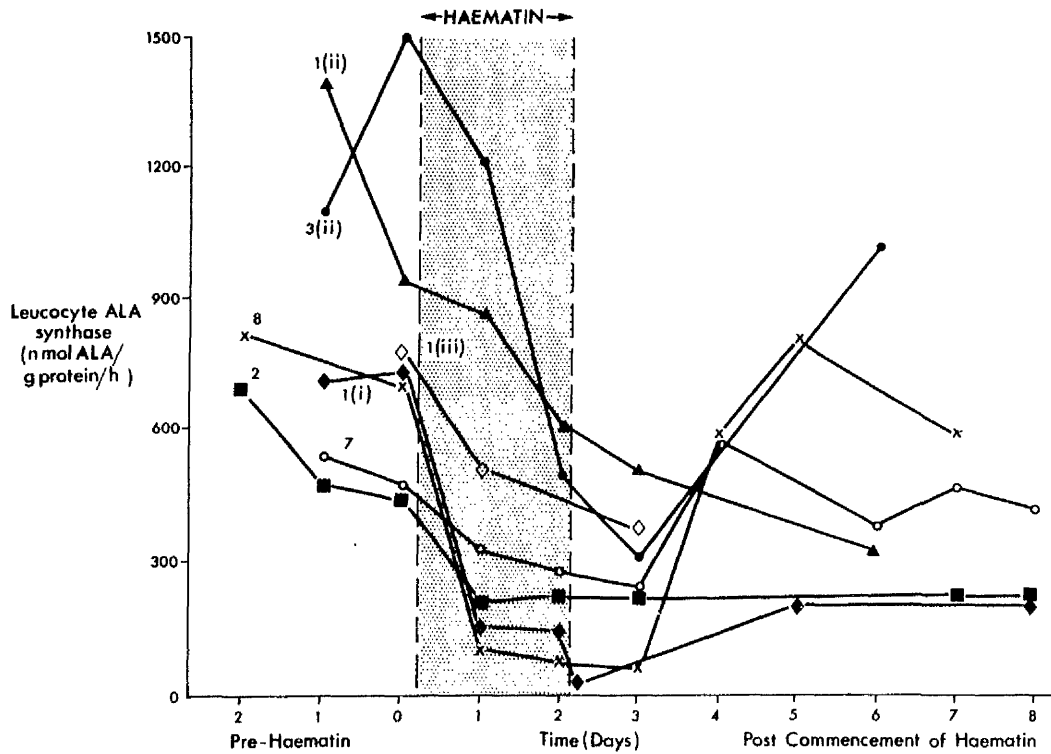


Figure 39

Effect of haematin therapy on leucocyte ALA synthase activity in 7 uncomplicated courses to 5 patients. Arabic numeral indicates case number and roman numeral course number.

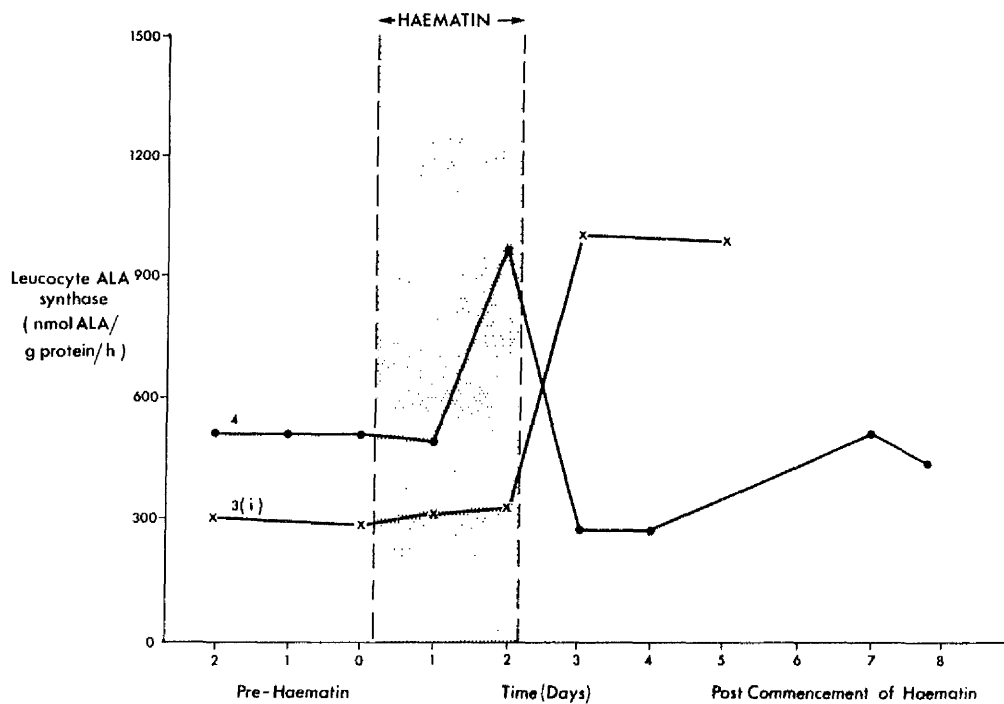


Figure 40

Leucocyte ALA synthase activity during 2 courses complicated by severe phlebitis.

	Pre - Haematin	During Haematin	Percentage change of activity
E. ALA. dehydratase ($\mu\text{mol ALA/IRBC/min}$)	19 (range = 15 - 24)	18 (range = 11 - 22)	- 5
E. URO. synthase (nmol URO/IRBC/h)	21 (7 - 28)	25 (16 - 35)	+19
E. URO. decarboxylase (nmol Copro/IRBC/h)	11 (4 - 25)	11 (4 - 21)	0
L. COPRO. oxidase ($\text{nmol Proto/g protein/h}$)	83 (40 -180)	108 (90 -140)	+25
L. Ferrochelataase ($\text{pmol mesohaem/g protein/h}$)	1.6 (0.3 - 5.0)	2.9 (0.4 - 6.2)	+81

Table 28

Effect of haematin administration on activities of enzymes of haem biosynthesis in peripheral blood cells of patients in a acute attack of AIP. The mean activity over the 3 days prior to haematin has been compared with the mean activity during haematin administration. Uroporphyrinogen-1-synthase was monitored during 5 courses and the other enzymes during only 3 courses.

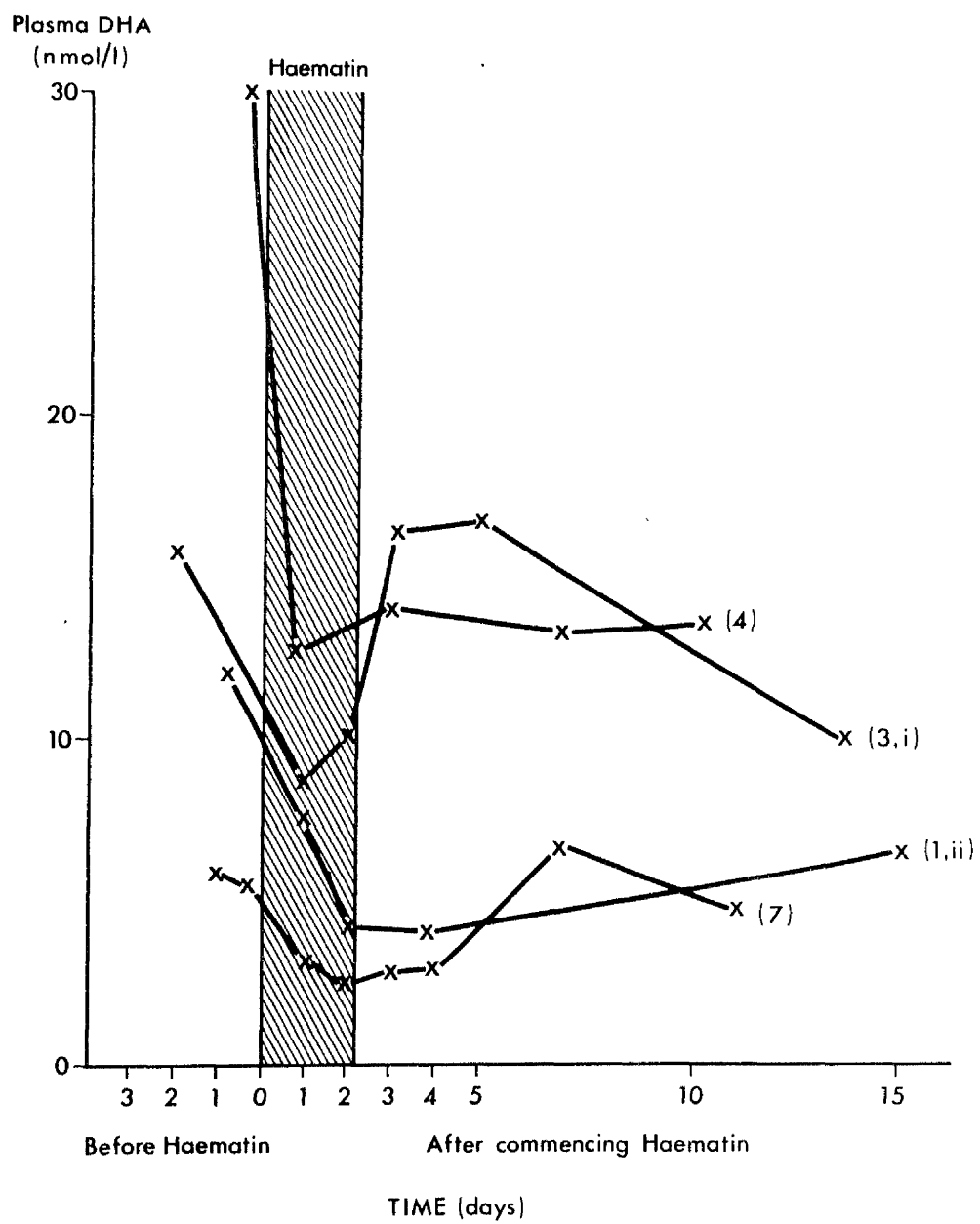


Figure 41

Effect of haematin on plasma levels of dehydroepiandrosterone in 4 patients in attack of acute intermittent porphyria.

in the other 2 patients for 2 weeks. The number of patients studied was insufficient to allow meaningful statistical analysis. We confirmed that porphyrins and their precursors did not interfere with the plasma hormone radio-immunoassay.

9.5.5. Clinical Response to Haematin Therapy

Clinical summaries of each of the 8 patients treated with intravenous haematin are presented in the Appendix.

Abdominal pain was the major symptom of the 12 attacks in which the patients remained conscious. Following haematin administration the pain was reduced in 7 attacks, unaltered in two and increased in three. Peripheral neuropathy was the major clinical sign in 10 attacks. Following haematin therapy the neuropathy objectively improved in 7 attacks, showed no change in two and continued to progress in one. When neurological improvement occurred this was observed within 7 days of commencing the haematin and slowly continued over the ensuing months. Two of the 14 attacks were fatal in spite of haematin therapy. Both of these patients had been completely paralysed and on assisted ventilation prior to the commencement of the haematin. A summary of the severity of each attack and the clinical response following haematin is shown in Table 29.

In an attempt to achieve a more objective assessment of the clinical response, the analgesic requirement was monitored in the 12 courses to conscious patients. This was reduced following five of the courses, unchanged following

	Age (years)	Sex	Clinical Class of attack *	Response to Haematin		
				Abdominal pain	Neuropathy	Outcome
Case 1						
course i	29	F	B	improved	improved	survived
course ii	29	"	A	improved	improved	"
course iii	30	"	B	improved	improved	"
course iv	30	"	B	worse	improved	"
course v	31	"	C	worse	improved	"
Case 2	26	F	B	improved	improved	survived
Case 3						
course i	23	F	A	improved		survived
course ii	25	"	A	no change		"
Case 4	19	M	A	no change		survived
Case 5	57	F	C	comatose	no change	died
Case 6	19	F	C	comatose	no change	died
Case 7	24	F	B	improved	improved	survived
Case 8	21	M	A	improved		survived

* Classification is as described by Watson et al 1978, i. e.

Class A = Includes gastrointestinal symptoms and signs, hypertension and tachycardia, psychiatric manifestations but absent or mild peripheral neuropathy

Class B = As above but severe peripheral neuropathy

Class C = Life threatening generalised neuropathy with respiratory failure

Table 29

Clinical response to haematin therapy.

three and increased following four (Table 30). The imperfect correlation between the reduction in analgesic requirement and improvement in abdominal pain is explained by the development of painful phlebitis following 2 courses of haematin. There was no discernible difference in clinical response between the two dose schedules employed.

No major side-effects were observed following haematin administration. Painful thrombophlebitis with associated mild pyrexia and leukocytosis occurred following 2 courses and mild phlebitis with no systemic upset was noted following a further 3 courses. In each case the haematin had been injected into an antecubital or more distal arm vein. Blood urea and creatinine concentrations were monitored daily in all patients and there was no evidence of any rise associated with the haematin administration. The two patients who died had moderately elevated concentrations of urea and creatinine, terminally. These, however, had been rising prior to the haematin administration and the haematin did not accelerate this rise. The cause of death in both these cases was considered to be severe chest infection and anoxia and not renal failure. At post-mortem both macroscopic and microscopic examination of their kidneys was normal. The patient who had received 6 courses of haematin over 2 years at no time showed evidence of renal impairment and post mortem examination showed normal renal histology.

	Pre-haematin analgesic requirement (mg pethidine/24h)	Post-haematin analgesic requirement (mg pethidine/24h)	Change in analgesic requirement (as % of pre- haematin dose)
Case 1			
course i	585	528	-10%
course ii	571	457	-20%
course iii	814	524	-36%
course iv	560	592	+ 6%
course v	514	635	+23%
course vi	600	600	0
Case 2	0	0	0
Case 3			
course i	571	542	- 5%
course ii	450 (+ diamorphine 8.5mg/24h)	900	?
Case 4	120	457	+280%
Case 7	300 (+ diamorphine 8.5mg/24h)	300 (+ diamorphine 17mg/24h)	increase
Case 8	335	307	- 8%

Table 30

Effect of haematin therapy on analgesic requirement in acute intermittent porphyria.

9.6. DISCUSSION

Our results confirm that intravenous haematin lowers the urinary excretion of ALA, PBG and uroporphyrin in patients in attack of acute hepatic porphyria. It has been assumed by previous workers that this is the result of haematin repressing hepatic ALA synthase activity. Our ability to demonstrate repression of this enzyme in leucocytes supports this hypothesis. In the two patients who developed marked phlebitis, leucocyte ALA synthase was not suppressed though there was a reduction in the excretion of porphyrin precursors. It is possible that the inflammatory reaction affected the leucocyte response. The clinical response was unfortunately not as consistent as the biochemical response, definite improvement accompanying only about half of the courses and in several of these it was temporary. The majority of acute attacks of porphyria resolve spontaneously and this makes it impossible to be certain that clinical improvement was due to haematin rather than spontaneous remission. Because of the rarity and nature of the condition a double blind controlled trial would be difficult to mount.

The only side-effect noted following haematin administration was inflammation and thrombosis of the veins into which the solution was injected. In a recurrent and sometimes life-threatening condition such as acute porphyria, it is important to conserve accessible peripheral veins in order to facilitate the patient's future management. The infusion of haematin through a central venous line spares the peripheral veins but patient acceptance of repeated insertion of central lines is very limited. The major toxic effect

noted following the administration of high doses of haematin to laboratory animals was generalized vascular damage. It is probable that the venous damage is similar to this, being due to the relatively high concentration of non-protein-bound haematin at the site of injection. If this is the case, phlebitis might be avoided by slower administration of a more dilute solution. The mixing of haematin with human albumin solution prior to injection might also help by reducing the non-protein-bound component. It is unfortunate that haematin in its present form has no biochemical or clinical effect when administered orally (Tschudy et al, 1979). The development of an effective oral therapy would have great advantages.

One theoretical complication of repeated courses of haematin is tissue iron overload, as every gramme of haematin contains approximately 100mg iron. In our patient who had received 6 courses over 2 years there was no evidence of hepatic siderosis at post mortem examination. The doses of iron are unlikely to be sufficient to cause tissue damage but even slight increases in hepatic iron stores might aggravate the porphyria process as is known to occur in cutaneous hepatic porphyria.

The dose of haematin currently used in the treatment of acute porphyria is largely arbitrary and may not be optimal. Animal toxicity studies indicate that there is little scope for any significant increase in dosage and indeed patients with acute porphyria, with their reduced protein binding capacity due to reduced plasma albumin concentration and occupation of binding sites by the high levels of circulating porphyrins, may be more prone to haematin toxicity (Steele et al, 1978; Morgan et al, 1980).

We found no difference in clinical or biochemical response between the 4mg/24h and the 8mg/24h regimes and it is possible that the dose could be reduced further without loss of efficacy. The use of lower doses could theoretically improve efficacy, as animal studies have demonstrated that large doses induce haem oxygenase activity which catabolizes both exogenous and endogenous haem (Srivastava et al, 1980 a, b). Studies are required of different doses and different rates of administration of haematin and may lead to considerable improvement of both the clinical and biochemical response.

Haematin therapy may help in the elucidation of the relationship between the biochemical disorder of haem biosynthesis and the clinical neuropathy. If the neuropathy is due to neuronal haem deficiency, then clinical response to haematin will depend on its ability to enter the nervous system. Lamon et al (1979) were unable to detect haematin in CSF of 2 patients with acute porphyric relapse being treated with haematin, though this may have been due to the relatively insensitive spectrophotometric method employed. Confirmation that haematin can produce clinical improvement without entering the nervous system, would disprove the neuronal haem deficiency theory. If the neuropathy is the result of the hepatic overproduction of porphyrin precursors, then clinical improvement can be expected to accompany the effective suppression of this by haematin.

One interesting observation following haematin administration was the fall in the plasma concentration of dehydroepiandrosterone. This hormone is known to be increased in the acute hepatic porphyrias due to deficient 5 alpha reductase activity (Paxton et al, 1974). The normalization of the dehydroepiandrosterone levels with haematin suggests that the abnormal steroid metabolism in the acute porphyrias is secondary to haem deficiency impairing 5 alpha reductase activity. Hepatic drug metabolism is also impaired in the acute porphyrias and this is believed to be due to deficiency of the haemoprotein enzyme cytochrome P450 (Anderson et al, 1976). Impaired hepatic metabolism in the acute porphyrias and its correction by haematin may be relevant to the aetiology of acute porphyric neuropathy. It is possible that the neuropathy is the result of deficient hepatic synthesis of a circulating neurotrophic factor or deficient hepatic catabolism of a circulating neurotoxic factor.

Our conclusion from our own studies and our review of the literature is that haematin therapy causes consistent biochemical improvement, with associated clinical improvement in approximately fifty per cent of cases. The only side-effect noted following the recommended dosage has been thrombophlebitis and this should be preventable. Because of the variable clinical response one cannot be dogmatic about its place in the management of acute attacks of porphyria. It should probably be considered in attacks which are unresponsive to the removal of precipitating factors and laevulose infusions, especially where there is evidence of developing neuropathy. It appears to have little place in the management of chronic attacks. Clearly, further research and development is indicated in order to improve the clinical response to this

potentially useful form of therapy.

One intriguing future development could be the supplementation of the hepatic haem pool with endogenous rather than exogenous haem. This could be achieved by inducing a degree of intravascular haemolysis. Haem released in this way is handled in the same way as exogenous haematin, being bound by haemopexin and transported to the liver. Haemoglobin synthesis is not impaired in the acute hepatic porphyrias and intravascular haemolysis might be a means of utilizing the considerable reserves of erythropoietic haem synthesis to correct hepatic haem deficiency. Clearly, numerous practical problems would be encountered, such as achieving controllable intravascular haemolysis, but research into the effects of haemolysis on hepatic haem biosynthesis may be fruitful.

CHAPTER 10

FINAL DISCUSSION AND CONCLUSION

10. FINAL DISCUSSION AND CONCLUSIONS

This thesis has concentrated on the management of the acute hepatic porphyrias examining, in turn, the detection of subjects with the genetic trait, the factors which may precipitate attacks in these subjects and the treatment of the established attack.

The measurement in peripheral blood cells of the activities of both the rate-controlling enzyme of haem biosynthesis, ALA synthase, and the appropriate genetically deficient enzyme has been shown to be a sensitive and specific means of detecting latent cases of acute porphyria. Enzymatic screening of affected families has confirmed that the genetic trait is inherited in an autosomal dominant, sex-independent manner. The family studies have also demonstrated that the vast majority of subjects with the genetic trait remain clinically latent, and the reason why a minority develop severe clinical manifestations was examined. There was no difference in the activity of the genetically-deficient enzyme in clinically latent and manifest cases. The activity of ALA synthase, however, was higher in patients who had experienced attacks of AIP and it is suggested that this may reflect higher circulating levels of endogenous inducing agents such as certain steroid hormones. A study comparing endogenous inducing agents in these two groups is indicated and may reveal a potential means of reducing the incidence of clinical attacks.

The effects on haem biosynthesis of each of the precipitating factors for acute porphyria, namely drugs, alcohol, hormones and fasting were

examined in peripheral blood cells of non-porphyrinic subjects. All, except fasting, increased the activity of ALA synthase, confirming the means by which they cause biochemical relapse in porphyric subjects. The mechanism by which these factors stimulate ALA synthase activity was investigated with particular reference to the role of induction of the mixed function oxidase enzyme system. The relevance of the observed changes in haem biosynthesis induced in peripheral blood of healthy subjects by porphyrinogenic agents extends outwith the field of the porphyrias. The stimulation of ALA synthase in leucocytes demonstrates the important fact that enzyme induction in human subjects is not restricted to hepatic tissue. The effects of alcohol on haem biosynthesis in blood cells is relevant to the biochemical basis of alcohol-induced sideroblastic anaemia, and the hormonal effects on blood ALA synthase to the hormonal regulation of erythropoiesis.

The two currently available means of treating attacks of acute porphyria have been assessed. The early administration of intravenous laevulose resulted in reduction of both duration and severity of attacks, though, in patients experiencing frequent attacks, venous access soon became compromised. Intravenous haematin therapy resulted in biochemical improvement, shown by repression of leucocyte ALA synthase and reduction in the urinary excretion of porphyrin precursors. The clinical response, however, was less consistent, with objective signs of improvement accompanying only half of the courses. The only side-effect noted following haematin therapy was

localized thrombophlebitis. Two of our patients died in acute attack in spite of receiving both laevulose and haematin, illustrating that current therapy remains unsatisfactory. Advancement of the therapy of acute hepatic porphyria is hindered by our lack of understanding of the pathogenesis of the neuropathy.

The elucidation of the association between the biochemical abnormality of haem biosynthesis and the neuropathy which characterizes the clinical presentation of the acute porphyrias, remains a major challenge. Our observation that the effect of porphyrinogenic factors on haem biosynthesis are not limited to hepatic tissue lends some support to the hypothesis that the neuropathy is due to disordered haem biosynthesis within nervous tissue, though, to date, it has not been possible to demonstrate that porphyrinogenic agents are modifying intra-neuronal haem biosynthesis. Further studies of neuronal haem biosynthesis and its response to porphyrinogenic factors are required. The theory that the clinical attack is due to neurotoxic effects of porphyrin precursors is, at present, widely discarded, due to the inability to reproduce such effects in animal models. The development, however, of biological models of metabolic neurological disease is fraught with technical difficulties and, therefore, the porphyrin-precursor neurotoxicity theory should not be abandoned too readily. It is conceivable that both theories are correct and that impaired neuronal haem biosynthesis makes the nervous system more susceptible to damage by the

high circulating levels of porphyrin precursors. The pursuit of the association between abnormal haem biosynthesis and neurological dysfunction is likely to contribute significantly to the understanding and treatment of other more common neurological and psychiatric disorders, and should be actively encouraged.

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- November 1979 "Enzyme induction in peripheral leucocytes of patients on phenytoin anti-convulsant therapy"
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- July 1980 "The Acute Porphyrias"
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- September 1980 "Haematin Therapy for Acute Hepatic Porphyria"
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- September 1980 "A Review of Haematin Treatment of the Acute Hepatic Porphyrias"
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A P P E N D I X

CLINICAL CASE HISTORIES
OF PATIENTS TREATED WITH HAEMATIN

CLINICAL CASE HISTORIES OF PATIENTS TREATED WITH HAEMATINCase 1

This 29 year old woman was admitted in May 1978 with severe abdominal pain, vomiting and constipation. She was known to have had AIP since 1971 and had suffered 13 previous attacks requiring hospital admission but only two of these had been complicated by peripheral neuropathy. On most previous admissions she had been treated with I. V. 20% laevulose, but this was no longer feasible as all suitable veins had been compromised. She was, therefore, treated with nasogastric infusions of the glucose polymer Caloreen (Roussel Laboratories Ltd) amounting to 2,000 kcal/24 h. Over the ensuing 6 weeks she gradually developed peripheral motor neuropathy and by early July there was marked distal paresis of upper and lower limbs and diminished respiratory function. On July 5th., her first course of intravenous haematin (4mg/kg/24 h for 3 days) was commenced. On the day following the final infusion she felt much improved. There was also improvement in her respiratory function which was maintained. Her peripheral motor function improved slowly over the following 7 weeks. At the end of August, however, she developed increasing abdominal pain and generalized muscle pains and evidence of peripheral sensory neuropathy. On 31st. August, her second course of haematin was commenced (8mg/kg/24 h for 3 days). The day following completion of this she again felt much improved and required less analgesia. Sensory discrimination returned to normal within 3 days.

Improvement was maintained and 4 weeks later she was able to be discharged home and able to walk with the aid of a zimmer. She was re-admitted 8 months later on 18th. April 1979 with a further attack. On the 3rd. May, there was evidence of motor and sensory peripheral neuropathy and some deterioration of respiratory function. On 6th. May, her third course of haematin (4mg/kg/24 h for 3 days) was commenced. Following this there was again improvement of respiratory function and a reduction in her analgesic requirement, and she was able to be discharged home 12 days later. The biochemical and clinical response to the first 3 courses of haematin are shown in Figure 42.

On 5th. July 1979 she was re-admitted in a further attack, and found to be 4 weeks pregnant. She was unwilling to consider a therapeutic abortion, and over the following weeks developed generalized neuropathy with severe impairment of respiratory function and bulbar paralysis and also severe hypertension. Eventually, she agreed to a therapeutic abortion and this was performed on 23rd. August. The day following, her fourth course of haematin (4mg/kg/24 h for 6 days) was commenced. She felt generally improved, and there was some objective evidence of improved motor function over the following month. On the 9th. October, however, the pain again became much more severe and, as she appeared to be going into a further attack, a fifth course of haematin (4mg/kg/24 h for 3 days) was commenced. She felt better on the final day of the course and again it was possible to reduce her analgesia. The blood pressure, however, remained high and 7 days after the haematin she

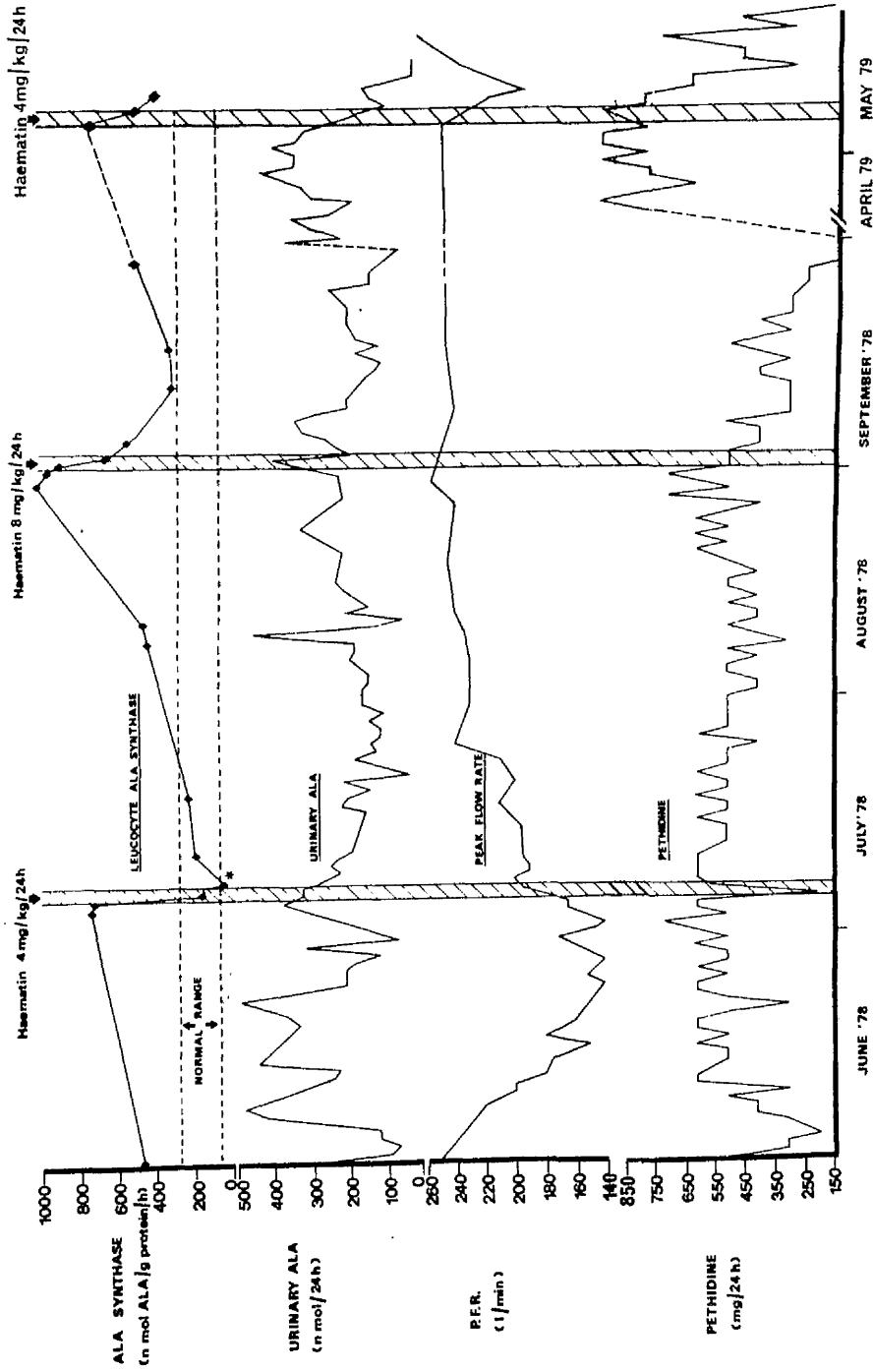


Figure 42

Effect of 3 courses of haematin on leucocyte ALA synthase activity, urinary ALA excretion, respiratory peak flow rate and analgesic requirement in 31 year old woman (Case 1) with prolonged attack of acute intermittent porphyria.

developed a hypertensive exudative retinopathy (Fig. 43). The blood pressure settled 2 weeks later and she was discharged home.

On 20th. May 1980 she was re-admitted with abdominal pain and weakness of dorsiflexion of both hands. Her pain and paralysis increased over the following week and on 3rd. June her sixth course of haematin (4mg/kg/24 h for 2 days) was commenced. On the second day of haematin therapy she felt and looked more ill. There was marked peripheral vasoconstriction. Her temperature was elevated at 38.5°C and the total white cell count was increased at $19.2 \times 10^9/l$ due to a neutrophilia. No infection could be isolated from the patient or the haematin solution. She was commenced on intravenous ampicillin and flucloxacillin. On 5th. June, she was transferred to the intensive care unit because of progressing paralysis and ventilatory embarrassment. The following day she had an episode of cardiac asystole lasting 20 minutes from which she was successfully resuscitated. There was no evidence of cerebral impairment following this but she required to be maintained on the ventilator for the following 10 weeks due to severe paralysis. In mid August she was weaned-off the ventilator. Two weeks later she developed a further attack with increasing pain and paralysis. On 28th. September, 1980, she had a further episode of asystole and resuscitation was unsuccessful.

Post mortem examination showed acute tracheitis extending down both bronchi. In addition there were several focal areas of pulmonary infarction. The heart and kidneys appeared normal. Detailed histological examination of the central nervous system revealed no abnormalities. The peripheral somatic and autonomic nerves have been submitted for special studies and the results are awaited.

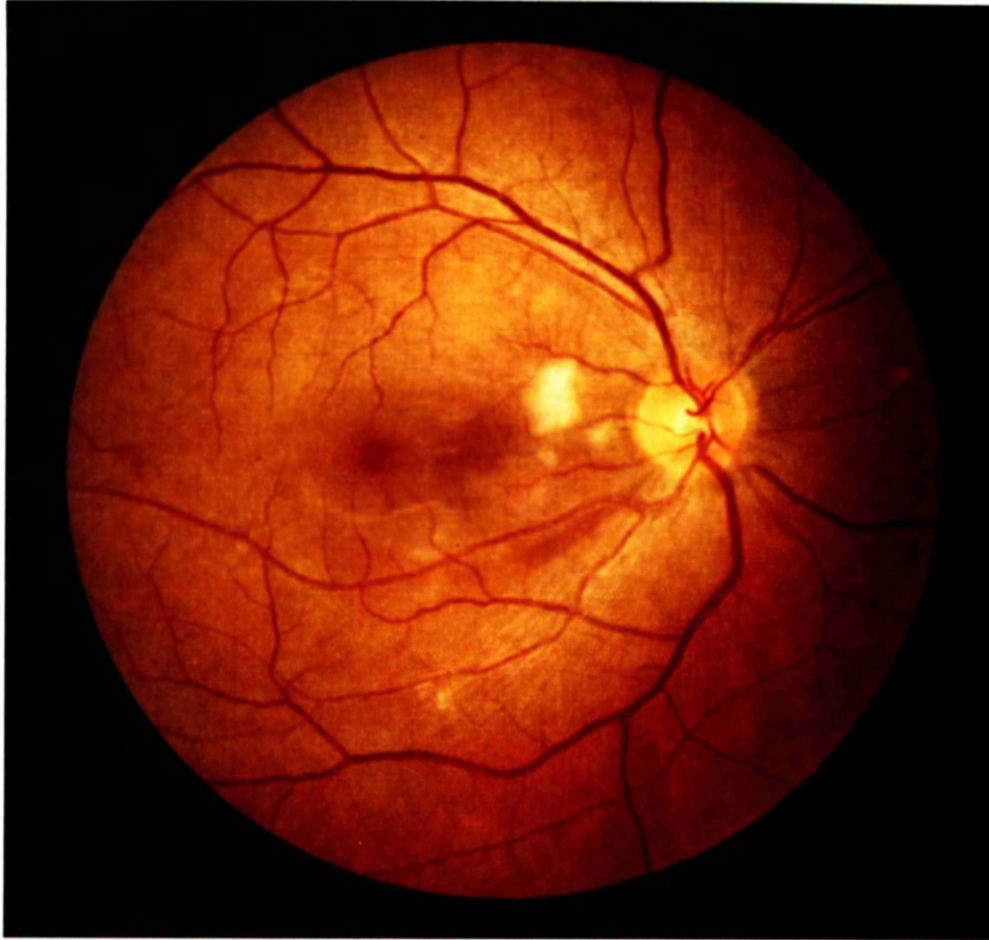


Figure 43

Hypertensive retinopathy due to attack of acute intermittent porphyria in 31 year old woman (Case 1).

Case 2

This 26 year old woman, with a family history of AIP, was admitted to Manchester Royal Infirmary in February 1978 with abdominal pain, weakness of the lower limbs and strange mental behaviour. She was confirmed to be in an attack of porphyria. She had a past history of severe bronchial asthma for which she had been receiving dexamethasone 2mg T. I. D. for the past 6 years. The steroids were continued and she was given a high calorie and protein diet. Over the following 5 months she continued to have frequent attacks of severe abdominal pain. Her mental state also became worse and she suffered from depression, paranoid delusions and auditory and visual hallucinations which made her in-patient care very difficult. In August 1978, she was transferred to our unit for assessment for haematin therapy. On arrival in Glasgow she complained of abdominal pain, nausea, constipation and painful weakness of all her limbs. Pulse and blood pressure were normal but there was marked peripheral motor neuropathy and she was unable to walk. She was convinced that the nurses were poisoning her food and only ate food in sealed containers and refused all oral medication. The consultant psychiatrist who assessed her was of the opinion that she was suffering from an exogenous psychosis without clouding of consciousness, secondary to her porphyria. Her albumin was low at 27g/l, cholesterol increased at 10.5 mmol/l, and urate, gamma-glutamyl transpeptidase, aspartate transaminase and alanine transaminase also moderately increased. Haematology was normal. An electroencephalogram was normal and nerve conduction studies showed prolongation of distal motor latencies in the feet.

On the 16th. August, she was commenced on a 4 day course of haematin in a dosage of 4mg/kg/day. This caused mild phlebitis of the peripheral vein but no other side-effects. On the final day of the haematin, she felt a marked improvement of her abdominal and generalized muscle pains. This improvement was short lived, her abdominal pain returning 2 days later and her muscle pains 7 days later. Over the 10 days following the haematin, there was objective improvement in motor function as assessed by our consultant neurologist. The haematin had no effect on her mental state. She was able to be discharged home 12 days after completion of the haematin therapy. When reviewed in Glasgow two months later, she felt generally improved and was able to walk unassisted.

Over the subsequent six months at home, she suffered from severe mental disturbance, which resulted in domestic strife. She became very paranoid and demanding towards her husband and committed a large number of petty criminal offences including, shoplifting and swindling mail order companies. She trained her children to steal library books and messages for her. On two occasions she went missing and was found by the police camping alone in the moors. On one occasion her husband arrived home to find their 6 hens with their heads cut-off and on another occasion to find their pet cat burnt alive having been soaked in paraffin. In April 1979, she was found unconscious in her back garden having attempted to commit suicide by taking an overdose of diazepam. After recovering from this she continued to exhibit bizarre mental behaviour and complained of intermittent abdominal pains. She refused any further medical attention. On 20th. January 1980, she tragically took a

further and fatal drug overdose. Interestingly, her mother had suffered severe psychiatric disturbance due to porphyria between 25-30 years of age but had no trouble thereafter and now occupies an important government position. The following poem was written by the patient 6 months before her death.

That man is coming here once again
I hope from chastising me he does refrain
For with great fear and trepidation I wait
Just what is to be that day's fate.

It could be new treatment
Of which I am in need
But who am I to decide this indeed
One day, however, I do know
That I shall die even
As the wind doth blow.

Unconscious sleep will come to me
Giving me peace from that everlasting gasp.

Oh, doctor Sir you must help me
Don't cross me off your books as a failure
For I do wish to respond
And be a credit to your work
thus making your job seem worthy of you.

Case 3

This 23 year old girl was admitted on 1st. November 1978 with severe abdominal and generalized muscle pains, which had developed following the consumption of a moderate quantity of alcohol. She had been diagnosed to have AIP in March 1976 and had had 10 previous attacks but never any evidence of peripheral neuropathy. Her abdominal and muscle pains did not respond to carbohydrate administration and she was requiring large doses of parenteral narcotic analgesia. Three weeks after admission on the 20th. November, intravenous haematin (4mg/kg/12 h for 3 days) was commenced. The final infusion, which was into a small peripheral vein, resulted in severe thrombophlebitis with associated pyrexia and leucocytosis. On the final day of the haematin she felt that the "porphyria pains" were relieved but continued analgesia was required because of the painful thrombophlebitis. Five days after completion of the haematin, the "porphyria pains" returned and she was unable to get home for a further 6 weeks.

Over the following year she experienced recurrent attacks of abdominal pain and was rarely free of pain. She was re-admitted on 19th. February 1980 with severe abdominal and generalized muscle pains, photophobia and vomiting. She was given large doses of diamorphine and high calorie nasogastric infusions. Two days later she was no better and had a tachycardia of 120/min but no evidence of peripheral neuropathy. On 20th. February she was commenced on a 3 day course of haematin (4mg/kg/day). Two days after completing the haematin, she felt slightly better and the pain was less severe. Overall,

however, the haematin had very little discernible effect on her clinical condition and she remained in hospital requiring intramuscular analgesia for a further 4 months. No phlebitis occurred with this second course which was administered into a central venous line.

Case 4

This 19 year old man with AIP was transferred to our unit for haematin therapy in March 1979. Over the previous 2 years he had suffered frequent attacks of abdominal pain and vomiting and had rarely been symptom free. No precipitating factors were identified. He had never suffered mental disturbance but there was some mild peripheral sensory neuropathy. The day following his transfer his symptoms became more severe and he developed tachycardia and hypertension. Two days later a 3 day course of haematin (4mg/kg/24 h) was commenced. Following the final infusion he developed severe phlebitis with associated low grade pyrexia and leucocytosis (Fig. 44). The haematin did not result in any subjective or objective clinical improvement.



Figure 44

Severe phlebitis due to haematin injection in right arm of 19 year old man (Case 4) with acute intermittent porphyria.

Case 5

This 57 year old woman with no significant past or family history was admitted to hospital in Birmingham in February 1979 with abdominal pain, vomiting and constipation. She was diagnosed as having cholecystitis, and a cholecystectomy performed on the same day. She was discharged 5 days later but over the following 2 weeks developed confusion, bizarre behaviour and peripheral neuropathy. On re-admission, investigations revealed acute attack of porphyria variegata with associated dilutional hyponatraemia. In spite of intravenous administration of 20% laevulose and correction of the hyponatraemia, her neuropathy progressed and she became quadriplegic and required mechanical ventilation. Ten days later there was no improvement and a 3 day course of haematin (8mg/kg/24 h) was commenced. In spite of this, her condition continued to deteriorate and she developed a chest infection, hypotension and mild renal impairment and eventually died two weeks later.

Post mortem examination showed extensive chest infection. Histological examination of the kidneys was normal.

Case 6

This 19 year old girl was admitted to hospital in Liverpool on 6th. May 1979 with severe lower abdominal pain. She was initially thought to have acute salpingitis or urinary tract infection and cotrimoxazole was prescribed. Two days later her pain became more severe and she developed dilutional hyponatraemia and convulsions. Urinalysis at this point confirmed an acute attack of AIP. In spite of stopping all contra-indicated drugs and administering laevulose infusions, she continued to deteriorate. She developed quadriparesis and required assisted ventilation. On 22nd. May, a 3 day course of haematin (8mg/kg/24 h) was commenced. Over the following 2 weeks she continued to deteriorate with extensive chest infection, hypotension and latterly deteriorating renal function. She died on 7th. June.

Post mortem showed extensive chest infection. Histological examination of the kidneys was again normal.

Case 7

This 24 year old woman was admitted to our unit on 26th. February 1979 with abdominal pain, vomiting and constipation. She had had 12 previous admissions for acute attacks of AIP over the preceeding 3 years. She was treated with high carbohydrate nasogastric infusions but, in spite of this, developed peripheral motor neuropathy of upper and lower limbs. On 2nd. March, a 3 day course of haematin (4mg/kg24 h) was instituted. On the final day of therapy she felt that the pain was much improved and 3 days later her peripheral reflexes had returned. Eight days after completion of therapy she relapsed with further progression of her neuropathy. She also complained of severe toothache and was found to have extensive caries and several septic foci. Dental clearance was performed and following this her neuropathy slowly recovered and she was able to be discharged home one month later. Over the subsequent year she was re-admitted on 5 occasions in attack but none of these were complicated by neuropathy and all settled with symptomatic therapy and high calorie intake.

Case 8

This 21 year old man (brother of case 7) was admitted to our unit on 21st. September 1979 with a 7 day history of abdominal pain, vomiting and constipation. There was no peripheral neuropathy but he had marked postural hypotension. He was treated symptomatically and given high oral carbohydrate intake. Seven days after admission he remained in severe pain and a 3 day course of haematin (4mg/kg24 h) was commenced. Two days after completion of haematin the pain settled and he was able to be discharged 10 days later. The haematin resulted in mild phlebitis.